GRIGORE T. POPA UNIVERSITY
OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

CLINICAL, BIOLOGICAL AND EXPERIMENTAL APPROACHES FROM ORTHODONTIC PRACTICE

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2019

CONTENTS

ABSTRACT

The habilitation thesis entitled "*Clinical, Biological and Experimental Approaches in Orthodontic Practice*" reveals the main scientific achievements after obtaining the title of Doctor of Medicine, the Orthodontic specialty. The thesis is structured in three sections according to the CNATCU recommendations.

Before the first section I briefly presented the academic, professional and scientific achievements of my teaching career (25 years), medical (31 years) and research.

The first section is dedicated to scientific research carried out during the post-doctoral period (1998-2019), being structured in 6 research fields (6 chapters). This section presents in 123 pages the results of 48 published articles (17 ISI and 31 IDB articles) of personal research and in collaboration with multidisciplinary teams of other universities from Iasi, exemplified in 31 tables and 59 figures.

The first chapter was aimed to assess aspects, behaviors and opinions of patients, practitioners and students about orthodontic practice in the context of dentistry practice. The results of the clinical and statistical studies and surveys presented were specific to the geographical area and the investigated period time. Thus, the common profile of the orthodontic patient in the public service was sketched; the marketing methods of competitive private dental clinics/offices were identified; the degree of patients' satisfaction has been established regarding the quality of private dental services; the degrees of patients' anxiety has been identified for some therapeutical procedures, depending on their age and social background; the disadvantages of MCQ tests for students in assessing their medical training were revealed; and a positive feedback from last-year students on their training for a future orthodontic career was identified.

The second chapter was conducted in the context of the general development of the child and teenager, which gains skills and behaviors through learning and education from parents, school and community. The presented studies were aimed to assess the degree of education for general and oral health, means of preventing illness and the impact of health on the self-reported physical, psychological and social well-being of children and teenagers. Survey results have shown that the induction of sanogenous behaviors in children is carried out in stages, corresponding to the general growth of the child. Also, parents, school and community have to work together through education. The physical, psychological and social well-being of schoolchildren depends on general and oral health, being maintained in the socio-economic status of the family and accessibility to health services.

The third chapter addressed dental anomalies that occurred during the development of permanent dentition due to general factors or during dental eruption due to local factors. Clinical studies aimed at assessing hypodontia and impacted teeth and the damage caused by arcades symmetry, dental occlusion and dental-facial aesthetics. The results showed that the absence of an arcade tooth produces dento-alveolar compensatory movements and occlusal, skeletal and joint imbalances. An important aspect was the presentation of the family inheritance of hypodontia and possible environmental mutagens. Various clinical forms of congenital missing teeth or impacted teeth required interdisciplinary and individualized treatments to restore the dento-maxillo-facial balance. Although these dental anomalies seem simple at first glance, they are frequent in the Romanian population and the complexity of orthodontic, surgical and restorative therapy reveals the severity of these anomalies.

The fourth chapter aimed to assess the clinical and therapeutical evaluation of dento-maxillary anomalies characteristic of the investigated population, as well as severe skeletal and

maxillo-facial anomalies. The results of the studies showed that there is a link between the time of identification (the patient's age at first consultation), the type of dentition, the severity of the dental crowding and/or crossbite and the type of orthodontic treatment instituted. Although these anomalies are visible, patients did not go to the orthodontist when the first signs of the anomaly appeared and so there were discrepancies in the jaw bases (Class II or Class III skeletal) and the treatment was complex. Orofacial clefts and dento-maxillo-facial asymmetries represent difficult orthodontic anomalies due to the complexity of intra- and inter-arches disorders and their treatment is interdisciplinary especially when associated with other general disorders.

The fifth chapter aimed to assess the clinical and biological study of the biocompatibility of some components of the orthodontic appliances in the context of the controversial results from literature. Clinical results have shown that although many localized gingivitis have been found in orthodontic patients studied, not all are reactions to the allergens in orthodontic appliances components. The orthodontic archwires made from nickel-titanium alloys retrieved from patients have been degraded in the oral environment, indicating that they become corrosive, reducing biocompatibility and releasing metal ions in saliva. Although this fact is proven by other researchers, we have identified nickel in the saliva of subjects without orthodontic treatments, which raises questions about the environment. All latex-based tested materials were cytotoxic to human fibroblast cultures, indicating that latex acts as an allergen.

The sixth chapter was intended for technological experiments that had the purpose to improve the quality of orthodontic biomaterials and antibiotics used in oral cavity infections during orthodontic treatments. Studies of titanium-based orthodontic mini/micro-implants used in orthodontic anchorages coated with hydroxyapatite, titanium and zinc oxides or silver doped have been presented to improve the osseo-integration process and to prevent implant loss and oral infections. Also, a new iron-based material was synthesized and the electrochemical corrosion rate in biological medium (simulated body fluid solution) was evaluated. The effect of nickel-titanium alloy shape memory was evaluated by designing a respiratory ventilation device for patients who suffer from sleep apnea. Improvement in the controlled release of antibiotics (amoxicillin and clavulanic acid, doxycycline, metronidazole and ampicillin) used in dental practice was evaluated by obtaining antibiotic-loaded chitosan particles, liposome-loaded chitosan hydrogels and antibiotic/ ascorbic acid intercalated hydrotalcites particles.

The second section describes the future directions of research that will continue the main topics presented in this thesis, from which new ones will develop as I get new results.

The third section contains the 811 bibliographical references used in the development of the habilitation thesis.

REZUMAT

Teza de abilitare cu titlul "*Abordări clinice, biologice și experimentale din practica ortodontică*" relevă principalele realizări științifice după obținerea titlului de Doctor în Medicină, specialitatea Ortodonție. Teza este structurată în trei secțiuni conform recomandărilor CNATCU.

Înainte de prima secțiune am făcut o scurtă prezentare a realizărilor academice, profesionale și științifice din cariera mea didactică (25 ani), medicală (31 ani) și de cercetare.

Prima secțiune este destinată cercetărilor științifice realizate în perioada postdoctorală (1998-2019), fiind structurată în 6 domenii de cercetare (6 capitole). Această secțiune prezintă în 123 de pagini rezultatele a 48 de articole publicate (17 articole ISI și 31 BDI) ale cercetărilor personale și în colaborare cu echipe de lucru multidisciplinare din alte universități ieșene, exemplificate în 31 de tabele și 59 figuri.

Primul capitol a avut ca scop evaluarea aspectelor, comportamentelor și opiniile pacienților, practicienilor și studenților privind practica ortodontică în contextul practicii medicinii dentare. Rezultatele studiilor clinico-statistice și anchetelor prezentate au fost specifice zonei geografice și a perioadei de timp investigate. Astfel, a fost schițat profilul obișnuit al pacientului ortodontic din serviciul public; au fost identificate metodele de marketing a clinicilor/cabinetelor stomatologice private competitive; a fost stabilit gradul de satisfacție a pacienților privind calitatea serviciilor stomatologice private; a fost identificat gradul de anxietate a pacienților pentru unele procedee terapeutice, în funcție de vârstă și mediul social; au fost relevate dezavantajele testelor MCQ pentru studenți în evaluarea pregătirii lor medicale; și a fost identificat un feed-back pozitiv de la studenții anilor terminali privind pregătirea lor pentru o carieră ortodontică viitoare.

Al doilea capitol a fost realizat în contextul dezvoltării generale a copilului și a adolescentului, dobândind abilități și comportamente prin învățare și educare de la părinți, școală și comunitate. Studiile prezentate au avut ca scop evaluarea gradului de educație pentru sănătatea generală și orală, mijloacele de prevenire a îmbolnăvirilor și impactul sănătății asupra bunăstării fizice, psihologice și sociale auto-raportate a copiilor și adolescenților. Rezultatele anchetelor au arătat că inducerea comportamentelor sanogene la copii se realizează în etape, corespunzătoare creșterii generale a copilului. De asemenea, părinții, școala și comunitatea trebuie să intervină împreună prin educație. Bunăstarea fizică, psihologică și socială auto-raportată de elevi depinde de sănătatea generală și orală, fiind menținută în contextul status-ul socio-economic al familiei și a accesibilității la serviciile de sănătate.

Al treilea capitol a abordat anomaliile dentare care au apărut în timpul dezvoltării dentiției permanente datorită factorilor generali sau în timpul erupției dentare datorită factorilor locali. Studiile clinice au avut ca scop evaluarea hipodonției și a incluziei dentare și prejudiciile aduse simetriei arcadelor, ocluziei dentare și a esteticii dento-faciale. Rezultatele au arătat că lipsa unui dinte de pe arcadă produce mișcări compensatorii dento-alveolare și dezechilibre ocluzale, scheletale și articulare. Un aspect important a fost prezentarea transmiterii familiale a hipodonției și posibilii factori mutageni de mediu. Formele clinice variate ale dinților absenți congenitali sau incluși au impus tratamente interdisciplinare și individualizate pentru restabilirea echilibrului dento-maxilo-facial. Deși aceste anomalii dentare par simple la prima vedere, ele sunt frecvente în populația românească, iar complexitatea terapiei ortodontice, chirurgicale și restaurative relevă severitatea acestor anomalii.

Al patrulea capitol a avut ca scop evaluarea clinică și terapeutică a anomaliilor dento-maxilare caracteristice populației investigate, cât și a anomaliilor severe scheletale și maxilo-faciale. Rezultatele studiilor au arătat că apare o legătură între momentul identificării (vârsta

pacientului la prima consultație), tipul dentiției, forma de severitate a înghesuirii dentare și/ sau a angrenajului invers și tipul de tratament ortodontic instituit. Deși aceste anomalii sunt vizibile, pacienții nu s-au prezentat la ortodont la primele semne ale anomaliei și astfel au apărut decalajele bazelor maxilare (clasa a II-a sau a III-a scheletală), iar tratamentul a fost complex. Despicăturile oro-faciale și asimetriile dento-maxilo-faciale reprezintă anomalii dificile pentru ortodont, datorită complexității tulburărilor intra- și inter-arcadice, iar tratamentul lor este interdisciplinar, mai ales când se asociază cu alte afecțiuni generale.

Al cincilea capitol a avut ca scop studiul clinic și biologic al biocompatibilității unor componente ale aparatelor ortodontice în contextul rezultatelor controversate din literatura de specialitate. Rezultatele clinice au arătat că deși s-au găsit multe gingivite localizate la pacienții ortodontici studiați, nu toate sunt reacții la alergenii proveniți din componența aparatelor ortodontice. Arcurile ortodontice din aliaje de nichel-titan recuperate de la pacienți au fost degradate în mediul oral, ceea ce indică că acestea devin corozive, reducând biocompatibilitatea și eliberând ionii metalici în salivă. Deși acest fapt este dovedit și de alți cercetători, am identificat nichelul în saliva subiecților fără tratamente ortodontice, ceea ce ridică întrebări asupra mediului înconjurător. Toate materialele testate pe bază de latex au fost citotoxice pentru culturile de fibroblaste umane, ceea ce arată că latexul acționează ca un alergen.

Al șaselea capitol a fost destinat unor experimente tehnologice care au avut ca scop îmbunătățirea calității biomaterialelor ortodontice și a antibioticelor utilizate în infecțiile cavității orale din cursul tratamentelor ortodontice. Au fost prezentate studii ale mini/micro-implantelor ortodontice pe bază de titan utilizate în ancorajul ortodontic, acoperite cu hidroxiapatită, oxizi de titan și zinc sau dopate cu argint pentru a îmbunătăți procesul de osteo-integrare și a preveni pierderea implantului și a infecțiilor orale. De asemenea, s-a sintetizat un nou material pe bază de fier și s-a evaluat rata de coroziune electrochimică în mediu biologic (soluție simulată a fluidului corpului). Efectul memoriei formei aliajelor din nichel-titan a fost evaluat prin proiectarea unui aparat de ventilație respiratorie pentru pacienții cu apnee în somn. Îmbunătățirea eliberării controlate a antibioticelor (amoxicilina și acidul clavulanic, doxiciclina, metronidazol și ampicilină) utilizate în practica stomatologică a fost evaluată prin obținerea particulelor de chitosan încărcate cu antibiotic, a hidrogelurilor de chitosan încărcate cu lipozomi și a particulelor de hidrotalcite intercalate cu antibiotic/ acid ascorbic.

A doua secțiune descrie direcțiile viitoare de cercetare care vor continua principalele teme prezentate în această teză, din care se vor dezvolta și altele noi pe măsură ce voi obține noi rezultate.

A treia secțiune conține 811 referințele bibliografice utilizate în elaborarea tezei de abilitare.

BRIEF PRESENTATION OF ACADEMIC, PROFESSIONAL AND SCIENTIFIC ACHIEVEMENTS

ZEGAN GEORGETA, DMD, PhD, Orthodontist
Professor at the Discipline of Orthodontics and Dento-facial Orthopedics, Department of Surgery, Faculty Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy of Iasi

ACADEMIC POSITIONS:

2016 – present	**Professor** at the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2005 – 2016	**Associate Professor** at the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
1999 – 2005	**Lecturer** at the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
1994 – 1999	**Assistant Professor** at the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi

PROFESSIONAL POSITIONS:

1999 – 2014	**Consultant in Orthodontics** at the Orthodontics Clinic of the Ambulatory of the "St. Spiridon" Universitary Emergency Hospital of Iasi
1994 – 1999	**Specialist in Orthodontics** at the Orthodontics Clinic of the Pediatric Dentistry Department of the "St. Spiridon" University Hospital of Iasi
1990 – 1994	**Postgraduate student in Orthodontics and Pedodontics** at the Orthodontics Clinic of the Pediatric Dentistry Department of the "St. Spiridon" University Hospital of Iasi
1988 – 1990	**Dentist** at the Cozmesti Office of the Raducaneni Hospital of the Health Department of Iasi
1985 – 1988	**Vocational Dental Training** at the "St. Spiridon" County Clinical Hospital of Iasi

COMPETENCES:

2012 June	**Academic Trainer** by the Ministry of Labour, Family, Social Protection and the Elderly, and the Ministry of National Education of Romania
2006 – present	**Postgraduate education instructor** of Master Degree in Orthodontics by the Ministry of Health of Romania
1999 March	**Consultant in Orthodontics** by the Ministry of Health of Romania
1997 – 1998	**Academic Specialization** in Psychology and Pedagogy by the "Alexandru Ioan Cuza" University of Iasi, and the Ministry of Education of Romania
1994 – 1998	**PhD in Medicine**, Orthodontics specialization by the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, and the Ministry of Education of Romania
1994 March	**Master Degree in Orthodontics** by the Ministry of Health of Romania
1980 – 1985	**Bachelor of Dental Medicine** by the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, and the Ministry of Education and Teaching of Romania

WORK EXPERENCE:

25 years	Teaching experience
31 years	Medical experience
21 years	Postdoctoral research experience

ACADEMIC WORK:

2012 – 1016	Member of the Council of the Faculty of Dental Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2008 – 2015	Coordinator of didactic activity at the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2008 – 2012	Head of Pedodontics-Orthodontics Department of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2011 – present	Member of the Board of inventory management of the Discipline of Orthodontics and Dento-facial Orthopedics of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2013	Projects evaluator of Grant intern research of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
2003 – 2007	Coordinator doctor of medical activities in the Botosani County of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
1999 – 2003	Coordinator doctor of medical activities in the Bacau and Vaslui Counties of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
	Doctoral committee member (2 referent, 7 instructor of scientific project) of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi
	President/member of exams and competitions committee (license, admission, job occupation, specialty) of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi

POSTGRADUATE COURSES ABSOLVED:

3 courses	of General and medical management
1 course	of Facial electromyography
21 courses	of Orthodontic techniques
5 courses	of Medical Statistics

MEDICAL WORK:

2015 – present	Member of the Council of College of Dentists of Iasi, Commission of specialty
2006 – present	Postgraduate education instructor of Master Degree in Orthodontics
2006 – present	Accredited lecturer of College of Dentists from Romania (annuals)

SCIENTIFIC RESEARCH WORK:

2009 – present	Editorial staff member at International Journal of Medical Dentistry (IDB)
2011 – present	Reviewer at Romanian Journal of Oral Rehabilitation (IDB)
2014 – present	Reviewer at Indian Journal of Dental Research (IDB)
2017	Reviewer at the 6th IEEE International Conference on e-Health and Bioengineering 22-24 Jun 2017 Sinaia, Romania (ISI)
2015	Reviewer at the 5th IEEE International Conference on e-Health and Bioengineering 19-21 Nov 2015 Iasi, Romania (ISI)
2013	Reviewer at the 4th IEEE International Conference on e-Health and Bioengineering 19-21 Nov 2013 Iasi, Romania (ISI)
2005 – 2009	Editorial staff member at Journal of Romanian Dentistry (CNCSIS)
2000 – 2004	Editorial staff member at Romanian Journal of Orthodontics and Dento-facial Orthopedics (CNCSIS)

SCIENTIFIC RESEARCH PUBLICATIONS:

14 Medical book
52 Medical book chapters
20 Full research articles in ISI Journals
63 Full research articles in IDB Journals
23 Full research articles in CNCSIS Journals
9 Abstract research articles in ISI Journals
84 Abstract research articles in abstract book with ISSN/ISBN

SCIENTIFIC RESEARCH PRESENTATIONS:

8 Invited speaker at International Congresses/Conferences
9 Invited speaker at National Congresses/Conferences
52 Speaker at International Congresses/Conferences
177 Speaker at National Congresses/Conferences
57 Poster presentations at International Congresses/Conferences
13 Poster presentations at National Congresses/Conferences

RESEARCH-DEVELOPMENT PROJECTS:

2015 – 2019   Coordinator of the National Network and Member of the Management International Committee: COST Action IS1406 „Enhancing children's oral language skills across Europe and beyond - a collaboration focusing on interventions for children with difficulties learning their first language"

2017 – 2021   Member of the National Network: COST Action CA16112 „Personalized Nutrition in aging society: redox control of major age-related diseases (NutRedOx)"

2014 – 2018   Member of the National Network: COST Action FA1402 „Improving allergy risk assessment strategy for new food proteins (ImpARAS)"

2001   Member of the National Project: Grant CNCSIS 7 „Research on obtaining and characterization of alloy Ti-Ni-Cu system with memory effect for biomedical applications"

EDUCATIONAL PROJECTS:

2007 – 2013   Short term Expert: POSDRU/90/2.1/S/63942 „Practical training for rapid integration into the labour market of students specializing in dental medicine"

2007 – 2013   Short term Expert: POSDRU/81/3.2/S/55651 „Ergonomics, prevention, performance management in dental medicine through alignment with European standards"

2007 – 2013   Target group Member: POSDRU/81/3.2/S/56573 „DOCTORS in Quality Medical Services"

2007 – 2013   Target group Member: POSDRU/86/1.2/S/63699 „Adapting the offer of underground education dental health to the needs of the labor market and of the society based on knowledge"

2007 – 2013   Target group Member: POSDRU/174/1.3/S/149155 „Teachers in higher-education and public undergraduate education - the promoter of lifelong learning"

SCIENTIFIC RESEARCH STUDENTS:

68 licenses   Scientific Coordinator, the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi

12 presenters   Scientific Coordinator at International Congresses/Conferences

11 presenters   Scientific Coordinator at National Congresses/Conferences

9 awards   Scientific Coordinator at Congresses/Conferences

POSTGRADUATE COURSES:

| 4 courses | Coordinator, the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi |
| 12 courses | Lector, the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi |

RECOGNITION AT NATIONAL AND INTERNATIONAL LEVEL:

Member in scientific professional societies:

International
  International Association of Pediatric Dentistry (IAPD)
  International Congress of Oral Implantologists (ICOI)
  European Orthodontic Society (EOS)
  World Federation of Orthodontists (WFO)
  L'Union Medical Balkanique (UMB)
  Balkan Stomatological Society (BaSS)

National
  The College of Dentists from Romania (CMDR)
  Union National of Stomatological Associations (UNAS)
  The National Association of Pediatric Dentistry in Romania (ANSPR)
  Society of Physicians and Naturalists (SSM) Iasi Branch
  Romanian Dental Association for Education (ADRE)
  Romanian National Association of Orthodontics (ANRO)

Honours/ Awards:

2018 December
  UEFISCDI Award for published article: "Electrochemical behavior of biodegradable FeMnSi–MgCa alloy". *Metals,* Jul 2018; 8(7): 541. IF=2.259

2005 – 2019
  Excellence Diploma/ Certificate of Appreciation at *international* congresses and conferences for conferences presentations

1997 – 2019
  Excellence Diploma/ Certificate of Appreciation at *national* congresses and conferences for conferences presentations

2002
  Silver Medal at the International Exhibition of Inventions, Research and Technology Transfer Iasi, Romania for the invention: "Device for measuring dental castings"

1984
  First Award at the XXIX Session of students from the "Gr. T. Popa" Institute of Medicine and Pharmacy "Gr. T. Popa" of Iasi for oral presentation: "Isometric contraction at the total edentulous"

1983
  First Award at the XXVIII Session of students from the "Gr. T. Popa" Institute of Medicine and Pharmacy of Iasi for oral presentation: "Research on the act of swallowing in the totally edentulous"

Citations:

83	in Web of Science Core Collection
108	in Google Scholar
10	in Medical Books
14	in PhD/Master Thesis
5	in Doctoral Scientific Projects
43	in Licenses

Member in committees of congresses/conferences:

International
  1 vice-president
  23 member

National
  9 president
  5 vice-president
  17 member

SECTION I
SCIENTIFIC ACHIEVEMENTS FROM THE POSTDOCTORAL PERIOD

CHAPTER 1
The potential of dental care services and the performance of dental medical undergraduate education

Nowadays, the dento-facial orthodontics and orthopedics have become an important service to the health of the population, restoring and maintaining the physical and mental harmony of patients. This service provides preventive procedures for anticipating the occurrence of dento-maxillary anomalies, interceptive procedures for eliminating etiological causes and correcting incipient dento-maxillary anomalies and corrective procedures for treating generated dento-maxillary anomalies (Bishara, 2001; Graber & Vanarsdall, 2005).

The orthodontist has to deal with a large amount of data concerning the patient's general and oral status of health, in order to establish the diagnosis of certainty and to recommend the best treatment. These data must be analyzed and integrated in the adequate context, in order to know the population's subgroup to which his patient belongs, thus finding the directions for the general treatment scheme, which will be individualized later.

Generally, patients with dento-maxillary anomalies do not directly address to an orthodontic service, but are sent by the dentists which detected their anomalies. The patients are informed and choose the dental office according to their financial possibilities, the complexity of the treatment, the professional quality of the practitioner and the medical equipment. Patients with low-income choose emergency dental care services that are settled by the National House of Health Insurance or public dental care services with a lower price that the private ones. Ensuring the quality of dental care services provided to patients is a systematic process focused on the performance of the physician, the team, the local network of practitioners and the health system (Starfield *et al.*, 2005; Badri *et al.*, 2009).

A part of the population with dental affections does not address to dental health services, because of the fear of dentist. Dental anxiety is a strong negative feeling associated with dental treatments, with consequences on oral health-related quality of life (Carter *et al.*, 2014). Dental fear is related to dental anxiety and is described as a normal emotional reaction to danger in a clinical dental situation (Cianetti *et al.*, 2017). Dental anxiety and dental fear are early signs of dental phobia, which is described as an unreasonable fear or excessive anxiety to the challenge or threat of dental examination and treatment (Sprouls, 1992).

Higher education as an integral part of the educational system can contribute directly and indirectly to the progress of society (Mara, 2011), providing the intellectual and moral standards of society, attitude codes and values of the guidelines (Neculau, 1997). The major objective of modern undergraduate education curricula does not only concern acquiring theoretical knowledge, but also concerns the equally development of critical thinking, the students' analysis, reasoning and decision skills and the formation of correct learning skills (Vaughan-Wrobel *et al.*, 1997). At graduation, dentists are competent in detecting new orthodontic cases (Popat *et al.*, 2010; Cowpe *et al.*, 2010), but this depends on the quality of their professional training. Usually, identified patients with dento-maxillary anomalies are sent for treatment in orthodontic care services. In current practice, the dentist treats simple orthodontic cases or collaborates with the orthodontist when the patient is from afar and cannot come to the appliance activation sessions. The current context of competitiveness in the higher education institutions creates multiple concerns from specialists in order to increase the quality of education in these institutions.

Deriving from these observations based on my clinical and didactic experience, I conducted a series of studies to assess these aspects and behaviors that are characteristic of patients, practitioners and students. The outcomes of these studies are specific to the socio-demographic parameters of the groups of subjects investigated and the time period in which they were performed.

1.1. The common profile of orthodontic patients from the North-Eastern region of Romania

In orthodontics, as well as in any other medical specialty, an important stage of the clinical activity is represented by the appropriate management of medical data and the achieving of periodic statistics which give helpful information not only about the economic efficiency of the clinic, but also from a medical point of view. Using this statistical information we can establish the patient's general profile, which can be used as a reference point to identify the diagnosis and the best treatment for new orthodontic cases.

This study aimed to identify the prevalence of types of dento-maxillary anomalies encountered in patients who have requested specialized services in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania (personal casuistry), serving the population from the North-Eastern region of Romania, in order to find the common profile of orthodontic patient, through statistical methods.

Patients and methods

This cross-sectional analytical observational study was performed on 375 orthodontic patients, 157 (41.9%) boys and 218 (58.1%) girls, with ages between 5-24 years, 321 (85.6%) patients from the urban area and 54 (14.4%) patients from the rural area, 269 (71.7%) patients from Iasi County 106 (28.3%) patients and other Moldavian Counties from Romania, who were clinically and paraclinically diagnosed with dento-maxillary anomalies and treated in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, during 2000-2010 period.

Studied parameters. The data was collected from orthodontic records and evaluated according to socio-demographic parameters (sex, age and environment area), Angle Classes of malocclusions, isolated dental anomalies, group anomalies, etiological causes, treatment types and therapeutic outcomes.

The statistical analysis was performed using the SPSS 16.0 software (Chicago, Illinois, SUA) for Windows (Field, 2005). Descriptive statistics and correlations were carried, in order to describe the influences between variables. To estimate the confidence intervals (CI) 95% corresponding to the calculated frequencies distributions, we performed bootstrapping with 1000 bootstrap samples. The 5% level of significance and the *p*-value<0.05 was regarded as significant. Also, in order to find the influences between variables we calculated the Spearman rho correlation coefficient, which is the appropriate choice for these used variables.

Results

The Angle Classes of malocclusions: 237 (63.2%) patients with Angle Class I; 106 (28.3%) patients with Angle Class II, including 42 (11.2%) patients with division 1 and 64 (17.1%) patients with division 2; 22 (5.8%) patients with Angle Class III, including 8 (2.1%) patients with maxillar retrognathism, 8 (2.1%) patients with mandibular prognathism and 6 (1.6%) patients with cleft lip and palate; 10 (2.7%) the patients presented transitory anomalies, which have adjusted with growth, without orthodontic treatment.

The isolated dental anomalies associated: 31 (8.3%) patients with number anomalies, 5 (1.3%) patients with shape anomalies, 33 (8.8%) patients with size anomalies, 311 (82.9%) patients with position anomalies, 55 (14.7%), patients with eruption anomalies, and 120 (32.0%) patients with structure anomalies.

The group anomalies associated: 21 (5.6%) patients with midline diastema and 253 (67.5%) patients with dental crowding.

The etiological causes of the dento-maxillary anomalies: 70 (18.7%) patients with general causes, 107 (28.5%) patients with dysfunctional causes and 198 (52.8%) patients with local causes.

The types of orthodontic treatments applied according to the age of patients: 12 (3.2%) patients with prophylactic treatments, 22 (5.9%) patients with interceptive treatments and 341 (90.9%) patients with corrective treatments.

The therapeutic outcomes depended on the patients' presence at the treatment sessions: 147 (39.2%) patients who received only the first consultation, 1 (0.3%) patient to whom the treatment was contraindicated, 9 (2.4%) patients who refused the treatment, 81 (21.6%) patients who finished the treatment and 137 (36.5%) patients who abandoned the orthodontic treatment.

The Spearman correlations calculated on the sample of patients showed: weak, directly proportional influence of the diagnosis of the dento-maxillary anomalies on the therapeutic results (rho=0.136, *p*=0.008); moderate, inverse proportional influence of the types of treatments used on the therapeutic results (rho=-0.306, *p*=0.000); strong, directly proportional influence of the types of orthodontic appliances applied on the therapeutic results (rho=0.635, *p*=0.000); weak, directly proportional influence of the surgical-orthodontic treatment carried out on the therapeutic results (rho=0.235, *p*=0.000).

Discussions

In this study we achieved a statistical assessment on a sample of patients from the North-Eastern region of Romania, who asked for orthodontic consulting, over a period of 10 years, in order to find the common profile of orthodontic patient.

The prevalence of dento-maxillary anomalies was in the order of the Angle Classes, the most common being the Class I malocclusion, which did not involve skeletal discrepancies and difficult treatment for orthodontist, which represents a positive aspect of the patient's orthodontic status and cost of treatment. These data are in consensus with those from literature (Proffit & Fields, 1986; Moyers, 1988). However, the increased prevalence of dental crowding and associated position anomalies imposed more steps for orthodontic treatment to correct these anomalies (creation of space on dental arches, alignment of teeth, levelling occlusion and retention). The increased prevalence of local etiological factors of anomalies showed that their identification and removal was easier than dysfunctional and general ones which require cooperation with other health services.

The main problem of those patients is the decreased frequency of finished orthodontic treatments, compared to the practitioner's expectations. Most of the patients did not want to wear an orthodontic appliance coming only to the first consultation or they abandoned the wearing of the appliances for various reasons, which made the treatment useless. Generally, the main reason is that these treatments are free of charge until 18 years, being provided by the National House of Health Insurance, but there may be other causes, such as: not showing up at the activation sessions of the appliances, the lack of time for parents, the long-time of the orthodontic treatment (1-3 years), or the patient's satisfaction of dental aesthetic self-condition, at a certain time. This situation is mostly caused by the low orthodontic education of the population who attends to the health public system, because the primary orthodontics is sporadically applied in communities (Vernescu, 1974), and seems to be the most important challenge for those in this system.

The correlations based on medical reasoning were carried out to verify if in the studied sample of patients there was a connection between the obtained therapeutic results and the diagnosis of the dento-maxillary anomalies, the types of orthodontic treatments used, the types of applied orthodontic appliances and we found positive aspects.

On the other hand, data collected over a 10-year period from this statistical study gave us significant information to establish the general profile of orthodontic patients, which will be further described. Therefore, the common patient of this clinic is a child or a young adult from the urban area, most probably from Iasi County, which presents a dento-maxillary anomaly Angle Class I with dental crowding and associated position and structure (dental decay) dental anomalies, to local etiology, that comes only to the first consultation. This patient profile is specific in time and space, being characteristic of the geographic region in which it originated and of the time it was addressed to this service.

By this perspective, the orthodontic profile of the patient does not demand great therapeutic problems for the orthodontist, because the therapy is limited only to a dental biomechanics (dental movements) and not skeletal (maxillo-mandibular displacements). Depending on the age of the patient, the dental movements can be performed with removable or multi-brackets fixed orthodontic appliances. This patient comes only at the first consultation, perhaps because of his mistrust in the orthodontic public services. Thus, he comes to find out the diagnosis and the strategy of treatment which is discussed with the patient and guardian at the first visit, and then they either give up or they go to a private orthodontic care service.

The statistical analysis of medical data as a stage in their management is important for both public healthcare system and the clinic as well as for practitioners, in order to highlight the most important aspects of the needs and difficulties, the goal being to improve the performances of the health services for patients.

Future studies can be performed on other patient groups over different time and geographic regions, so the presented outcomes can be compared.

Conclusions

In this study we established the patients' orthodontic profile from the geographical area and the investigated time and we found that the severity of dento-maxillary anomalies is mild, treatment planning is not very difficult, but patients do not follow this therapy or they abandon it. The implementation of a national orthodontic education program would be a solution for public awareness about the consequences of dento-maxillary anomalies on dental, facial and psysical health. The study suggests that motivation and compliance of the patient are important aspects for accepting and finishing the orthodontic treatment.

1.2. The importance of ways to promote the activity of the private dental office

During the last decade, there have been a series of changes and restructuring in the Romanian public health system, in line with the requirements of the European Union (WHO, 2013). While public dental clinics are no longer performing, serving the population only in emergency, and the medical universitary institutions remain only bases for education, the public dentistry market is replaced by the private dental care services (Carausu *et al*., 2017; Forna, 2019). In this context, the patient is the one who chooses his office and practitioner according to his performance, equipment and visual identity.

This study aimed to identify the preferences of graduates and young dentists regarding the ways of promoting dental clinics/offices regulated by law in Romania, to attract future patients, through an opinion questionnaire.

Carausu EM, Dascalu CG, Lupu IC, Burlea LS, Feier RD, **Zegan G**. Marketing in dentistry: opinion survey on promotion of the dental office. *Revista de Cercetare si Interventie Sociala,* Dec 2018; 63/2018: 346-358.

Material and methods

This cross-sectional descriptive observational study was performed on 360 voluntaries, 177 (49.17%) males and 183 (50.83%) females, aged between 25 and 34 years, divided in 186 (53.30%) graduate of the Faculty of Dental Medicine and 174 (46.70%) young dentists with licence to free practice from Iasi. They answered to a questionnaire on how the visual image of the dental clinic/office will be promoted, after signing the informed consent.

The structure of the questionnaire that assessed the preferences of graduates and young dentists regarding the promotion of dental clinics/offices consisted of 5 items with multiple answers, regarding the preferred/used methods for: to promote the dental clinic/office, to present therapeutic solutions, the patients' impression at the first visit to the dental office, to keep in touch with the patient after finishing the dental treatment and to optimize the dentist-patient relationship.

The statistical analysis was performed using the SPSS 19.0 (Chicago, Illinois, SUA) for Windows. In order to perform the statistical processing and analysis of data we used descriptive statistics. We used the Chi-square (χ^2) Pearson test to assess the statistical significance of differences which was considered at *p*-value<0.05.

Results

The promotion of the dental office through: direct recommendation to former patients (34.17% answers of graduates and 45.83% answers of dentists); outdoor advertising (11.67% answers of graduates and 5.00% answers of dentists); business card (42.50% answers of graduates and 22.50% answers of dentists); flyers (51.39% answers of graduates and 21.94% answers of dentists); promotions (50.83% answers of graduates and 24.17% answers of dentists); web page (51.11% answers of graduates and 46.39% answers of dentists); social page (Facebook) (50.55% answers of graduates and 45.00% answers of dentists).

The presentation of therapeutic solutions through: oral presentations (51.11% answers of graduates and 48.06% answers of dentists); adapting the medical terms (50.83% answers of graduates and 48.06% answers of dentists); casts (30.83% answers of graduates and 44.17% answers of dentists); drawing/ sketches (49.17% answers of graduates and 35.00% answers of dentists).

The main elements taken into account by patients at first impression of the dental office are: the team's attitude (50.83% answers of graduates and 48.05% answers of dentists); the atmosphere in the dental office (26.67% answers of graduates and 23.33% answers of dentists); politeness (50.00% answers of graduates and 46.67% answers of dentists); the appearance of the dental office (23.33% answers of graduates and 24.17% answers of dentists); professional outfit (8.33% answers of graduates and 23.33% answers of dentists); the approach to the patient (28.33% answers of graduates and 35.00% answers of dentists).

Keeping in touch with the patient after finishing the dental treatment through: the programming ticket (40.00% answers of graduates and 35.00% answers of dentists); call from the nurse (9.17% answers of graduates and 7.50% answers of dentists); e-mail (5.83% answers of graduates and 3.33% answers of dentists).

Optimization the dentist- patient relationship through: forms of address (26.67% answers of graduates and 13.33% answers of dentists); patient follow-up (16.67% answers of graduates and 15.00% answers of dentists); session revalidation (2.50% answers of graduates and 12.50% answers of dentists); motivating the patient (bonuses, free samples) (10.83 answers of graduates and 4.17% answers of dentists); patient satisfaction (by questionnaire) (0.00% answers of graduates and 1.67% answers of dentists).

The statistical correlations show significant differences between most answers of the two groups of respondents and genders (*p*<0.05).

Discussions

In Romania, the promotion of the dental clinic/office was regulated by law (MS, 2017), and the Code of Ethics of Dental Practitioners limited and standardized the ways to promote the activity of dental care services to the population (CMDR, 2010).

In this study, the opinion questionnaire complied with the legal rigors in Romania in the making of the multiple answers and the respondents showed a proactive behavior towards the importance of the methods and means of promoting the dental clinic/office. They mostly appreciated the media's digital resources for promoting the office, and oral presentations with the appropriate approach of medical terms using drawings to promote therapeutic solutions. Respondents considered that the attitude and politeness of the team's office and also the approach to the patient are the most important aspects to create a positive imagine on his first visit. Also, the programming ticket is considered the best way to keep in touch with the patient after the dental treatment has finished. Respondents did not have a majority opinion regarding methods to optimize

the dentist-patient relationship, which means that they do not know how to do it. Respondents' opinions were significantly different by respondents and genders.

Kent and Jackson (2012) reported that the competitive product, prices and services of dental practice can attract new patients. Clarkson and Bhtia (2008) showed that the purpose of the visual identity of the dental office is to transmit a visual display of its main features to attract new patients. Richard (2009) revealed that the distinction, fast memory assurance, easy reproduction of components, the use of appropriate colour and graphics and the benefits of the dental clinic/office are the requirements that the correct visual identity must meet.

This study has a practical importance because it showed the opinion and attitude of the young dentists from the Romanian private dental medical system, which will promote their activity in their clinic/office and will ensure the optimal dental health of the population, both in urban and rural areas. Also, their ways and methods used for the visual identity of the dental clinic/office are important for future patients which will choose the most competent dental practice. Thus, these dentists will ensure their cooperation with other dental specialties in order to finish interdisciplinary dental treatments.

Conclusions

This study identified the preferences of graduates and young dentists regarding the ways to promote dental clinics/offices to be competitive on the private dentistry market. Their effectiveness and success are mainly the result of the quality of dental care services and the patient's satisfaction. Those depend on their professional training and the modern equipment of the dental offices. Optimizing the dentist-patient relationship is important for their professional and financial medium and long term success. The implementation of the optional and postgraduate courses with these themes for students and younger dentists would be desirable to improve these aspects of dental care services, in order to successfully compete with more experienced dentists.

1.3. The assessment of the degree of patients' satisfaction regarding the quality of private dental care services

Quantification of patient satisfaction regarding private dental care services is an important indicator of their quality and provides information on accessibility, effectiveness, efficiency and social accessibility (Maxwell, 1984; Donabedian, 2003).

This study aimed to know the patient's satisfaction degree regarding the quality of private dental care services from Iasi, in order to assess the level of addressability, professionalism, financial accessibility, infrastructure and good communication, using an original anonymous questionnaire.

Carausu EM, Dascalu CG, Lupu IC, Anistoroaei D, **Zegan G.** The quality of dental care services provided by the private offices in Iasi - pilot study results. *Romanian Journal of Oral Rehabilitation,* Apr-Jun 2019; 11(2): 132-138.

Patients and methods

This cross-sectional descriptive observational study was performed on 144 patients, 58 (40.28%) males and 85 (59.02%) females, aged between 12 and 74 years old, from the waiting room of a private dental office in Iasi, during January to December 2018 period. They responded to an opinion questionnaire on impressions about the quality of the dental office's activity, after having given informed consent.

The structure of the questionnaires. The anonymous questionnaire about the patient's satisfaction degree regarding the dental care services consist in 14 items that provide the following information about: socio-demographic status (gender, age, environment area and occupation); addressability to dental offices (1 item); clinical quality of dental care services (3 items); accessibility to dental care services (7 items); quality of the dental care services infrastructure (2 items); and quality of professional communication (1 item). The questionnaire has multiple answers

and the chosen variant (v1-5) was evaluated with score from 1-5, according Likert scale (Jamieson, 2004).

The statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, IL) for Windows, using descriptive statistics and the Chi-square (χ^2) Pearson test with statistical significance at $p<0.05$.

Results

The socio-demographic characteristics of the sample: 27 (18.75%) patients are from the age range bellow 20 years, 55 (38.19%) patients aged 20-29 years, 29 (20.14%) patients aged 30-39 years, 14 (9.72%) patients aged 40-49 years, 11 (7.64%) patients aged 50-59 years, and 8 (5.55%) patients aged over 60 years; 79 (54.86%) patients were from urban area and 65 (45.14%) patients in rural area; 58 (40.27%) are schoolchildren, 51 (35.42%) workers, 18 (12.50%) pensioners, 17 (11.81%) intellectuals and 10 (4.86%) unemployed.

The addressability of patients to dental offices: 111 (77.08%) patients addressed a dental office, of which 43 (72.88%) males and 68 (80%) women.

The assessment of clinical quality in dental care services: 104 (74.31%) patients indicated that the dentist was involved in their examination and treatment; 82 (56.95%) patients are satisfied of received treatments, with statistically significant gender differences ($p<0.019$); 37 (25.69%) patients indicated that the dentist sometimes works superficially.

The accessibility of patients to dental care services: 95 (65.97%) patients showed that private dental care services are financially accessible; 89 (61.80%) patients prefer expensive dental care services; 86 (59.72%) patients have access to emergency dental care services; 91 (63.19%) patients have access to specialist in dental medicine; 82 (56.95%) patients have temporary access to dental care services; 50 (39.72%) patients agree with the waiting time; 100 (69.44%) patients follow the planning of treatment sessions.

The assessment of quality of the dental care services infrastructure: 91 (63.19%) patients considered the good quality of infrastructure; 94 (65.28%) patients considered that infrastructure should be improved.

The quality of professional communication in dental care services: 74 (51.39%) patients have a good communication with medical staff.

The statistical correlations did not find other significant differences between patients' responses and socio-demographic characteristics ($p>0.05$).

Discussions

This study evaluated the degree of satisfaction of patients regarding the quality of private dental care services, who received treatments in a calendar year, using an original anonymous questionnaire. The presented results are related to some aspects of addressability of patients by gender, clinical quality assessment in dental medicine, population accessibility to local dental care services, assessment of local dental infrastructure and evaluation of dental professional communication.

In the present study, most patients addressed to a dental office and were satisfied with the professionalism with which their dentist consulted and treated them. The degree of satisfaction toward the treatments received was different for the two genders. A small number of patients considered that they sometimes received superficial, inadequate treatment from their dentist.

The study revealed that patients' financial accessibility to usual dental care services was increased and only a small proportion of patients could not afford expensive treatments. Also, the population's accessibility to local dental care services in an emergency situation and to dental specialist was increased. Most patients followed the planning of treatment sessions and do not agree with the waiting time. The quality of the infrastructure was considered good, but many patients believed that the infrastructure of dental office needs improvement. Half of the patients had a good communication with medical staff in dental care services.

There is a causal relationship between the quality of the dental health care, the patient's satisfaction and the profitability of the office (ADA, 2016). In the competitive environment of the dental care services market, the patient's dissatisfaction toward the quality of care received leads to the ulterior behaviour. If the patient is satisfied, the probability that he will return to the same dentist to solve subsequent oro-dental health problems is high (Mills & Batchelor, 2011). One of the most important ways to highlight and promote a private dental care service is based on a better quality compared to competition (Tickle & Campbell, 2010). To achieve this, he must provide dental health care to the level of quality desired by the patient and even overcome it. Patients' expectations (Duma, 2011) regarding the quality of dental health care are determined by their previous experiences, the reputation of the dental office, and the quality of the services, as perceived by the patient.

Starting from these simple findings about patient behaviour and how it responds after receiving inadequate dental care, management specialists have recommended to private providers the introduction of quality management in health services (Duma, 2011). Thus, the dentist will be able to provide for each patient a good quality dental health care and will respond to the level of exigency requested by the patient.

This study is a good sensor about the patient's satisfaction degree regarding the quality of private dental care services from Iasi, and is based on the subjectivity of they interviewed patients. Future studies should be made compared to malpractice complaints from the College of Dentistry Iasi, in order to know exactly how many patients were unhappy because of these dental care services.

Conclusions

This study showed that generally, the patients surveyed were satisfied regarding the quality of the private dental care services, but there are some dysfunctions that can be remedied later, if they are reported by the patients.

1.4. The assessment of the degree of patients' anxiety towards dental treatments

Dental anxiety is a global public health problem, spread around the world and has been extensively studied. The prevalence of high dental anxiety varies from 2% to 30% worldwide depending on the study population, the methods applied, and the scores used (Klingberg & Broberg, 2007; Svensson *et al.*, 2016). It is estimated that about 3-16% of adults suffer from dental phobia (Appukuttan, 2016).

The purpose of the study was to determine the prevalence of dental anxiety before treatment, and to assess the perception of the risks to dental treatment according to socio-demographic characteristics.

Material and methods

This cross-sectional descriptive observational study was performed on 210 patients, 69 (32.9%) males and 141 (67.1%) females aged between 11-70 years old (mean age 26.57 ± 13.378 years) from the North-eastern region of Romania, who asked for dental treatment in a private dental office in Iasi, during March - December 2018 period. The study included patients on their first visit to the dental office and without any dental treatments already begun. Patients with ongoing dental treatments and those who refused informed consent were excluded.

The structure of the questionnaires. The subjects completed three multiple-answers questionnaires during the time they spent in the waiting room, in order to measure their anxiety caused by their visit to the dental office. (1) Corah's Dental Anxiety Scale (DAS), Revised (DAS-R) has 4 items with 5 possible answers. Each answer has a score, and the total score (possible 4-20)

evaluated moderate (score 9-12), high (score 13-14) and severe anxiety or phobia (score 15-20) (Corah, 1969; Corah *et al*., 1978; Schuurs & Hoogstraten, 1993). (2) The Modified Dental Anxiety Scale (MDAS) is the most used today. Corah's DAS was used as base and it has 5 items with 5 possible answers. Each answer has a score, and the total score (possible 5-25) evaluated severe anxiety or phobia (score $\geq$19) (Humphris *et al*., 1995; King *et al*., 2010). (3) Dental Concerns Assessment (DCA) or anxiety over the dental procedures, Revised (DCA-R) has 26 items with 4 possible answers, for the patient to assess their levels of concern: low, medium, high or don't know (Humphris *et al*., 1995; Ronis *et al*., 1995; Drachev *et al*., 2018).

The statistical analysis was performed using the SPSS 16.0 package (Chicago, Illinois, SUA) for Windows. The overall scores that contained the responses to the specific items of the DAS-R and MDAS questionnaires and frequency of MDAS questionnaire responses were calculated. The Chi-square (χ^2) Pearson test was used to compare scores and anxiety levels of DAS-R and MDAS with socio-demographic variables (sexes, age ranges, environment area of origin, education level, and family economic status) and also with the DCA-R questionnaire answers. The statistically significant value $p<0.05$ was considered.

Results

The socio-demographic characteristics of the sample: 145 (69%) patients are from the age range of 11-30 years, 59 (28.1%) patients aged 31-55 years and 6 (2.9%) patients aged 56-70 years; 155 (73.8%) patients were from urban area and 55 (26.2%) patients in rural area; 29 (13.8%) patients with secondary school, 71 (33.8%) patients with high school, 29 (13.8%) patients with bachelor's degree and 81 (38.6%) patients with postgraduate degree; 11 (5.2%) patients have a low family economic status, 113 (53.8%) patients with middle economic status and 86 (41%) patients with high economic status. We found statistically significant differences between age groups and the level of education of patients ($p=0.000$).

The overall scores of the DAS-R questionnaire indicated moderate anxiety in 195 (92.9%) of patients, high anxiety in 9 (4.3%) of patients and severe anxiety or phobia in 6 (2.9%) of patients.

The overall scores of the MDAS questionnaire indicated 13 (6.2%) patients not at all anxious, 119 (56.7%) patients slightly anxious, 62 (29.5%) patients fairly anxious, 11 (5.2%) patients very anxious and 5 (2.3%) patients extremely anxious or possibly dentally phobic.

The answers of the DCA-R questionnaire indicated high anxiety for injection (82 – 39% answers) and extraction (101 – 51.9% answers) and moderate anxiety for sound or vibration of the drill (109 – 39% answers), that will not be enough anesthetized (87 – 41.4% answers), probing to assess periodontal disease (71 – 33.8% answers) and for root canal treatment (75 – 35.7% answers).

The statistical correlations show significant differences between the level of anxiety, age ranges ($p=0.001$) and environment area ($p=0.016$) of patients (tab. 1). 14 items shows the statistically significant differences between answers DCA-R and scores and levels anxiety of DAS-R and MDAS (tab. 2).

Discussions

In this study we identified the prevalence of dental anxiety and its reasons in a group of patients before treatment. Using three questionnaires (DAS-R, MDAS and DCA-R) we compared scores and levels of anxiety with socio-demographic characteristics and the concerns of future patients, in order to determine patients' perception of the dental treatment risks.

In the studied group, high dental anxiety and phobia of dental patients had a low prevalence, following the total scores of DAS-R ($\geq$13) and MDAS ($\geq$19). Most patients had moderate anxiety to certain dental stress-specific factors that could be discussed and managed with the dentist. Anxiety levels were significantly different depending on age ranges and the environment area of patients. Previous studies reported higher prevalence of dental anxiety with present study, values varying according to the studied population. Thus, Drakiev *et al*. (2018) reported a prevalence of dental anxiety of 13.7% in a Russian population, Biggs *et al*. (2003) 9.56% in a Texan population and Jain *et al*. (2018) 8% to an Indian population. Also, significant differences between anxiety levels and

socio-demographic characteristics were specific to the studied groups. Thus, some authors found differences between genders (Humphris *et al*., 1995; Biggs *et al*., 2003; Humphris & Dyer, 2009; Drachev *et al*., 2018; Caltabiano *et al*., 2018), age ranges (Humphris *et al*., 1995; Humphris & Dyer, 2009), environment area (Drachev *et al*., 2018; Zukanovic *et al*., 2018), or educational levels (Drachev *et al*., 2018), and other authors did not find statistically significant correlation (Campos *et al*., 2013; Saatchi *et al*., 2015; Jain *et al*., 2018; Zukanovic *et al*., 2018).

Table 1. Correlations between scores and levels of anxiety DAS-R and MDAS with socio-demographic variables

Survey	Variables									
	Gender		Age intervals		Environment area		Level of education		Economic status	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Corah's Dental Anxiety Scale, Revised (DAS-R)										
Item 1	5.204	0.267	15.330	0.053	5.691	0.223	23.374	0.025*	1.827	0.986
Item 2	5.257	0.154	18.530	0.005*	3.523	0.318	6.452	0.694	9.720	0.137
Item 3	1.703	0.790	18.371	0.019*	1.225	0.874	9.105	0.694	7.878	0.445
Item 4	5.237	0.264	22.249	0.004*	13.136	0.011*	17.243	0.141	18.246	0.019*
Score	9.154	0.761	48.497	0.005*	23.122	0.040*	31.015	0.815	31.683	0.204
Anxiety	0.734	0.693	19.580	0.001*	8.279	0.016*	5.735	0.454	3.280	0.512
The Modified Dental Anxiety Scale (MDAS)										
Item 1	3.905	0.419	20.420	0.009*	6.599	0.161	5.197	0.951	2.296	0.971
Item 2	2.710	0.607	17.638	0.000*	3.518	0.475	12.102	0.438	5.696	0.681
Item 3	1.209	0.877	15.030	0.059	6.764	0.149	13.685	0.321	8.479	0.388
Item 4	0.743	0.946	23.973	0.002*	10.164	0.038*	18.100	0.113	13.246	0.104
Item 5	4.101	0.392	13.522	0.095	9.439	0.051	17.696	0.125	7.149	0.521
Score	29.060	0.034*	62.081	0.002*	20.799	0.236	37.636	0.918	21.943	0.945
Anxiety	7.868	0.097	19.762	0.011*	11.366	0.023*	14.354	0.279	7.682	0.465
*statistically significant differences when *p*<0.05										

In the present study, the most common concerns of patients were regarding injections and teeth extraction. Previous studies recommended the use of a local anesthetic (20% benzocaine) at the injection site (Al-Samadani & Gazal, 2015) or a preoperative dental extraction video (Gazal *et al*., 2016) to reduce the anxiety of these patients.

The levels of studied patients' concerns were significantly different depending on the reasons: sound or vibration of the drill; injections; the sound or feel of scraping during teeth cleaning; panic attacks; the inability to stop the dentist; the smell in the dental office; the need of a long dental treatment; the number of appointments and the necessary time for treatment; time away from work, or the need for childcare or transportation; feeling constrained or not being in control. Bolla *et al*. (2017) have reported the highest anxiety scores for teeth extractions, costs of the procedures, sound and vibration of drilling procedures on teeth and root canal treatments, and Caltabiano *et al*. (2008) for local anaesthesia and tooth drilled.

Moderate dental anxiety can be reduced by a pleasant dental office environment, a calm atmosphere and medical staff that support the patient, a relationship of trust established through good communication between the patient and the practitioner (Caltabiano *et al*., 2008). Certain information about the treatment procedures received from the practitioner would anticipate the sensation the patient might expect and would see his preferences (Biggs *et al*., 2003; Hamasaki *et al*., 2011). Also, initiating rest breaks at the patient's request during the therapeutic procedures would increase his confidence that he is in control (Humphris *et al*., 1995; Humphris & Dyer, 2009; Newton *et al*., 2012).

In children, high dental anxiety or phobia occurs due to fear of the unknown, and in adults it can be activated by the fear stimulus from the negative past experiences in the dental office (Butler, 1989). To manage these situations during treatment, the authors recommend interventions and

cognitive behavioral psychotherapy (psychological education, relaxing therapy and modelling technique) (Biggs *et al*., 2003; Lahmann *et al*., 2008; Armfield & Heaton 2013; Bucur & Pacurar, 2014; Appukuttan, 2016; Gan *et al*., 2017; Plesea Condratovici *et al*., 2018) and pharmacological sedation interventions.

Table 2. Correlations between answers of DCA-R and scores and levels of anxiety of DAS-R and MDAS

Dental Concerns Assessment	DAS-R				MDAS			
	Overall score		Anxiety scale		Overall score		Anxiety scale	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Item 1	55.116	0.084	21.375	0.011*	84.277	0.005*	48.721	0.000*
Item 2	55.079	0.697	11.832	0.459	67.496	0.628	26.401	0.333
Item 3	59.473	0.039*	10.754	0.293	88.187	0.094	33.391	0.096
Item 4	40.743	0.526	7.012	0.636	95.564	0.033*	45.030	0.006*
Item 5	59.302	0.256	18.072	0.114	93.585	0.045*	29.614	0.198
Item 6	94.376	0.028*	22.560	0.094	97.713	0.024*	42.992	0.010*
Item 7	46.393	0.296	5.078	0.827	73.863	0.417	20.975	0.640
Item 8	26.309	0.972	3.047	0.962	71.406	0.498	11.980	0.980
Item 9	54.933	0.515	8.893	0.712	48.994	0.983	22.433	0.553
Item 10	43.202	0.420	4.391	0.884	37.207	0.960	13.936	0.733
Item 11	43.726	0.884	18.220	0.109	66.639	0.656	15.814	0.895
Item 12	27.929	0.953	9.497	0.393	98.974	0.721	19.517	0.989
Item 13	46.935	0.277	15.857	0.070	196.322	0.000*	41.377	0.498
Item 14	54.458	0.094	13.476	0.142	97.910	0.271	36.724	0.185
Item 15	58.346	0.048*	9.328	0.408	65.716	0.686	28.265	0.249
Item 16	86.292	0.000*	20.715	0.014*	137.778	0.000*	48.645	0.002*
Item 17	111.279	0.000*	54.709	0.000*	168.460	0.000*	65.657	0.000*
Item 18	63.768	0.222	3.202	0.994	94.518	0.039*	20.080	0.692
Item 19	25.245	0.981	12.515	0.186	47.814	0.988	8.555	0.998
Item 20	37.410	0.672	3.764	0.929	77.651	0.019*	15.358	0.637
Item 21	118.684	0.000*	44.600	0.000*	97.098	0.000*	33.206	0.016*
Item 22	55.936	0.074	14.989	0.091	71.994	0.051	33.155	0.016*
Item 23	50.539	0.172	16.217	0.062	84.771	0.636	17.877	0.960
Item 24	45.356	0.334	18.106	0.034*	69.911	0.548	21.617	0.602
Item 25	49.089	0.210	12.194	0.203	65.471	0.136	24.255	0.147
Item 26	60.229	0.325	27.020	0.008*	124.514	0.000*	34.860	0.070
*statistically significant differences when $p<0.05$								

The anxiety of patients towards dental practice is an unpleasant emotion that can affect the quality of treatment. Knowing the patient's anxiety before treatment can be useful to the practitioner. He can become aware of what to expect from the patient, and he can take the necessary measures to decrease the patient's anxiety.

Conclusions
In this study we found a high dental anxiety in 4.3% of patients, severe anxiety in 2.9% of patients and phobia in 2.3% of patients for injection and tooth extraction, being significantly different depending on age range and environment area of patients. Dental anxiety and phobia in dental practice is a significant problem for both the patient and the dentist. These patients need to be identified, and their concerns should be addressed at the earliest, in order not to prejudice dental treatment subsequently applied.

1.5. The performance level of the students in public dental medical undergraduate education

In pedagogical theory there are three main categories of assessment: self-assessment, group assessment and peer assessment. The first two are useful during the learning process, while the last is necessary during the final assessment stage. The non-standard forms of assessment, especially those computer-based, are very popular in the USA, in ascension in UK and more and more popular among students (Ventouras *et al.*, 2010).

The MCQ tests (Multiple Choice Questions) can be used in any type of assessment, in order to evaluate the student's capacity to remember exact data, to interpret data or to analyze a proposed material. The MCQ tests must cover the six purposes of education process, specific for cognitive sciences and described in Bloom's taxonomy (Higgins & Tatham, 2003*). The study's purpose* was to evaluate the advantages and the drawbacks of MCQ tests as assessment forms at the end of specific modules from the public dental medical undergraduate educational system.

> Dascalu CG, Enache AM, Mavru RB, **Zegan G.** Computer-based MCQ assessment for students in dental medicine - advantages and drawbacks. *Book Series: Procedia Social and Behavioral Sciences,* May 2015; 187: 22-27.

Material and methods

This cross-sectional analytic observational study was performed on 285 undergraduate students, 111 boys and 174 girls, with ages between 19-26 years old, which were divided in two groups: 153 students (52 boys and 101 girls) from the 1st year, examined at the end of a preclinical discipline; and 132 students (59 boys and 73 girls) from the 5th year, examined at the end of a clinical (orthodontics) discipline, from the Dental Medicine Faculty curriculum. The participation was voluntarily, and the students were informed about the aims and the nature of the research.

Before the assessment, the students were asked about their preferences regarding the type of written evaluation. The students' *theoretical knowledge* was evaluated through the final written MCQ test, and the *practical knowledge* through oral evaluation, in different days. The assessments' results were quantified through marks from 1 to 10.

The statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. We calculated the parameters of descriptive statistics and used the Wilcoxon Signed Ranks test for paired samples, the Mann-Whitney test and the *t*-test for independent samples when the normal law was verified. For all the statistical tests we used the significance level $p \leq 0.001$ corresponding to a confidence interval of 95%.

Results

At the question „Which is the most relevant method to evaluate the theoretical knowledge?" the students answered that they preferred the MCQ tests: 56% of students from the 1st year and 73.6% of students from the 5th year.

We found statistically significant differences of students' marks between the two types of assessments for both preclinical and the clinical disciplines, reported on years of studies and genders (tab. 3).

Regardless the assessment type (MCQ tests or oral evaluation), the differences between the marks obtained by the students in the 1st and the 5th year of study are statistically significant, while, within each year of study, the differences between the marks obtained by girls and boys are not significant (tab. 4).

Discussions

This study compared the undergraduate students' performance level in the public dental medical undergraduate educational system, measured by marking the knowledge of two groups of students from a preclinical and a clinical (orthodontics) discipline after two types of assessments, in order to evaluate the advantages and the drawbacks of MCQ tests.

Table 3. Significant differences of students' marks at the two types of assessments

Variables	MCQ tests		Oral assessment		Wilcoxon Signed Ranks test	
	Mean ± SD	SEM	Mean ± SD	SEM	Z value	*p* value
Preclinical discipline	5.16 ± 1.058	0.086	8.53 ± 1.595	0.129	10.540	0.000*
boys	5.07 ± 1.211	0.168	8.53 ± 1.742	0.242	5.993	0.000*
girls	5.20 ± 0.974	0.097	8.52 ± 1.524	0.152	8.678	0.000*
Clinical discipline	6.31 ± 1.342	0.117	8.14 ± 1.327	0.115	9.113	0.000*
boys	6.21 ± 1.153	0.150	8.03 ± 1.348	0.175	5.880	0.000*
girls	6.39 ± 1.480	0.173	8.22 ± 1.312	0.153	6.952	0.000*
*statistically significant differences when *p*<0.001						

Table 4. The statistical comparison of students' marks at the two types of assessments

Compared groups	MCQ tests		Oral assessment	
	Independent *t*-test		Mann-Whitney test	
	t value	*p* value	U value	*p* value
Groups 1st year and 5th year students	7.981	0.000*	7731.500	0.001*
Group 1st year students: boys and girls	0.689	0.492	2497.500	0.614
Group 5th year students: boys and girls	0.764	0.446	1914.000	0.268
*statistically significant differences when *p*<0.001				

The presented outcomes derive from certain aspects regarding the students' preferences for specific written assessment methods, the distribution of obtained marks at MCQ test and their comparison with the marks obtained at the oral evaluation. Although most students preferred the MCQ test instead the essay for the written assessment, the marks they obtained were low, at the preclinical as well as the clinical discipline. The presented outcomes confirm previous studies which showed that the students perceive the MCQ tests as a simple form of evaluation and practice a surface learning, obtaining consequently low results (Scouller, 1998; Furnham *et al*., 2011). Other authors have studied other variables; outlining the student's profile correlated with his preferences for certain assessment forms: the emotional students prefer the MCQ tests, while those less emotional choose other methods of assessment (Struyven *et al*., 2005). The introverted prefer the MCQ tests while the extroverted prefer the oral assessments, as well as the conscientious (Furnham *et al*., 2008). The "Openness-to-Experience" personalities prefer the essays and the oral evaluation, rejecting instead the MCQ tests. There are no identified correlations between the intelligence level or the students' university performances and the preferred assessment method (Chamorro-Premuzic *et al*., 2005).

The emotional stress level was increased during the oral evaluation, because of the direct questions and the examiner's presence, but the students' results were far better than those obtained at MCQ tests.

The MCQ assessment type presents an increased objectivity, ease of filling and speed of obtaining the results, which explains the students' preference, a fact that was also noted by Hammond *et al*. (1998) and Brady (2005). Also, some advantages of MCQ tests are: checks a large area of knowledge in a relatively short period of time (Brady, 2005); tests and stimulate of students' critical thinking (Leung *et al*., 2008); the time is significantly shortened for computerized tests evaluation, which simplifies the teachers' work (Ventouras *et al*., 2011); the student's performance is not influenced by the speed of his handwriting (Higgins & Tatham, 2003). Higgins and Tatham (2003) and Ventouras *et al*. (2010) point out the advantages of MCQ tests and especially the disadvantages, citing the risk of "dumbing down". That is why some authors recommend combining different methods of assessment for the students in medical undergraduate education, in order to train good practitioners (Hayes & McCrorie, 2010).

Conclusions

The present study revealed that all students were disfavored by the MCQ test compared with the oral assessment, obtaining weak results regardless the discipline, year of study or gender. Despite their advantages, the use of MCQ tests in the medical undergraduate education is not enough, if a formative evaluation is desired for a process of analysis allowing the diagnosis, prognosis and the assessments' adjusting, because the most important attributes of a successful career in this domain are the capacities of analysis, synthesis and quick applying of knowledge in solving new, complex and individualized practical problems. A further research should target what are the implications of using MCQ in the medical undergraduate education, given the fact that this method is applied empirically, without an analysis of structure, reliability, validity and efficiency of the tests applied, especially in the medical field.

1.6. The orthodontic undergraduate education effectiveness for serving future patients

The most visible mission of dental medical education is to develop future specialists. With the progress in science and technology and the increased needs of the patient, there is a change of responsibility towards society. That is why teachers have to take the responsibility to train dentists to provide care to patients. The absence of competent dental education can lead to a lack of confidence in future dentists in their ability to effectively treat patients, which could affect the availability and quality of health care (Terrell & Beaudreau, 2003; Dao *et al*., 2005; Smith *et al*., 2006).

The objective of this study was to determine the relationship between the quality of the orthodontic undergraduate education and the professional attitude and practical skills reported by the students of the last two years of a public dental medicine faculty. *The aim of this study* was to assess the students' interest in orthodontic practice, their views on the structure of the orthodontic curriculum and the agrees evaluation methods of the theoretical knowledge, compared to the final marks obtained, in order to appreciate the standard orthodontic undergraduate education.

> **Zegan G,** Golovcencu L, Anistoroaei D. Undergraduate orthodontic education and dental students' performances. A survey. *International Journal of Medical Dentistry*, Apr-Jun 2019; 3(2): 237-243.

Material and methods

This cross-sectional descriptive observational study was performed on 273 students, 94 boys and 179 girls, aged 23-35 years, divided into 143 students (49 boys and 94 girls) from the 5th year and 130 students (45 boys and 85 girls) from the 6th year of the public Faculty of Dental Medicine, „Grigore T. Popa" University of Medicine and Pharmacy Iasi Romania, during a university year. Participation was voluntarily, and students were informed about the goal and aim of the study.

The structure of the questionnaire. After studying about the orthodontics modules (practical works and courses) in the curriculum, the students were invited to answer an anonymous questionnaire on orthodontic discipline. The questionnaire consisting of 16 items provided the following information about: socio-demographic status of the students (age, gender and study year) (3 items); the interest in orthodontic practice (4 items); the evaluation of the structure orthodontic curriculum (6 items); effectiveness of knowledge assessment methods (3 items). The questionnaire has multiple answers and the chosen variant (v1-5) was evaluated with score from 1-5, according Likert scale (Jamieson, 2004).

The students' *practical knowledge* was evaluated through oral exam and *theoretical knowledge* through final written exam (students of 5th year through Multiple Choice Questions - MCQ test and students of 6th year through essay). The examinations results were quantified through marks from 1 to 10.

The statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. We identified the score of answers to each of the questionnaire's items and we have cumulated the items score based on theme of questions. We used the Chi-square (χ^2) Pearson test to

compare the students' answers with socio-demographic variables. We considered the $p \leq 0.05$ value as statistically significant.

Results

Students' opinions about orthodontic practice were appreciated by the answers to questions 4-7. The answers versions to question 4 were: v1=64 (23.44%) answers, v2=132 (48.35%) answers, v3=71 (26.01%) answers and v4=6 (2.20%) answers. To question 5 were the following versions of answers: v1=65 (23.80%) answers, v2=161 (58.98%) answers, v3=44 (16.12%) answers and v4=3 (1.10%) answers. The students had to question 6 the following versions of answers: v1=5 (1.83%) answers, v2=175 (64.10%) answers, v3=89 (32.50%) answers and v4=4 (1.47%) answers. Question 7 had the following answers versions of students: v1=200 (73.26%) answers, v2=67 (24.54%) answers, v3=6 (2.20%) answers and v4=0 answers (tab. 5).

Students' views about the topics and structure of orthodontic curriculum were assessed by answers to questions 8, 9, 13-16. The versions of the answers to question 8 were: v1=109 (39.93%) answers, v2=148 (54.21%) answers, v3=13 (4.76%) answers and v4=3 (1.10%) answers. To question 9 were the following versions of answers: v1=142 (52.01%) answers, v2=4 (1.47%) answers and v3=127 (46.52%) answers. The students had the following versions of answers to question 13: v1=79 (28.94%) answers, v2=154 (56.41%) answers, v3=37 (13.55%) answers and v4=3 (1.10%) answers. Students had the following versions of answers to question 14: v1=10 (3.66%) answers, v2=96 (35.16%) answers, v3=108 (39.56%) answers and v4=59 (21.62%) answers. The students register to question 15 the following versions of answers: v1=164 (60.08%) answers, v2=99 (36.26%) answers, v3=7 (2.56%) answers and v4=3 (1.10%) answers. The versions of answers to question 16 were: v1=86 (31.50%) answers, v2=166 (60.80%) answers, v3=21 (7.70%) answers and v4=0 answers (tab. 5).

The effectiveness of assessing methods of the theoretical knowledge acquired at orthodontics courses and practical work was appreciated by answers to questions 10-12. The versions of answers to question 10 were: v1=177 (64.83%) answers, v2=85 (31.14%) answers and v3=11 (4.03%) answers. The following versions of answers were at question 11: v1=132 (48.35%) answers and v2=141 (51.65%) answer. The students had the following versions of answers to question 12: v1=72 (26.38%) answers, v2=178 (65.20%) answers and v3=23 (8.42%) answers (tab. 5).

The statistical correlations show significant differences between some answers according to gender and study years of the students (tab. 5).

The distribution of students' finale marks means at examinations follows the Gauss curve. Students in the 5st year had 39.40% marks means 8-10 (fig. 1a), and students in the 6th year had 60.16% marks means 8-10 (fig. 1b).

a) the 5st year   b) the 6th year
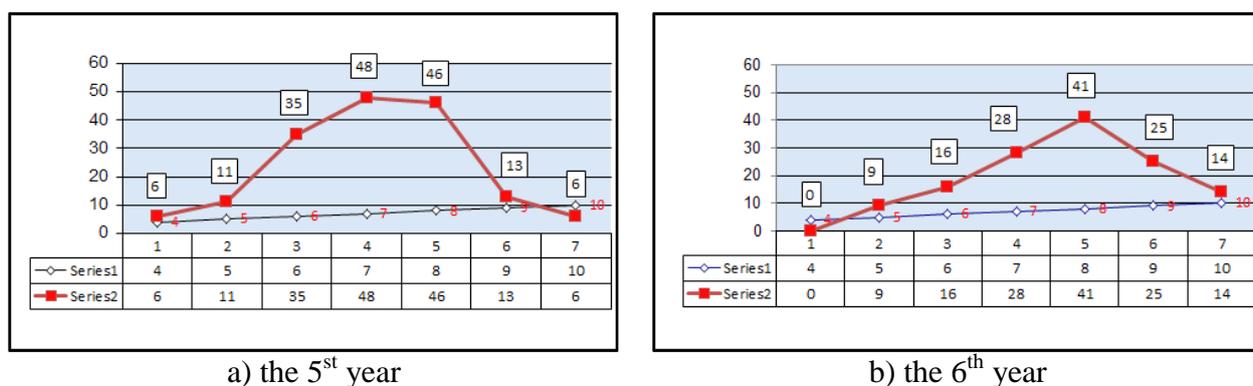
Fig. 1. Distribution of final marks for students

Discussion

This research has assessed the views of the students in the final years of a public faculty on orthodontic practice and curriculum to check the effectiveness of undergraduate education strategies

in the field of orthodontics. Students' views are also assessed on the methods of examining knowledge gained during a university year and quantified their final results.

Table 5. Correlations between student's answers and statistical variables

Theme	Questionnaire				Gender		Years of study	
					χ^2	p	χ^2	p
Orthodontic practice	4. My interest for orthodontic practice is:							
	very high	high	low	not at all				
	64	132	71	6	2.005	0.571	16.216	0.001*
	5. My collaboration as a dentist with orthodontist will be:							
	very frequency	frequency	sometimes	not at all				
	65	161	44	3	4.972	0.174	14.907	0.002*
	6. Orthodontic practice seems to me:							
	easy	acceptable	difficult	very difficult				
	5	175	89	4	6.020	0.111	5.485	0.140
	7. The contribution of the dentist in early detection of malocclusions is:							
	very high	high	low	not at all				
	200	67	6	0	6.456	0.040*	6.674	0.036*
Orthodontic curriculum	8. The best time for the orthodontic course is:							
	9.30-11.30	11.30-13.30	13.30-15.30	15.30-17.30				
	109	148	13	3	4.375	0.224	55.961	0.000*
	9. The most useful method of teaching courses is:							
	PowerPoint	to the board	both					
	142	4	127		3.214	0.201	5.669	0.059
	13. The structure of the orthodontic course is:							
	very good	good	satisfactory	satisfactory				
	79	154	37	3	5.945	0.114	26.965	0.000*
	14. I want the orthodontic course to contain:							
	more theoretical notions	more clinical cases	both	I am satisfied				
	10	96	108	59	9.027	0.029*	20.282	0.000*
	15. The orthodontics course has clarified my notions:							
	much	little	barely	not at all				
	164	99	7	3	4.311	0.230	17.108	0.001*
	16. The theme of the orthodontic course seems to me:							
	very interesting	interesting	acceptable	uninteresting				
	86	166	21	0	1.026	0.599	25.131	0.000*
Assessment of knowledge	10. Periodic testing of knowledge in practical work helps me:							
	much	little	not at all					
	177	85	11		2.972	0.226	25.131	0.000*
	11. The most effective method of teaching in practical work is:							
	individual training and discussions		teaching and seminar					
	132		141		1.345	0.246	0.203	0.652
	12. The most relevant method for evaluating theoretical knowledge is:							
	essay type	single choice questions	multiple choice questions					
	72	178	23		6.709	0.035*	0.006	0.997
*statistically significant differences when $p<0.05$								

The presented results are related to some aspects of orthodontic undergraduate education in students' preferences, using an original anonymous questionnaire. Thus, most of the students' opinions on orthodontic practice were positive, with variations between gender and the study years, indicating: a great interest in this, a frequent collaboration with the orthodontist as a future dentist, an acceptable difficulty of the workouts (especially the students of the 5th year) and a great role attributed to dentistry in the early detection of dento-maxillary anomalies in the population (especially girls).

The patient's orthodontic examination for diagnosis is learned in the clinical setting and represents a combination of didactic teaching and practical experience under close supervision of the teacher (Johnson & Mays, 2019). The increase in the number of students in our faculty compared to the number of teachers reduced the teacher-student contact, so the ratio is 1:8 (10) (1 teacher at 8-10 students at clinical trials).

Under such conditions, the literature proposed as Compensation Assisted Computer Assisted Learning (CAL) (Turner & Weerakone, 1993; Hobson *et al.*, 1993) and Objective Structured Clinical Examination (OSCE) (Lai *et al.*, 2018) as an adjunct in classical clinical delivery and traditional seminar. Some authors believe that these methods are more beneficial for advanced orthodontic education during residency program (Fields *et al.*, 2017).

Dento-maxillary anomalies are usually identified by the dentist, who refers the patient to the orthodontist on the basis of his diagnosis, the dental graduate having this competence (Popat *et al.*, 2010; Cowpe *et al.*, 2010). Managing the orthodontic emergency (anterior crossbite) by assisting and referring to a specialist is another competence of the graduate of dental medicine. The dentist becomes an active team member with the orthodontist regarding the orthodontic emergency represented by the damage to a mobile or fixed orthodontic appliance. These final orthodontic competencies are established by our faculty. Several previous studies showed that graduates of dental medicine are not convinced that they can successfully manage orthodontic patients (Yiu *et al.*, 2011) and their emergencies (Adamidis *et al.*, 2000; Fleming & Dowling, 2004; Oliver &, Hingston, 2006; Jones *et al.*, 2016). The study by Schueler *et al.* (2018) promotes an effective method of increasing student performance and individual guidance in learning through individual structured and qualified feed-back during clinical classes.

In the present study, most students had positive impressions about the subject and structure of the orthodontics curriculum, which were in consensus with day-to-day reality. Thus, the presentation of the courses was indicated in the middle of the day and the best method of teaching the courses was the presentation of PowerPoint (especially for the students of the 5th year). The orthodontics courses were well structured, interesting and clear, and in the future students want more theoretical notions (especially girls) as well as more presentations of clinical cases (especially students from the 6th year).

In our curriculum, the 5th year courses provide basic orthodontic knowledge, from terminology and etiopathogenicity to the orthodontic treatment of the child with simple orthodontic appliances. In the 6th year, the courses provide in-depth orthodontic and interdisciplinary orthodontic knowledge about the complex oral rehabilitation of the adolescent and the young adult, based on knowledge accumulated in the previous year. This orthodontic undergraduate education strategy has benefited from the learning process and agrees with the study by Yilmaz *et al.* (2019). The students' requirement for different theoretical notions is not justified, because in orthodontics there is a residency programme that will help them accumulate more notions. This way, future studies should assess the options of our graduates in choosing orthodontics as a career specialty, as other schools did in the world (Shin *et al.*, 2015; Halawany *et al.*, 2017). The fact that students want more case presentations at courses is justified because dentistry is a practical field and by this method the students could develop their knowledge and skills in the orthodontic planning of the cases, as shown by the study of Azeem *et al.* (2018). Opposite to this study, Heath *et al.* (2017) showed that supplementary training has a beneficial influence only on orthodontists who have acquired in time the necessary knowledge to recognize the complexity of the cases.

In the present study, most of the students of the terminal years have considered that the most effective methods of evaluating acquired theoretical knowledge are those currently used namely teaching new notions and seminars, but also individual learning and seminar discussions, as well as periodic testing of knowledge at practical work. At the written exam, most students chose for MCQ tests (especially boys) with just one correct answer. The specialized literature outlined the student's profile correlated with the preference of the form of knowledge examination (Struyven *et al.*, 2005; Brady, 2005; Furnham *et al.*, 2008).

In this study, although most students preferred the MCQ tests at the written test, the average marks of the final grades (grades 8-10) of 5th year students (who had MCQ tests) were lower than those of 6th year students (who had the essay). This situation may be due to either MCQ tests that check a broad range of knowledge over a relatively short period of time (Brady, 2005) (1.55 minutes/answer), or to the essay that allows the student to organize his ideas on a well-defined subject, and concentration time is short (10 minutes/topic x 3 topics). In specialty literature, an evaluation system has been proposed to detect students' maturity skills and competences acquired during their training, the speed of response, the ability to improvise to a new problem and the ability to concentrate (Ferrer-Martin *et al.*, 2014).

The European Higher Education Area (EHEA) created in 2005 at Bergen facilitated the mobility of students and graduates between institutions, to promote international cooperation exchange, as well as for the overall development of Europe. Their objectives are to facilitate the mobility of students and graduates and to promote cooperation between higher education institutions. To achieve these goals is necessary that the degrees of different countries are comparable and the skills acquired in higher education are well established. Thus, all countries of the EHEA have redefined competency-based degree programs, where the total student work is estimated in European Credit Transfer and Accumulation System (ECTS credits) (Ferrer-Martin *et al.*, 2014).

Through the feed-back from students, the presented study is important for verifying our standards of excellence in teaching, learning and training of practical skills in orthodontics and provides guidance for improving the quality of this process.

Conclusions

In this study, the orthodontic practice, the orthodontic curriculum theme and structure and the theoretical knowledge evaluation methods received positive feed-back from the students of the final years of our faculty, with variations between gender and years of study. The average marks of the final grades earned by students in the 6th year were better than the students of the 5th year. The students' suggestions, submitted through the anonymous questionnaire were justified from the point of view of their future orthodontic career. Future studies can be carried out on other topics related to the orthodontic underground education, aimed at improving the didactic process for increasing the graduates' professional performance.

CHAPTER 2
Attitudes and behaviors of children and teenagers for oral health and their impact on the quality of life

Childhood is the period ranging from birth to adolescence (0-12/14 years) and is divided into early childhood (play age) and middle childhood (school age) (Collins Dictionary, 2019). At the beginning of early childhood, the child learns to walk, speak, and around the age of 3 becomes less dependent on parental assistance for basic needs. Early childhood continues approximately until the age of 7. At this stage children are learning through play, observing, experimenting and communicating with others. Parents supervise and support the child's process of development, which then will lead to the child's autonomy and also, a strong emotional bond is created between the child and the adults who care for him. During this period, children begin kindergarten and their social lives. Middle childhood begins around the age of seven, when the child enters school and begins his social and mental development. During this period, children make new friends and acquire new skills, become more independent, increase their individuality, have responsibilities, start formal education (learning and teaching from teachers) and social play (learn and teach from each other) (Konner, 2010; Berger, 2017).

Acquisition of communication skills begins early in childhood and allows the development of knowledge in the community and the educational area. Most children acquire speech and language by a seemingly automatic process. Typically, basic communication skills are developed by parents until the time a child enters kindergarten, enabling the child to begin learning from teachers and interacting fluently with peers (Oller *et al*., 2006). Good communication skills of the child are crucial to the development of thinking ability, a sense of self and full participation in society. Language Impairment occurs when the child's language skills are judged to be significantly delayed relative to those of children of the same age. It may appear as a primary and/or secondary disorder. A primary disorder occurs in the absence of any apparent etiology (Newcastle University, 2019). A secondary disorder occurs due to causes, such as autism, hearing impairment, behavioral and emotional difficulties, or trauma and other developmental disorders (Down syndrome, fragile X syndrome, Williams syndrome, etc.). 5-10% of all preschool children have language impairment (Bishop & Leonard, 2000). Parent-reports from questionnaires showed that 10% of children between 6 and 10 years old had language impairment (Hollung-Møllerhaug, 2010).

Oral health is defined as an oral status free of diseases, which makes people look more beautiful (Kumar *et al*., 2017), involving the ability to smell, touch, taste, chew, swallow, smile, speak, and convey a lot of emotions through facial expressions with confidence, without discomfort, pain and disease of the cranio-facial region (Glick *et al*., 2016). According to estimates by the World Health Organization, more than 3.58 billion people in the world are affected by tooth decay in permanent teeth (WHO, 2018). The onset of tooth decay can begin immediately after teeth eruption, and preschool age may be a risk for the child's oral health and for overall health of the future adult (Athanasiu, 1998). The specialists emphasize that the attitude to any condition, including oro-dental diseases is determined by internal factors related strictly to a person and over which we cannot interfere, but also by external factors that can be modelled through education (Pasca, 2006). The risk factors of oral diseases are related to lifestyle for prevent the most important non-communicable diseases (NCDs) (cardiovascular diseases, diabetes mellitus, cancer and chronic obstructive pulmonary diseases) and dietary habits significant to the development of NCDs and dental caries (WHO, 2019), such as sugar excessive consumer (Patel, 2012).

In Europe, oral diseases are among the most important tasks of the health system with a socio-economic impact on quality of life and constitute a significant public health problem. In the last 30 years, the prevalence of dental caries decreased in children and young adults, and the prevalence of periodontitis increased in the same time with diabetes mellitus (Patel, 2012). Most of the evidences in oral health promotion relates to dental caries and periodontal diseases prevention, through topical fluorides (toothpaste, varnish and mouth rinses) and by good personal oral hygiene practices, including tooth brushing (Marinho *et al*., 2003).

The dento-maxillary anomaly is not a disease, but a deviation from normal, which causes the alteration of functions (mastication, speech, etc.) and can influence the quality of life of the affected subjects (WHO, 2019). The dento-maxillary anomalies have been associated with the appearance and severity of dental caries, especially in children aged 11-14 years (Feldensa *et al*., 2015) and the development of periodontal disease, although it has no direct etiology (Bollen, 2008). The treatment of dento-maxillary anomalies enhances the social and psychological well-being of the subjects, through improvements of the facial and dental appearance (WHO, 2019).

The impact of oral health on the quality of life is measured by sanogenous, functional, social or psychological determinants and is usually known in the literature as OHRQoL – the oral health related quality of life. OHRQoL is recognized as a multidimensional concept, comprising the presence/absence of oral pathology and psychological aspects of oral health (Gift *et al*., 1997) and is an extension of HRQoL – the health related quality of life. OHRQoL is used to assess the impact of oral disease severity on the child's physical, psychological and social well-being. Allen (2003) stated that one of the main limitations of the biomedical paradigm of health is that this model deals only with the disease, adding the social and environmental model of health which also involves cultural, environmental and psycho-social influences. There have been developed instruments to evaluate OHRQoL the individual's own perspective and its impact on the daily well-being, emphasizing especially on functional parameters and less on the clinical dental parameters (Locker & Miller, 1994; Gherunpong *et al*., 2004). Within all quality of life evaluations, the Child Oral Health Impact Profile (COHIP) questionnaire is multidimensional and could be applied cross-culturally (Broder & Wilson-Genderson, 2007), evaluating those aspects of oral health described by the World Health Organization, not merely the absence of disease, but also the social, psychological and functional well-being (Slade & Reisine, 2007).

In this chapter I will present some studies on these topics, in which the dento-maxillary anomaly is implicitly included in oral health prophylaxis, because its presence can be a cause for worsening/ occurrence of oral diseases. Good verbal communication and harmonious facial and dental appearance are important aspects of their physical, psychological and social well-being of people.

2.1. The role of the family in the development of the sanogenous behaviors at preschool children

The period of childhood can be defined by biological criteria completed by the psychological and the social environment of schooling. The child's concrete and animistic thinking is less distinct than fantasy and playing completes this picture of psychological characteristics (Margineanu, 1944).

Based on these characteristics, this study aims to answer three essential questions regarding the shaping of sanogenous behaviors in preschool children: (1) How important is the role of the mother? (2) How important is the influence of mother's socio-economice status? (3) Can education in schools compensate for the deficiency of information? The study is focused on the conception of illness-health, in this case oro-dental disease and the variables of age, gender, education and financial condition in the preschool group and their mothers. *The aim of this study* was to identify psychosocial factors of the family that may contribute to the development of sanogenous attitudes and behaviors of the preschool child, in order to ensure a proper oral health.

Zegan G, Dascalu CG, Enache AM, Mavru RB. Psychosocial factors in acquiring sanogenous behaviors in preschoolers. *Book Series: Procedia Social and Behavioral Sciences,* May 2015; 187: 16-21.

Material and methods
This longitudinal descriptive observational study was performed on 90 preschool children (45 boys and 45 girls), aged 4-6 years and their mothers, during January-April 2013. They were asked to answer an anonymous oral health questionnaire. Informed consent was obtained from mothers of

the children. Children were enrolled in kindergartens in three socio-demographic different neighbourhoods from Iasi. Children were divided into two groups: 30 (33.33%) children from a kindergarten with nurses (group A) and 60 (66.66%) children from two kindergartens without nurses (group B). Kindergarten nurses previously conducted oral health education lessons for children.

The structure of the questionnaire that assessed the level of knowledge of oral prevention measures consisted of 18 items (e.g., "Did you know why we brush our teeth?", "Do you know when we may eat sweets?", "Did you know that decayed teeth could hurt your heart?"), to which the answer was rated as "yes - has knowledge on the subject of the question" (score=1) or "no - no knowledge on the subject of the question" (score=0). It provided the following information: (1) socio-demographic status (age, gender, education and financial situation of the mother); (2) knowledge of oral hygiene; (3) knowledge of food hygiene; (4) knowledge of preventing dental caries; (5) knowledge of preventing dento-maxillary anomalies; (6) knowledge of prevention periodontal disease; (7) knowledge of preventing disease outbreak.

The questionnaire was applied to mothers, once, at the beginning of the study and separately, to the children, in three stages: pre-test, initially; inter-test, after five sessions of oral health education (lectures and practical demonstrations); post-test, at the end of oral health education sessions. The questionnaire was applied at all stages face to face by one calibrated operator, previously trained, both in terms of medical knowledge and the knowledge of communication with preschool children.

The statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. We identified the score of correct answers to each of the questionnaire's items and we aggregated the items score based on their subject. We used the Chi-square (χ^2) Pearson test to compare the mothers' correct answers and between the groups of children and gender. For all the statistical tests we used the significance level of $p \leq 0.05$ corresponding to a confidence interval of 95%.

Table 6. Relations between mothers' correct answers and the socio-economic status

Variables	Oral hygiene		Nutrition hygiene		Dental decay prevention		Dental anomalies prevention		Periodontal disease prevention		Disease outbreak prevention	
	n	%	n	%	n	%	n	%	n	%	n	%
Correct answers	465	64.6	135	75.0	216	60.0	99	55.0	49	54.4	45	50.0
20-25 years (n=24)	111	57.8	30	62.5	45	46.9	18	37.5	14	58.3	12	50.0
26-30 years (n=33)	186	70.5	48	72.7	84	63.6	42	63.6	16	48.5	21	63.6
31-35 years (n=33)	168	63.3	57	83.4	87	65.9	39	59.1	19	57.6	12	36.4
Chi-square		7.93		8.73		9.54		8.37		0.75		4.91
p value		0.019**		0.013**		0.009**		0.015**		0.688		0.086
Medium studies (n=36)	168	58.3	48	66.7	72	50.0	33	45.8	20	55.6	15	41.7
Superior studies (n=54)	297	68.8	87	80.6	144	66.7	66	61.1	29	753.7	30	55.6
Chi-square		7.75		3.73		9.32		3.48		0.03		1.16
p value		0.005**		0.053*		0.002**		0.062		0.863		0.281
Middle incomes (n=48)	219	57.0	66	68.8	105	54.7	51	53.1	26	54.2	24	50.0
High incomes (n=42)	246	73.2	69	82.1	111	66.1	48	57.1	23	54.8	21	50.0
Chi-square		19.82		3.60		4.38		0.15		0.03		0.04
p value		0.0001***		0.057*		0.036*		0.698		0.955		0.842
the significance level of *p*<0.05; **p*<0.01; ***p*<0.001.												

Results

The answers given by mothers. All mothers had over 50% correct answers to the questionnaire topics. Statistical significant differences occurred amongst the responses of mothers: by age interval regarding the knowledge on oral hygiene and nutrition, prevention of dental caries and dento-maxillary anomalies; by level of education and income regarding the knowledge on oral hygiene and nutrition and the prevention of dental caries (tab. 6).

The answers given by preschool children. The children's correct answers of the pre-test questionnaire according to the socio-demographic status of their mothers are only limited to the knowledge about oral hygiene and nutrition which are highly significant different for children with mothers of different ages, and also to the knowledge about preventing dento-maxillary anomalies for children with mothers with different income state (tab. 7).

The children in group A had more correct answers than the group B children concerning knowledge about oral hygiene and nutrition and the prevention of dento-maxillary anomalies at the pre-test questionnaire. Those concerning the prevention of other oro-dental diseases are non-existent for both groups of children. During learning, the children's knowledge significantly improves, as tested by the inter-test questionnaire. However, at the final testing, the children's knowledge are not fairly 100%, but different for the two groups, group A having better results, except those regarding the prevention of periodontal disease (fig. 2a) and between the two genders (fig. 2b).

Table 7. Relations between children's correct answers to the pre-test questionnaire and the socio-economic status of mothers

Variables	Oral hygiene		Nutrition hygiene		Dental decay prevention		Dental anomalies prevention		Periodontal disease prevention		Disease outbreak prevention	
	n	%	n	%	n	%	n	%	n	%	n	%
20-25 years (n=24)	99	51.6	12	25.0	0	0.0	6	12.5	0	0.0	0	0.0
26-30 years (n=33)	111	42.0	21	31.8	0	0.0	15	22.7	0	0.0	0	0.0
31-35 years (n=33)	111	42.0	9	13.6	0	0.0	12	18.2	0	0.0	0	0.0
Chi-square	5.88		6.20		-		1.94		-		-	
p value	0.0529*		0.0450*		-		0.3791		-		-	
Medium studies (n=36)	132	45.8	15	20.8	0	0.0	9	12.5	0	0.0	0	0.0
Superior studies (n=54)	186	43.1	27	25.0	0	0.0	24	22.2	0	0.0	0	0.0
Chi-square	0.43		0.22		-		2.12		-		-	
p value	0.5120		0.6390		-		0.1454		-		-	
Middle incomes (n=48)	180	46.9	21	21.9	0	0.0	9	9.4	0	0.0	0	0.0
High incomes (n=42)	138	41.1	21	25.0	0	0.0	24	28.6	0	0.0	0	0.0
Chi-square	2.60		0.10		-		9.78		-		-	
p value	0.1069		0.7518		-		0.0018***		-		-	
the significance level of *$p<0.05$; **$p<0.01$; ***$p<0.001$.												

Statistical correlations. We found statistically significant differences comparing the groups of children's results: the three questionnaires for oral hygiene knowledge; inter-test and post-test questionnaires for knowledge concerning the prevention of dental caries; post-test questionnaire for knowledge about disease outbreak prevention ($p<0.05$) (tab. 8). We found statistically significant differences between the two genders, comparing the children's results: the three questionnaires for oral hygiene knowledge; inter-test and post-test questionnaires for knowledge about disease

outbreak prevention; post-test questionnaire for knowledge about food hygiene, prevention of dental caries, of dento-maxillary anomalies, periodontal disease and disease outbreak ($p<0.05$) (tab. 9).
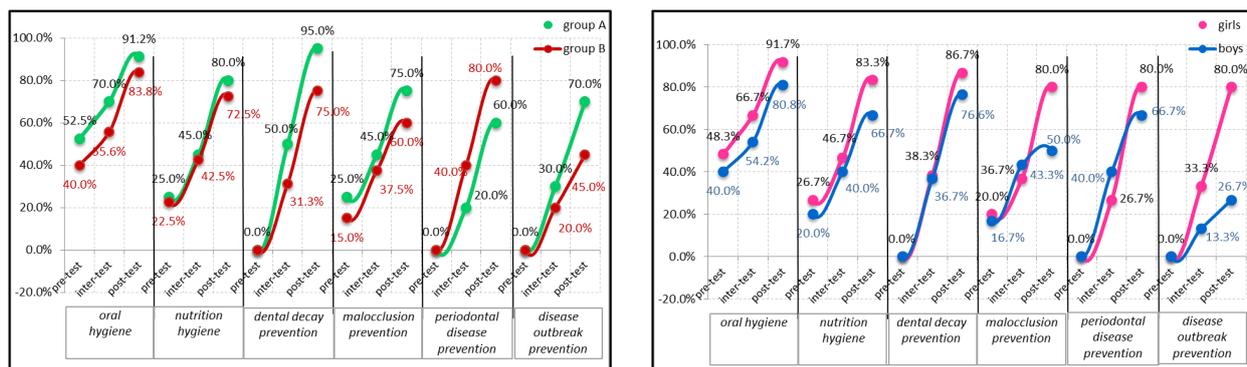
a) on groups   b) on genders

Fig. 2. The evolution of children's correct answers

Table 8. Statistical results of the three tests from the two groups of children

Variables	Oral hygiene		Nutrition hygiene		Dental decay prevention		Dental anomalies prevention		Periodontal disease prevention		Disease outbreak prevention	
	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value
Pre-test	9.64	0.0019***	0.03	0.8625	-	-	2.05	0.1522	-	-	-	-
Inter-test	13.23	0.0003***	0.03	0.8625	11.21	0.0008***	0.65	0.4201	2.76	0.0966	0.63	0.4274
Post-test	6.97	0.0083**	0.83	0.3623	20.06	0.0001***	3.32	0.0684	3.13	0.0769	4.07	0.0437*
the significance level of *p<0.05; **p<0.01; ***p<0.001.												

Table 9. Gender related statistical results of the three tests on children

Variables	Oral hygiene		Nutrition hygiene		Dental decay prevention		Dental anomalies prevention		Periodontal disease prevention		Disease outbreak prevention	
	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value
Pre-test	4.74	0.0295*	0.78	0.3771	-	-	0.15	0.6985	-	-	-	-
Inter-test	11.24	0.0008***	0.57	0.4503	0.05	0.8231	0.58	0.4463	1.25	0.2636	3.98	0.0460*
Post-test	16.91	0.0001***	5.81	0.0159**	5.36	0.0206*	16.51	0.0001***	1.42	0.2334	23.62	0.0001***
the significance level of *p<0.05; **p<0.01; ***p<0.001.												

Discussions

This study dealt with the identification of deep and nuanced information that lead us to an understanding of the importance of complex psychosocial gradients in shaping strategies for oral health education of preschool children. The presented study is distinguished by the originality of the method and the specificity of the study group.

The presented results are related to some aspects of the formation of attitudes and behaviors towards oral health of preschool children. Thus, there were differences in age, education level and income of mothers in relation to their knowledge of oral hygiene and nutrition and the prevention of dental caries. These topics accessible to common knowledge were observed in younger mothers with higher education and high income, which would be reflected in the sanogenous behavior of their children. On the same demographic variables, only the maternal age was significantly involved in the children's knowledge and was only related to knowledge about oral hygiene and nutrition. Mother's education level proved statistically significant and had no influence on the level of knowledge of children in any of the six categories of the studied items. There were differences between the incomes of mothers of children in relation to their knowledge about prevention of

dento-maxillary anomalies, a condition that brings a critical prejudice to facial aesthetics. Mothers' knowledge about prevention of dento-maxillary anomalies, periodontal disease and disease outbreak were sketchy, regardless of age, income or education, which emphasizes the idea that this information is not accessible to common knowledge. The same poor percentage of knowledge was also recorded in of preschool children, at the first application of the questionnaire.

Oral health education and acquisition of sanogenous behavior significantly depended on the information received in education classes, the quality of information and their presentation, by means of comparative statistical analysis of children's responses to the pre-test and post-test questionnaires. Nurse's presence turns out to have significant implications regarding the children's knowledge, only in the case of oral hygiene and nutrition and the prevention of dental caries. Concerning dento-maxillary anomaly prevention, periodontal disease and disease outbreak there were differences in the acquisition of knowledge between the groups with and without medical assistance in the institution, which lead to the conclusion that for such specialty topics specialized staff is needed in order to be explained and understood. During learning, there was noted an increased responsiveness, with a better assimilation of knowledge in case of girls rather than boys.

The present study highlights the search of a group of American specialists, who developed a conceptual model of the most important result of social epidemiology studies in the last 25 years, comprising five key determinants parts in the oral health of children: genetics and biology, social environment, physical environment, health-influencing behaviors and medical care (Fisher-Owens *et al*., 2007). Also, the presented results are in consensus with another U.S. study of a multidisciplinary team, which performed dynamically at 3, 8 and 14 years, measurements of demographic variables, medical and psychosocial and appreciated that maternal psychosocial factors can significantly influence oral health of future teenagers (Nelson *et al*., 2012).

The presented results fall in the direction that the World Health Organization promoted in terms of improving the oral health of children by adopting effective education programs at all levels of education, as early as possible, by showing that health education classes carried out in schools by trained personnel can correct family deficiencies caused by psychosocial factors (Kranz & Rozier, 2011).

Conclusions

This study demonstrated that the family alone cannot induce desirable sanogenous behaviors, though it is important in the early education for the child's oral health. The need to introduce some oral health educational programs in all kindergartens in Romania comes about, providing the necessary information, supplied by trained staff with medical and pedagogical knowledge to ensure proper and adequate assimilation since preschool age.

2.2. The role of the community in detection of the communication deficiency at preschool children

Language impairment is a common developmental problem in young children (Heim & Benasich, 2006). Language impairment is a delay in the appearance and development language of some children up to the age of 3 years, an inability to form simple sentences (Paunescu, 1984). Some of the major consequences of language impairment are hindrance in interpersonal and social communication (McCormack *et al*., 2011), learning difficulties and emotional and behavioral problems (Liiva *et al*., 2005; Yew *et al*., 2015; Snowling *et al*., 2016). Speech and language disorders can have wide-ranging and adverse impacts on the ability to communicate, to acquire new knowledge and participate fully in society (Van Daal *et al*., 2007). Early detection by specialists and therapy of language impairment is a priority for the child, and parents and educators have a decisive role (Sameroff, 2009; Rvachew & Bernhardt, 2010; Miltenberger, 2011; Karmiloff-Smith *et al*., 2014).

Based on these realities, this study aims to answer four essential questions regarding public awareness about childhood language impairment: (1) Do people have information about childhood language impairment? (2) Do people have knowledge about language therapy services? (3) What

role do parents have in the identification and therapy of language impairment? (4) How do people inform to improve the lives of children with language impairment? The study is focused on the children's communication skills, which have a direct impact on their psychological, behavioral and social development.

The purpose of this study was to investigate the level of awareness of a group of Romanian population regarding the childhood language impairment and them implications, depending on the socio-demographic factors of the sample, through public survey.

Material and methods

This cross-sectional descriptive observational study was performed on 75 Romanian volunteers (37 males and 38 females), aged 18-78 years, during March-May 2018. They answered an anonymous survey about childhood language impairment, after obtaining informed consent from all volunteers.

The structure of the questionnaire. This multilingual public survey (Newcastle University, 2019) was formed in 5 sections of questions: (1) socio-demographic information; (2) knowledge regarding childhood language impairment; (3) management/ therapy/ intervention; (4) the role of parents in intervention; and (5) information for improving the quality of life of these children. Multiple answers was rated as "yes - has knowledge on the subject of the question/ the statement is true" (score=1), "no - no knowledge on the subject of the question/ the statement is not true" (score=2), "don't know - did not hear about this subject" (score=3) or unanswered question (score=0).

The statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. We identified the score of answers to each of the questionnaire's items and the score of the correct answers to theme of questions. We used the Chi-square (χ^2) Pearson test to compare the volunteers' answers with socio-demographic variables. We considered the $p \leq 0.05$ value as statistically significant.

Results

Section 1: socio-demographic information: the age and gender groups have a uniform distribution; 44.0% of respondents have bachelor's degree and 66.7% of respondents have a middle economic status; 73.3% of respondents are monolingual and 60% of respondents do not have children under 18 years.

Section 2: knowledge about childhood language impairment: 58.7% of respondents know the term childhood language impairment and 28.0% know someone with this problem; these children have various problems; 33.3% of respondents believe that it is due to psychological (33.3%), behavioral (30.7% of respondents) origin and autism (33.3% of respondents); language impairment can be solved with hard work (46.7% of respondents) and through education (42.7% of respondents); 29.3% of respondents believe that people would help a parent who would have a child with language impairment.

Section 3: management/ therapy/ intervention: 42.7% of respondents have heard about language therapy and 22.7% of respondents know children who have undergone this therapy; 41.3% of respondents believe that the logopedist is the specialist who identifies and applies therapy (38.7% of respondents) and that parents should address to him (34.7% of respondents) (fig. 3); 14.7% of respondents believe that all children have access to language therapy services.

Section 4: the role of parents in intervention: 44% of respondents believe that parents have the role to identify and to be responsible (37.3% of respondents) toward their child with language impairment; 40.0% of respondents believe that parents need to engage in teamwork with the therapist and use strategic techniques (37.3% of respondents); 18.7% of respondents believe that the

language impairment can be prevented by informing parents; 30.7% of respondents do not think that childhood language impairment is related to bilingualism.

Section 5: information for improving the quality of life of children with language impairment: all ways of spreading information about childhood language impairments are good (53-77.3% of respondents); 89.3% of respondents have heard about autism, 58.7% of respondents about dyslexia, 65.3% of respondents about ADHD and 86.7% of respondents about speech impairment, from common sources.

The statistical correlations between answers and age groups, level of education, the economic status of the volunteers and the parents of the children under 18 have shown statistically significant differences ($p<0.05$) (tab. 10). We have not found statistically significant correlations between respondents' answers and gender and with bilingualism ($p>0.05$).
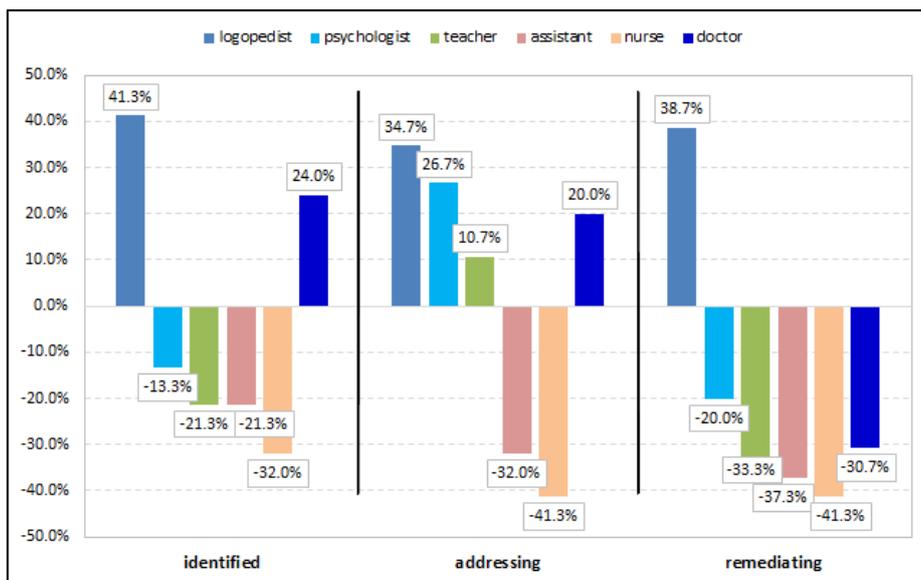

Fig. 3. Who identifies children with language impairment

Table 10. Correlations between responses and socio-demographic variables

Survey	Variables							
	Age intervals		Level of education		Economic status		Parents	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Item 11	*Have you heard of the term (childhood) language impairment?*							
	21.038	0.021*	30.394	0.000*	17.325	0.002*	5.501	0.064
Item 13	*Do you know anyone who has childhood language impairment?*							
	23.015	0.084	33.774	0.000*	20.015	0.003*	6.300	0.098
Item 20	*Have you ever heard of language therapy/intervention for children?*							
	25.716	0.041*	33.449	0.000*	19.023	0.004*	6.597	0.086
Item 21	*Do you think language therapy/intervention for children is helpful?*							
	19.925	0.001*	17.124	0.000*	16.843	0.000*	5.195	0.023*
Item 21a	*Have any of your children or children you know attended speech and language therapy/intervention?*							
	28.408	0.019*	19.652	0.003*	21.617	0.001*	10.150	0.017*
Item 23	*Can all children with childhood language impairment in your country access services/intervention?*							
	33.414	0.004*	25.654	0.000*	23.189	0.001*	9.553	0.023*
Item 26	*Do you think that childhood language impairment can be prevented?*							
	25.953	0.039*	19.994	0.003*	23.536	0.001*	8.274	0.041*
statistically significant differences when $p<0.05$								

Discussions

This research assessed the information of a population group in Romania about the knowledge and the therapy of childhood language impairment, the role of parents and improving the quality of life of these children, using an anonymous public survey.

Child rearing is the process of promoting and supporting the physical, emotional, social, and intellectual development of a child, from infancy to adulthood (Brooks, 2012). Usually, parents are the ones who take care of the child, and their skills vary (Ashish, 2014) depending on the historical time period, race/ ethnicity, social class and other social features (Witt, 2017) and they have a strongly influence on child development (Grienenberger *et al*., 2005; Lieberman *et al*., 2005; Schechter & Willheim, 2009). At the beginning of the school, children are medically and psychologically examined and some disorders (language impairment, autism, dyslexia and ADHD) can be detected.

In the present study, the socio-demographic characteristics of the respondents included in section 1 of the questionnaire served the purpose of the study, in order to know the particularities of the representative categories of the studied group. From section 2 of the questionnaire, more than half of the respondents (1.42:1 ratio) have heard about the child's retardation of language, especially from the media and/or from a person with this language disorder, which they know. The following questions in this section required more in-depth knowledge of the disorders, causes, and solving the childhood language impairment, as well as people's attitudes towards these children. The correct answers varied according to the respondents' knowledge. The rest of the respondents who did not hear about the subject had the opportunity to omit all the questions up to section 5.

In the present study, section 3 of the questionnaire revealed that almost half of all respondents had heard of language therapy and they believe that the logopedist is the one who identifies and makes therapy with the child with language impairment to which parents should address. Few respondents know children who did this therapy and believe that in Romania not all children with language impairment have access to these services for different reasons. The rest of the respondents who did not hear about the subject had the opportunity to omit all the questions up to section 5.

In Romania, some children go to kindergarten/ school with undiagnosed speech and language disorders, because parents are unaware of their deficiency or do not know that there are speech and language therapy supports available. The specialist who identifies children with speech and language disorders could be the family physician, paediatrician or early-childhood educator. They guide the child's parents towards a paediatric neuropsychiatrist, speech therapist or a psychologist. Parents or legal guardians may request public or private services.

Schoolchildren identified with speech and language disorders are guided towards the Inter-School Logopedic Centres in the public education system for assessment and therapy (MECTS, 2011). The intervention plan for the child with language impairment is designed by the logopedist and psychologist, individualized according to each child's speech and language disorder. The logopedist, psychologist, psycho-pedagogue, school educator and support teacher are involved in delivering interventions. Some parents prefer private language therapy services (Law *et al*., 2019).

In the present study, section 4 of the questionnaire revealed that almost half of all respondents make parents responsible for identifying the child with language retardation, which is a misconception. After identifying this problem by a specialist, parents must engage in teamwork with the therapist to help develop the child's language. Few respondents believe that language impairment can be prevented by informing parents and I do not think that the child's language impairment is related to bilingualism, which is a correct opinion. From section 5 of the questionnaire, it emerged that the majority of respondents believe that all the ways of spreading information about the child's impairment language are good for improving the quality of life of these children.

Parents must supervise children's health (physical, mental and social well-being) through offering them healthy food, insuring enough hours of sleep, exercising, and protecting their safety (MedlinePlus, 2019), found in the legislation that protects children's rights. Parents are responsible

for taking decisions for minors. Parents have an essential role in raising children, assuring them not only material existence, but also an affective and moral family climate (Baciu, 2006).

The presented results are related to some aspects of awareness of a group of Romanian population regarding childhood language impairment and represent a part of the results obtained by Working Group 3 of Cost Action IS1406. Some themes were accessible to the common knowledge of the Romanian population and should be reflected in the behavior towards children with this problem, and others were not accessible to common knowledge. The responses were different for age groups, education levels, economic status and those with/without children under 18 years.

Speciality literature in the field does not provide data on the population's views on child language disorders, and the data from present study cannot be compared. In this respect, further studies are needed on other populations.

Through this feed-back given by the Romanian population, this study is important for verifying the knowledge about childhood language impairment. Also, this study is relevant for verifying standards in information, prevention, identification and therapy of children with this issue, helping to know about public implementation measures with the purpose of raising the awareness of the population.

Conclusions

This survey has identified the status of the awareness of Romanian population regarding childhood language impairments, with an impact upon the effectiveness and efficiency of early treatment. There was a gap between people who can recognize the child's language impairment, depending on their age, level of education and economic situation and those with/without children.

2.3. The role of the school in the development of the sanogenous behaviors at schoolchildren

There are inequalities between countries regarding the prevalence and treatment of oral diseases (Patel, 2012). In Romania, the DMFT score (the number of Decayed teeth, Missing teeth due to caries and Filled teeth) decreased from 3.1 in 1986 to 2.8 in 2000 (WHO, 2018). Oral health care treatment is funded by public health insurance system and free treatment is available for children under the age of 18 (Amariei & Eaton, 2009). Prior to 1989, oral health care for children was provided by public health services. After 1989, these services were privatized, and most public health programs were stopped and had a negative impact on population (Petersen *et al.*, 1995; Petersen & Rusu, 2002). In recent years, oral health promotion is done most in private dental offices than in school dental office (Podariu *et al.*, 2017).

Based on these characteristics, this study aims to answer four essential questions regarding the shaping of health behaviors in schoolchildren: (1) Can health education in schools compensate for the deficiency of information? (2) Have schoolchildren correct habits of oral and nutrition hygiene? (3) Can schoolchildren appreciate their oral health status and self-image? (4) How important is the role of the scholar dentist? The study is focused on the conception of illness-health, in these case oro-dental diseases in the schoolchildren sample. *The aim of this study* was to identify the level of knowledge of schoolchildren regarding the sanogenous attitudes and behaviors, in order to ensure a proper oral health.

Material and methods

This cross-sectional descriptive observational study was performed on 166 schoolchildren, 71 boys and 95 girls, aged 7-18 years (mean age 14.51±3.312 years), from four public schools in Neamt County, from the North-Eastern region of Romania, who received oral health education in dental school office, during March-May 2018. They were asked to answer an anonymous oral health questionnaire, after obtaining informed consent from all schoolchildren or their parents.

The structure of the questionnaire that assessed the level of knowledge of oral prevention measures and their oral health condition consisted of 50 items (e.g. "Did you know that decayed teeth could hurt your heart?", "Do you use fluoride toothpaste?"), to which the answer was rated as "yes - has knowledge on the subject of the question/ the statement is true" (score=1) or "no - no knowledge on the subject of the question/ the statement is not true " (score=0).

The questionnaire provided the following information about: socio-demographic status (age, gender and school); oral hygiene (10 items); nutrition hygiene (5 items); prevention of teeth decayed (3 items); preventing dento-maxillary anomalies (7 items); prevention periodontal disease (3 items); prevention outbreak disease (3 items); their oral health condition (7 items); self-image and social impact of facial and dental aesthetics (8 items); the role of the dentist in providing oral health (4 items).

The statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. We have identified the score of the correct answers to each of the questions in the questionnaire and we have cumulative score of the answers according to their theme. Chi-square (χ^2) Pearson tests were used to compare the schoolchildren' answers with socio-demographic variables. We considered the *p*≤0.05 value as statistically significant.

Results

The level of knowledge about oral hygiene measures was appreciated after the answers to items 3, 5, 16, 23, 25, 28, 30, 32, 34 and 39. The schoolchildren had 67.90% correct answers. More than 50% of the correct answers were found in items 3 (97.60%), 5 (97.00%), 23 (80.10%), 28 (71.70%), 30 (65.10%), 34 (92.80%) and 39(86.10%). A percentage of less than 50% correct answers was in items 16 (7.80) and 32 (30.10%).

The level of knowledge about nutrition hygiene measures was appreciated after finding the answers to items 6, 19, 33, 36 and 40. Schoolchildren had 87.10% correct answers. Correct answers were recorded in a percentage greater than 50% for items 6 (91.00%), 19 (95.20%), 33 (97.00%), 36 (94.60) and 40 (57.80%).

The level of knowledge about dental decays prevention measures was appreciated after the answers to items 11, 17 and 21. Schoolchildren had 53.21% correct answers. Thus, correct answers in a percentage of over 50% were in question 21 (92.80%) and in less than 50% in questions 11 (45.20%) and 17 (21.70%).
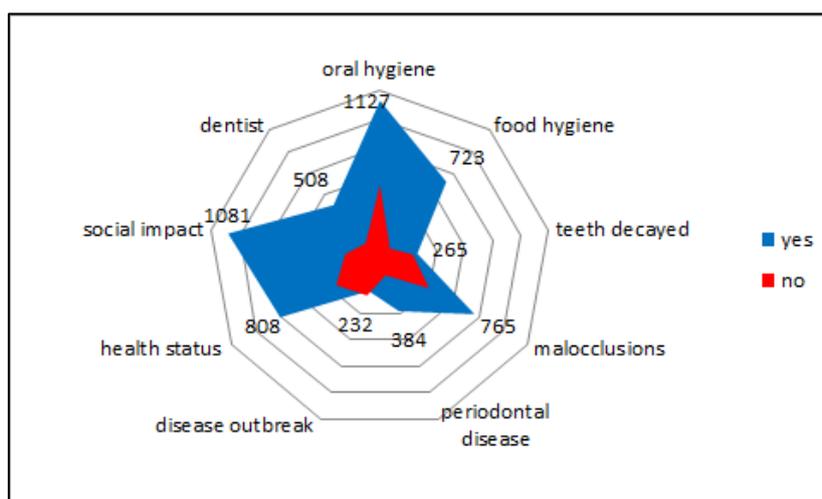

Fig. 4. The overall score of the schoolchildren' correct answers

The level of knowledge about dento-maxillary anomalies prevention measures was assessed according to the answers to items 12, 14, 15, 18, 26, 29 and 35. Schoolchildren had 65.83% correct answers. Thus, correct answers were found in a percentage of over 50% for items 12 (75.90%), 15 (86.70%), 18 (69.90), 26 (68.10%) and 29 (84.90%), while items 14 (39.20%) and 35 (36.10%) were correct answers in a percentage below 50%.

The level of knowledge about periodontal disease prevention measures was assessed according to the answers to items 7, 10 and 31. Schoolchildren had 77.10% correct answers. Correct answers in more than 50% were in items 7 (85.50%), 10 (58.40%) and 31 (87.30%).

Table 11. Statistically significant correlations between schoolchildren answers and age

Items	Survey	Answers		χ^2 test	p value
		yes	no		
Oral hygiene measures					
23	Do you brush your teeth before going to bed?	133	33	22.696	0.045*
32	Do you rinse your mouth with fluoride solutions?	50	116	32.474	0.002*
Dental decays prevention measures					
11	Did you know that gum disease could cause tooth decay?	75	91	29.297	0.006*
17	Did you know that you can keep your teeth healthy if you eat hard food?	36	130	24.862	0.024*
Malocclusion prevention measures					
12	Did you know that temporary teeth are extracted only if they are highly mobile?	126	40	29.658	0.005*
14	Did you know that the backpack should be carried in the back?	65	101	40.766	0.000*
35	Did you know that during classes you have to sit back with your back resting instead of supporting your head in your hand?	60	106	56.798	0.000*
Outbreak disease prevention measures					
2	Did you know that teeth and gums diseases could hurt your body?	109	57	23.020	0.041*
8	Did you know that decayed teeth could hurt your heart?	66	100	47.068	0.000*
Self-assessment's oral health status					
20	Are your teeth without caries?	98	68	28.952	0.007*
44	Do you have straight teeth and do not need dental appliance?	135	31	23.310	0.038*
47	Does your face and teeth look good and your colleagues do not avoid you?	157	9	43.723	0.000*
Self-image and social impact					
43	Does your face and teeth look good?	125	41	23.677	0.034*
*statistically significant differences when $p<0.05$					

The level of knowledge about the measures to prevent the outbreak disease was appreciated after the answers to items 2, 8 and 38. Schoolchildren had 46.59% correct answers. Thus, the correct answers in more than 50% were in item 2 (65.70%) and below 50% in items 8 (39.80%) and 38 (34.30%).

The self-assessment's oral health status was assessed after the answers to items 1, 9, 20, 22, 27, 41 and 44.69.54% of answers were positive feed-back about oral health: items 1 (65.10%), 9 (75.30%), 20 (59.00%), 22 (72.30%), 27 (70.50%), 41 (63.30%) and 44 (81.30%).

The self-image and social impact of facial and dental aesthetics was assessed after the answers to items 4, 42, 43, 45, 46, 47, 49 and 50. 81.40% of answers were positive feed-back about self-image and of the social impact: items 4 (81.90%), 42 (93.40%), 43 (75.30%), 45 (80.10%), 46 (80.70%), 47 (94.60%), 49 (69.30%) and 50 (75.90%).

The level of knowledge about the role of the dentist in providing oral health was appreciated after the answers to items 13, 24, 37 and 48. Schoolchildren had 76.50% correct answers. Correct answers to more than 50% were noted in items 13 (91.60%), 24 (66.90%), 37 (60.80%) and 48 (86.70%).

Statistical correlations. The overall score of the schoolchildren's correct answers was 71.00% (fig. 4). We found statistically significant correlations between some answers and age of the schoolchildren (tab. 11), as well as between schoolchildren answers of the four schools (*p*<0.05) (tab. 12).

Table 12. Statistically significant correlations between schoolchildren answers and schools

Items	Survey	Answers		χ^2 test	*p* value
		yes	no		
Oral hygiene measures					
25	Do you use a fluoride toothpaste?	84	82	15.899	0.001*
30	Do you use the toothpick seldom after you eat?	108	58	8.871	0.031*
32	Do you rinse your mouth with fluoride solutions?	50	116	10.356	0.016*
Malocclusion prevention measures					
14	Did you know that the backpack should be carried in the back?	65	101	17.822	0.000*
35	Did you know that during classes you have to sit back with your back resting instead of supporting your head in your hands?	60	106	35.989	0.000*
Outbreak disease prevention measures					
2	Did you know that teeth and gums diseases could hurt your body?	109	57	12.473	0.006*
8	Did you know that decayed teeth could hurt your heart?	66	100	29.794	0.000*
Self-assessment's oral health status					
1	Are your teeth beautiful and healthy?	108	58	8.201	0.042*
20	Are your teeth without caries?	98	68	8.392	0.039*
27	Are your teeth without spots?	117	49	14.222	0.003*
44	Do you have straight teeth and do not need dental appliance?	135	31	10.492	0.015*
Self-image and social impact					
42	Does your face look good and you do not have a nickname that your colleagues use?	155	11	11.335	0.010*
The role of the dentist					
37	Do you go to the dentist every year, even if you do not have any toothache?	101	65	11.487	0.009*
*statistically significant differences when *p*<0.05					

Discussions

This research has evaluated schoolchildren information in outlining oral health education strategies. This study is original through the questionnaire applied to the respondents and by the specificity of the group of studied schoolchildren. The anonymous questionnaire method enabled schoolchildren to respond as effectively as possible to the questions asked.

The presented results are related to some aspects of the knowledge and the formation of schoolchildren's attitudes and behaviors towards oral health and the prevention of oral diseases. The knowledge about oral hygiene was adequate, but the schoolchildren used less fluoride pills or mouth solutions for dental caries prophylaxis. There were differences between schoolchildren

answers according to age, on tooth washing at bedtime and use of fluoride solutions. The knowledge about nutrition hygiene was excellent.

The knowledge about dental caries prevention were poor and different according to age, schoolchildren did not have sufficient knowledge about the role of the bacterial plaque in the etiology of dental caries and the effect of saliva in tooth washing by vigorous mastication of hard consistency foods.

The knowledge about dento-maxillary anomalies prevention was adequate and different according to age and schools. Schoolchildren did not have sufficient knowledge about the etiopathogenicity of repetitive abnormal postural attitudes and the early extraction of temporary teeth in the production of dento-maxillary anomalies.

The knowledge about periodontal disease prevention was excellent. The knowledge about outbreak disease prevention was poor and different by age and schools, schoolchildren did not have sufficient knowledge about joints and heart illness through bacteraemia caused by chronic dental infection.

Most schoolchildren had a positive image of oral health and the social impact of facial and dental aesthetics, but there were differences in age and schools, in terms of facial and dental appearance and impact with colleagues.

Schoolchildren also knew about the dentist's contribution to oral health. There were differences between the answers of schoolchildren of the four schools regarding the annual appointment to the dentist.

The overall score of schoolchildren's correct answers showed an increased responsiveness and a well assimilation of knowledge. Oral health education and acquisition of health behaviour significantly depended on the information received in education classes, the quality of information and their presentation. Some topics were accessible to common knowledge and should be reflected in schoolchildren's health behaviors and others were not accessible to common knowledge.

Oral health problems affect children and teenagers' quality of life by impairing their physical and psychological and social functioning well-being. Several previous studies investigated the role of oral health of children and adolescents and showed that the main problem is related to toothache that can affect the processes of sleep and learning and can cause difficulty in chewing that can contributes to malnutrition (Oliveira *et al*., 2010). In adolescence, the risk of juvenile periodontal diseases and caries increase and also their possible effects, which will result in poor school performance and school absenteeism (Ananaba *et al*., 2008; Filgueira *et al*., 2016; Ruff *et al*., 2019).

The consumption of sugar-sweetened products begins early in childhood and is an important risk factor that targets primarily the parents (Laitala *et al*., 2018). Dental caries is progressive, and the loss of permanent first molars is a reality in Romania (Raducanu *et al*., 2009), leading to the most difficult orthodontic treatments when associated with a dento-maxillary anomaly. Personal oral hygiene in the absence of fluoridation has failed to show a benefit in terms of reducing the incidence of dental caries (Hujoel *et al*., 2018).

Health promotion strategies for the child and teenager are a responsibility not only of individuals, but of families and communities. Oral health promotion should address the entire population, focusing on diet, hygiene and the prevention of oral diseases, but promoted by nursery and schools as a matter of priority by medical staff and teachers (Oliveira *et al*., 2010). Family physicians and paediatricians are in frequent contact with patients, and their contribution to oral health promotion programs could be applied more effectively (Mohebbi *et al*., 2018).

The presented results are in the direction that World Health Organization promoted in terms for 2020 in Europe, regarding oral health promotion in schools, by adopting effective education programs at all levels of education, by trained personnel, for decreased DMFT index to 1.5 for children of age 12 years (Petersen, 2019).

Conclusions

This study demonstrated that school cannot induce alone desirable health promoting behaviors, although early education on oral health of schoolchildren is important. There is also a need to introduce oral health education programs in all kindergartens and schools in Romania to offer the necessary information provided by specially trained personnel with medical and pedagogical knowledge to ensure proper and appropriate training of sanogenous behaviors.

2.4. The correlation between the self-reported oral health of schoolchildren and quality of life

Studies have reported that children with visible right-left facial asymmetries have lower quality of life, similar to that of children with other chronic disorders (Topolski *et al*., 2005). Children and adolescents have been found to provide reliable information regarding their own OHRQoL when appropriate questionnaire techniques are used although few scales have been developed specifically for these surveys (Jokovic *et al*., 2002; 2004; Filstrup *et al*., 2003). COHIP – questionnaire for assessing the impact of children's OHRQoL, has proved through its testing that it has excellent reliability and validity (Broder & Wilson-Genderson, 2007).

This study's objective is to identify the impact of OHRQoL, by quantifying the COHIP scores on a sample of schoolchildren with Romanian ethnicity from two neighboring Moldavian regions. *The aim of this study* was to calculate the scores of the COHIP questionnaire, with the answers of children from three urban public schools from the North-Eastern region of Romania (Iasi and Suceava) and Republic of Moldova (Chisinau) and to compare the mean values of these scores according to age ranges, genders, cities and countries, in order to assess the correlation between the self-reported oral health and the quality of life.

Material and methods

This cross-sectional analytic observational study was performed on 216 schoolchildren, 113 (52.31%) boys and 103 (47.68%) girls, aged 7-15 years, from three urban public schools of different socio-demographic areas, located in the North-Eastern region of Romania (Iasi and Suceava) and Republic of Moldova (Chisinau), during April-June 2015. Informed consent was obtained from parents of schoolchildren. The schoolchildren were divided into two samples corresponding to the countries (Romania=116 schoolchildren, Rep. Moldova=100 schoolchildren), and into three groups corresponding to the cities of origin (Iasi=28 schoolchildren, Suceava=88 schoolchildren, Chisinau=100 schoolchildren). The subjects were asked to independently answer the standardized questionnaire COHIP. The selection criteria of the participants were based on the childhood's age intervals (middle phase 7-11 years old = 138 schoolchildren, and late phase of childhood/puberty 12-15 years old = 78 schoolchildren) and in a homogeneous ethnic geographic area (white population, Romanian ethnicity).

The COHIP questionnaire (Broder & Wilson-Genderson, 2007) translated into Romanian was used as working tool to measure the OHRQoL. Data was collected from schoolchildren's answers to the 38 items of the questionnaire, grouped into seven sub-scales: (1) oral health, (2) functional well-being, (3) socio-emotional well-being, (4) school-environment (5) self-image, (6) treatment expectancy and (7) global health. The COHIP sub-scales' scores were calculated, which totalled the answers to the specific questions and the overall OHRQoL score (the first 34 items), which totalled the scores of the first five sub-scales (limit values range 0-136).

The statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, IL) for Windows. The numerical values were characterized using the following descriptive statistical parameters. In order to compare the average values of COHIP scores between genders, age intervals, cities and countries of origin, we used the Kolmogorov Smirnov fitting with normal

distribution, *t*-Student test (t) and the nonparametric Mann-Whitney test (U). The *p*≤0.05 value was considered statistically significant.

Results

The schoolchildren answered to all questions of the questionnaire and COHIP scores were calculated individually and their mean values on groups.

The group of schoolchildren from Iasi with ages between 7-11 years old showed statistically significant differences between genders, comparing the mean scores of the oral health (*p*=0.034), the treatment expectations sub-scales (*p*=0.027) and the overall OHRQoL score (*p*=0.050).

The group of schoolchildren from Suceava with ages between 7-11 years old and between 12-15 years old did not show statistically significant differences between age intervals and genders, comparing the COHIP average scores (*p*>0.05).

Table 13. Correlations of the COHIP scores of schoolchildren between the Romanian cities

Scores	Interval age (7-11 years)						Tests
	Iasi (n=28)			Suceava (n=64)			
	Mean	SD	SEM	Mean	SD	SEM	t / U
Oral health	25.14	6.151	1.162	27.59	6.246	0.781	0.085
Functional well-being	20.46	3.939	0.744	20.09	3.711	0.464	0.546
Socio-emotional well-being	29.07	2.918	0.551	26.31	5.433	0.679	0.032*
School-environment	15.32	1.056	0.200	14.84	2.571	0.321	0.768
Self-image	17.21	3.542	0.669	13.81	3.427	0.428	0.000****
Overall OHRQoL	107.21	12.351	2.334	102.66	15.336	1.917	0.185
Treatment expectancy	6.75	1.531	0.289	5.56	1.489	0.186	0.000****
Global health	5.46	1.105	0.209	6.19	1.379	0.172	0.006**
the significance level of *p*<0.05; **p*<0.01; ***p*<0.001; ****p*<0.0001.							

Table 14. Correlations of the COHIP schoolchilren's scores in the middle phase of childhood between countries

Scores	Interval age (7-11 years)						Tests
	Romania (n=92)			Rep. Moldova (n=46)			
	Mean	SD	SEM	Mean	SD	SEM	t / U
Oral health	26.85	6.286	0.655	27.80	4.806	0.709	0.366
Functional well-being	20.21	3.764	0.392	19.15	3.091	0.456	0.019*
Socio-emotional well-being	27.15	4.959	0.517	24.33	5.342	0.788	0.001***
School-environment	14.99	2.226	0.232	13.26	2.871	0.423	0.000****
Self-image	14.85	3.785	0.395	12.80	5.451	0.804	0.058
Overall OHRQoL	104.04	14.578	1.520	97.35	13.788	2.033	0.001***
Treatment expectancy	5.92	1.591	0.166	5.09	1.860	0.274	0.010**
Global health	5.97	1.338	0.139	5.20	1.869	0.276	0.018*
the significance level of *p*<0.05; **p*<0.01; ***p*<0.001; ****p*<0.0001.							

The group of schoolchildren from Chisinau with ages between 7-11 years old and between 12-15 years old showed statistically significant differences between age intervals, comparing mean scores of the functional well-being (*p*=0.009), the socio-emotional well-being (*p*=0.014), the school-environment sub-scales (*p*=0.008) and the overall OHRQoL score (*p*=0.002). We have also found statistically significant differences between genders, comparing average scores of self-image (*p*=0.007) for the age interval of 7-11 years, oral health (*p*=0.013), functional well-being (*p*=0.035), socio-emotional well-being sub-scales (*p*=0.000) and the overall OHRQoL score (*p*=0.001) for the age interval of 12-15 years old.

The statistical correlations of the COHIP mean scores between the Romanian cities for schoolchildren aged 7-11 years old (tab. 13) and between countries for children from the two age stages of the childhood (tab. 14 and 15) show statistically significant differences ($p<0.05$).

Table 15. Correlations of the COHIP schoolchilren's scores in the late phase of childhood between countries

Scores	Interval age (12-15 years)						Tests
	Romania (n=24)			Rep. Moldova (n=54)			
	Mean	SD	SEM	Mean	SD	SEM	t / U
Oral health	26.21	5.672	1.158	28.56	4.820	0.656	0.064
Functional well-being	19.42	4.491	0.917	20.57	3.196	0.435	0.323
Socio-emotional well-being	21.00	7.132	1.456	26.67	5.154	0.701	0.000****
School-environment	15.04	2.196	0.448	14.52	2.187	0.298	0.049*
Self-image	14.00	4.263	0.870	14.57	5.175	0.704	0.668
Overall OHRQoL	95.67	19.662	4.013	104.89	14.170	1.928	0.035*
Treatment expectancy	5.50	1.532	0.313	5.76	1.913	0.260	0.488
Global health	5.42	1.283	0.262	5.44	1.734	0.236	0.643
the significance level of *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.							

Discussions

This study investigated the self-reported oral health and its impact on schoolchildren's quality of life from three cities of the neighboring Moldavian regions, using the COHIP questionnaire, comparing the mean values of scores between age intervals, genders, cities and the two countries.

The COHIP questionnaire was developed by Broder (2007) to assess the orofacial well-being of schoolchildren aged between 8-15 years old across different ethnicities, health systems and different living conditions. The COHIP questionnaire is based on the initial item pool developed by Jokovic (2002) and was used in the development of the Child Perception Questionnaire and it was created as a generic sensitive tool to the oral health's impact on children, to which positive elements that focused on health were added (Broder, 2007).

In the present study, the oral health sub-scale measured specific oral disease symptoms and recorded a good qualifier for schoolchildren of urban centers and significant differences were found between genders for children from Iasi (7-11 years) and Chisinau (12-15 years). The functional well-being sub-scale related to specific everyday activities and had a good qualifier for schoolchildren from urban centers, but there were significant differences between genders and age intervals (Chisinau), for the two countries (7-11 years). The socio-emotional well-being sub-scale related to peer interactions and mood states recorded a very good qualifier for schoolchildren from Iasi and good for those from Suceava and Chisinau and significant differences were found between genders and age ranges for children from the Romanian cities (7-11 years) and for the two countries (7-11 years and 12-15 years). The school-environment sub-scale addressed the school and social tasks and recorded a very good qualifier for schoolchildren from Iasi and Suceava and good for those from Chisinau, but there were significant differences between schoolchildren's age ranges from Chisinau and between the two countries. The self-image sub-scale addressed positive feelings about self and recorded a good qualifier for schoolchildren from Iasi and medium for those from Suceava and Chisinau, and significant differences were found between children from the Romanian cites (7-11 years).

The interpretation of the overall OHRQoL scores of schoolchildren from three urban schools corresponded to a very good quality of life in terms of self-reported health, but there were significant differences between schoolchildren's genders from major urban centers (Iasi and Chisinau) and from the two countries and between age intervals, which means that there were socio-economic differences between schoolchildren, but the oral health and psycho-emotional status did not influence the functionality of schoolchildren's everyday activities.

The treatment expectancy sub-scale was not included in the overall OHRQoL score, as indicated by the authors (Filstrup *et al*., 2003; Broder *et al*., 2007), and in the present study showed a good qualifier for schoolchildren from urban centers and were found significant differences between schoolchildren from the Romanian cities and between the two countries (7-11 years), which could be a positive factor for the two countries' health systems.

The global health sub-scale assessed the feelings about schoolchildren's oral and systemic health and was also not included in the overall OHRQoL score, and the present study showed a good qualifier for schoolchildren from the urban centers and significant differences were found between schoolchildren from the Romanian cities and between the two countries (7-11 years), which means that Romanian ethnic inheritance and environmental conditions in this geographical area are appropriate to maintain a good health state.

Specialty literature cites studies with controversial results, but also with results similar to the presented study. Studies carried out by Chen *et al*. (2010) and Sergl *et al*. (2000) showed a modest impact of malocclusions on the quality of life. Several studies found no associations between OHRQoL and gender, but Paula *et al*. (2012) and Castro *et al*. (2011) reported a greater psychological impact in girls of oral health, because they showed higher care than boys (Freddo *et al*., 2008). Sigelman and Shaffer (1995) reported problems with self-image in subjects with ages between 12-14 years old and more evident in girls, because they are at puberty, characterized by a series of physical changes, for which they do not possess satisfactory psychological skills.

The study confirms that oral health represents a determinant of the quality of life of schoolchildren, measured by functional, psychological, social and economic dimensions.

Conclusions

In this study, the studied schoolchildren with ages between 7-15 years old, of Romanian ethnicity, with the same culture and eating habits from three different urban public schools located in the North-East of Romania and Republic of Moldova had a good self-reported oral and general health status. Statistically significant differences between genders, age intervals, cities and countries were found in some sub-scales of the COHIP questionnaire. Health status, well-being, inheritance, environmental conditions, self-confidence and confidence in the health system had a positive impact on the quality of life of schoolchildren from this geographical area.

2.5. The correlation between socio-economic status of family and the impact of self-reported OHRQoL of schoolchildren

A human community is characterized by a geographical area, certain life conditions, and a profile of health status and risk of disease, caused by specific social and economic indicators. Health concepts suggest that dental health must be defined in terms regarding the physical, psychological and social well-being, related to dental status (Cohen & Jago, 1976). Various measurements for the quality of life and the socio-economic factors were developed and used to evaluate the social, dental well-being and the impact on OHRQoL (Slade, 1997; Allen, 2003). Thus, the COHIP questionnaire was developed to measure OHRQoL at children (Broder & Wilson-Genderson, 2007) and the Quick European Socio-Economic Status Questionnaire (SESq) was developed to evaluate the families' socio-economic status (Gonzales-Gross, 2014).

The purpose of this study was to establish the correlations between socio-economic status of family and the impact of self-reported OHRQoL on a sample of schoolchildren from several urban public schools from the North-Eastern region of Romania (Iasi and Suceava cities), using two standardized questionnaires.

Dascalu CG, **Zegan G***, Mavru RB, Cernei ED. The influence of socio-economic status on oral health of schoolchildren from the Moldavian region of Romania. *Romanian Journal of Experimental Applied Psychology* Aug 2016; 7(Special Issue 1): 216-219.

Material and methods

This cross-sectional analytic observational study was performed on 184 schoolchildren, 99 (53.8%) boys and 85 (46.2%) girls, aged 8-15 years, from the urban public schools of two major cities from the North-Eastern region of Romania (Iasi and Suceava), during May-June 2015. Informed consent was obtained from parents of schoolchildren. The participants' selection criterion was the children's age. The schoolchildren independently responded to the COHIP and SESq standardized questionnaires, translated into Romanian.

The structure of the questionnaires. The COHIP questionnaire (Broder & Wilson-Genderson, 2007) with seven sub-scales was used to measure self-reported OHRQoL of schoolchildren. The SESq questionnaire was to evaluate the self-reported socio-economic status of their families, being composed of 8 items grouped into five domains: (1) living conditions, (2) family structure, (3) employment status of both parents, (4) education level of both parents, and (5) family income according to the occupation of both parents.

Table 16. Correlations between COHIP scores and the families' socio-economic status

Scores	Overall OHRQoL			Treatment expectancy			Global health		
	Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	*p* value
Living conditions			0.014*			0.001**			0.089
good	138.55	14.259		9.07	1.438		7.86	1.505	
medium	132.50	13.111		7.85	1.861		7.30	1.501	
Family structure			0.028*			0.693			0.068
functional	133.78	13.842		8.03	1.821		7.43	1.511	
dysfunctional	130.39	8.603		8.17	2.176		7.00	1.495	
Employment status of mother			0.162			0.209			0.131
high	135.77	16.074		8.13	2.045		7.48	1.749	
medium	132.31	13.969		8.14	1.710		7.46	1.217	
low	136.00	13.771		8.55	1.549		7.76	1.431	
undefined	132.23	10.573		7.58	2.014		7.04	1.720	
Employment status of father			0.000****			0.000****			0.575
high	141.00	12.419		8.98	1.611		7.67	1.443	
medium	134.80	13.114		8.23	1.592		7.17	1.654	
low	131.18	12.467		7.91	1.929		7.16	1.758	
undefined	129.03	12.884		7.38	1.836		7.49	1.247	
Education level of mother			0.297			0.000****			0.923
college	135.09	15.225		8.31	1.997		7.40	1.666	
high school	132.28	12.967		7.63	1.823		7.40	1.497	
without	135.74	13.009		9.18	1.193		7.35	1.433	
Education level of father			0.000****			0.000****			0.108
college	140.23	13.301		8.89	1.701		7.64	1.448	
high school	131.67	11.822		7.64	1.771		7.41	1.585	
without	130.29	15.542		8.20	1.937		7.03	1.317	
Family income			0.006**			0.005**			0.004**
high	140.27	13.748		8.58	1.858		7.62	1.941	
medium	132.41	12.916		7.80	1.820		7.47	1.420	
low	131.75	14.632		9.00	1.686		6.55	1.276	
the significance level of *p*<0.05; **p*<0.01; ****p*<0.0001.									

Statistical analysis was performed in SPSS 16.0 (SPSS Inc., Chicago, IL), for Windows. The numerical values were characterized using the parameters of descriptive statistics. We used the Kolmogorov-Smirnov fitting test with normal distribution, the nonparametric Mann-Whitney (U) test (U) and Kruskal-Wallis test (K-W) in order to compare the COHIP scores between genders and

the SESq domains and the Chi-square (χ^2) Pearson test in order to compare the SESq answers between genders. We considered statistically significant a value of $p \leq 0.05$. We used a binary logistic regression model (B) in order to identify the socio-economic predictors and the validity of the model was determined with the Hosmer-Lemeshow goodness-of-fit test (HL test), at a significance level $p > 0.05$. To eliminate the multicollinearity problems, the multiple correlations among factors were analyzed. Statistical analysis was conducted at a significance level of 5% and $p < 0.05$.

Table 17. Socio-economic predictors for schoolchildren's quality of life

Predictors	Yes		No		Test		OR	95% CI	p
	n	%	n	%	%	HL			value
Very good oral health	47	25.5	137	74.5	75.5	1.000			
Family has a private car	25	53.2	72	52.6			0.117	0.039-0.345	0.000
Family has at least two private cars	15	31.9	13	9.5			0.301	0.126-0.719	0.007
Very good functional well-being	136	73.9	48	26.1	73.9	0.225			
Family has a private car	78	57.4	19	39.6			0.427	0.150-1.212	0.110
Family has at least two private cars	22	16.2	6	12.5			1.120	0.399-3.144	0.830
The child has a private room	90	66.2	23	47.9			0.486	0.243-0.972	0.041
Very good emotional well-being	144	78.3	40	21.7	78.3	1.000			
Family has a private car	83	57.6	14	35.0			0.280	0.086-0.915	0.035
Family has at least two private cars	24	16.7	4	10.0			0.988	0.297-3.282	0.984
Very good self- image	51	27.7	133	72.3	75.5	0.515			
Family has two children	25	49.0	69	51.9			0.213	0.071-0.637	0.006
Family has more than two children	15	29.4	19	14.3			0.570	0.241-1.349	0.201
The child lives with both natural parents	27	52.9	88	66.2			1.043	0.456-2.387	0.920
The child lives with one natural parent	11	21.6	33	24.8			3.990	1.259-12.652	0.019
The child lives with one natural parent and one step parent	8	15.7	11	8.3			22.527	2.168-234.037	0.009
Very good overall OHRQoL	76	41.3	108	58.7	67.4	1.000			
Low family income	8	10.5	12	11.1			0.123	0.044-0.347	0.000
Medium family income	47	61.8	91	84.3			0.159	0.042-0.596	0.006
Very good treatment expectancy	85	46.2	99	53.8	67.9	0.565			
Family has a private car	46	54.1	51	51.5			0.117	0.038-0.358	0.000
Family has at least two private cars	20	23.5	8	8.1			0.331	0.125-0.882	0.027
The child lives with both natural parents	52	61.2	63	63.6			2.430	1.076-5.489	0.033
The child lives with one natural parent	26	30.6	18	18.2			0.097	0.016-0.572	0.010
The child lives with one natural parent and one step parent	3	3.5	16	16.2			7.267	1.109-47.624	0.039
Low family income	15	17.6	5	5.1			0.532	0.204-1.389	0.198
Medium family income	54	63.5	84	84.8			8.789	1.447-53.400	0.018
Very good global health	34	18.5	150	81.5	82.1	0.988			
The child has a private room	29	85.3	84	56.0			0.257	0.089-0.742	0.012
Family has two children	15	44.1	82	54.7			0.261	0.087-0.779	0.016
Family has more than two children	19	55.9	68	45.3			0.321	0.116-0.887	0.028
Low family income	0	0.0	20	13.3			0.355	0.134-0.938	0.037

Results

The schoolchildren answered to all questions of the questionnaire and the COHIP scores were calculated individually as well as average values. No statistically significant differences were found between the COHIP scores on genders ($p > 0.05$).

We compared the children's families socio-economic levels deriving from the SESq questionnaire and we found statistically significant differences between genders regarding the

family's structure (p=0.008). We found multiple statistically significant differences between the values of overall OHRQoL, treatment expectancy and global health scores reported to the socio-economic levels of children's families (p<0.05) (tab. 16).

We used a binary logistic regression model in order to identify the socio-economic predictors for very good values of the COHIP scores (HL test, p>0.05) (tab. 17).

Discussions

This study investigated the influence of socio-economic status of family on the OHRQoL on a sample of schoolchildren from several urban public schools from the North-Eastern region of Romania (Iasi and Suceava cities) by comparing the mean values of COHIP scores between socio-economic status levels and identified their families' socio-economic predictors, using a cross-sectional survey based on standardized questionnaires.

All mean scores of COHIP sub-scales and schoolchildren's general OHRQoL were classified as "very good" quality of life, in the terms of self-reported health state, and no significant differences were found between genders, meaning that the living conditions from the studied geographic area were adequate to maintain health status. Generally, the studied schoolchildren's families' socio-economic status level was classified as "medium".

The statistical correlations between the means of COHIP scores and socio-economic status revealed highly statistically significant differences, which means that the family's socio-economic level influences the schoolchildren's quality of life. We have also identified with an acceptable precision, the socio-economic predictors for a very good schoolchildren's quality of life, such as: family owning 1-2 private cars, family's composition of two or more children, child owning his own private room, children being cared by natural parents, having a medium level of income. The literature has very limited studies of this kind for the European countries (Vereecken *et al*., 2009; Baran & Nalcaci, 2011).

Conclusions

This study proved that the socio-economic factors of schoolchildren's families from the investigated geographical area affects the self-reported oral health status related to the personal well-being and quality of life, measured by functional and psychological dimensions. Similar future studies can be extended to young adults from different geographical areas.

2.6. The correlation between the impact of self-reported OHRQoL of teenage students and their reasons for starting the orthodontic treatment

The World Health Organization defined quality of life as the perceptions of an individual's position in life in the context of culture and value system in which he lives, reported to the objectives, expectations, standards, and its concerns (WHOQOL, 1995). The quality of life is acknowledged as a valid parameter in the evaluation of human subjects in almost all areas of physical and mental health care, including oral health (Clancy & Eisenberg, 1998; Nelson *et al*., 1998).

This study aimed to assess the impact of self-reported OHRQoL on a sample of teenage students with/without orthodontic treatment, from a public urban school from the North-Eastern region of Romania (Suceava) and investigate their reasons for starting the orthodontic treatment, using two standardized questionnaires.

Material and methods

This cross-sectional analytic observational study based on two standardized questionnaires was conducted in May-June 2015, on 114 teenage students, 29 (25.4%) boys and 85 (74.6%) girls, aged 13-18 years, 80 (70.2%) teenage students from urban area and 34 (29.8%) teenage students from rural area, which they attended a public urban college from the North-Eastern region of Romania (Suceava). Informed consent was obtained from parents of schoolchildren. The selection criterion was based on the teenage students' age interval of adolescence. The sample of teenage students was divided into two groups: 43 teenage students with present/previous orthodontic treatments and 71 teenage students without orthodontic treatment.

The structure of the questionnaires. The COHIP questionnaire (Broder & Wilson-Genderson, 2007) with seven sub-scales was used to measure self-reported OHRQoL of all teenage students. The orthodontic questionnaire (Wedrychowska-Szulc & Syryńska, 2010) was used only for teenage students with orthodontic treatments, being consisted of 7 items, to which the answer was "yes" (score=1) or "no" (score=0), in order to find out their motivation for choosing the orthodontic treatment.

The statistical analysis was performed using the SPSS 16.0 (SPSS Inc., Chicago, IL) for Windows. Numerical values were characterized using the average value and the standard deviation. We used the Kolmogorov-Smirnov fitting test with normal distribution, *t*-Student test (t) and the nonparametric Mann-Whitney test (U) in order to compare the COHIP scores between the groups, genders and area of origin. We used the Chi-square (χ^2) Pearson test to compare the answers for the orthodontic questionnaire between genders and teenage students' area of origin. We considered the $p \leq 0.05$ value as statistically significant.

Results

Teenage students answered all items of the questionnaire and the COHIP scores were calculated individually and as well as the average values on groups.

We found statistically significant differences between the two groups, comparing the average scores of the oral health (*p*=0.001), functional well-being (*p*=0.004), socio-emotional well-being (*p*=0.000), school-environment sub-scales (*p*=0.019) and the overall OHRQoL score (*p*=0.000) (tab. 18). No statistically significant differences were found between genders and environment of origin (*p*>0.05).

Table 18. Correlations between groups of adolescent students for COHIP scores

Scores	Total (n=114)		With treatment (n=43)		Without treatment (n=71)		Tests
	Mean	SD	Mean	SD	Mean	SD	t / U
Oral health	24.39	4.725	26.42	5.128	23.17	4.028	0.001***
Functional well-being	8.26	2.778	9.33	3.393	7.62	2.107	0.004**
Socio-emotional well-being	10.85	4.549	12.53	4.793	9.83	4.102	0.000****
School-environment	3.77	1.276	4.21	1.641	3.51	0.908	0.019*
Self-image	14.74	2.717	15.12	2.452	14.51	2.858	0.248
Overall OHRQoL	62.02	12.389	67.60	13.881	58.63	10.069	0.000****
Treatment expectancy	4.64	1.325	4.65	1.307	4.63	1.344	0.923
Global health	7.59	1.225	7.58	1.239	7.59	1.226	0.640
the significance level of *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.							

Comparing the numerical values of the levels of intensity of COHIP scores, we found statistically significant differences between the groups regarding the socio-emotional well-being (*p*=0.031), school-environment sub-scales (*p*=0.002) and the overall OHRQoL score (*p*=0.015).

The group of teenage students with orthodontic treatment showed statistically significant differences between genders, comparing the average score of self-image sub-scales (*p*=0.050) and

showed no statistical significance between environments of origin (*p*>0.05). Furthermore, this group of teenage students also answered the orthodontic questionnaire and there were found no statistically significant differences between genders and environments of origin (*p*>0.05).

Discussions

This study investigated the impact of self-reported OHRQoL on a sample of teenage students with/without orthodontic treatments, from an urban public school from North-Eastern Romania (Suceava), by comparing the average for scores and intensity levels of self-reported OHRQoL on groups, genders and environments of origin, using the COHIP questionnaire. The reasons why teenage students started the orthodontic treatment, using an orthodontic questionnaire were also investigated. We calculated, compared and interpreted the COHIP questionnaire scores for the two groups of teenage students with/without orthodontic treatments. The study's discrimination validity was supported by the finding of significant differences in the COHIP scores between the two groups of teenage students. Teenagers with orthodontic treatments presented high mean scores of self-reported OHRQoL at all subscales of the COHIP questionnaire compared to those without treatments, without differences between genders and environment of origin, which means that the carried out treatment had a positive impact effect on functional and socio-emotional wellbeing and on self-esteem. Sigelman and Shaffer (1995) reported some problems with self-image of teenagers, more obvious in girls, because they pass through a series of physical changes, for which they do not have satisfactory psychological skills.

The overall OHRQoL scores of teenage students had a "middle" qualifier, but significant differences between the groups were found, the teenage students with orthodontic treatments having a high average score, which means that the orthodontic treatment performed had a modest impact on the quality of life, as also reported in scientific literature (Chen *et al*., 2010). The treatment expectancy sub-scale measured the expectations of the therapeutic outcomes and was not included in the overall OHRQoL score, as indicated by the authors (Broder, 2007) and there were no significant differences between groups, the teenagers getting the qualifier "middle", which illustrates teenager students' perception on the medical services of the health system. The global health sub-scale assessed feelings about teenage students' oral and systemic health and was not included in the overall OHRQoL score, as indicated by the authors (Broder *et al*., 2007) and no significant differences were found between groups, teenagers getting the qualifier "very good", which means that heredity and environmental conditions in this geographic area are adequate to maintaining a good health status.

The second questionnaire was applied only to teenage students with present/previous orthodontic treatments. The most important reason for starting the orthodontic treatment was the aesthetic factor and parents' reasons along with the doctors' advice that had decreasing percentages of motivations, as some authors reported (Rajagopal *et al*., 2011; Marques *et al*., 2009). The effect of social stereotypes based on facial looks as a main factor in life adjustment is one reason for choosing orthodontic treatment. Dental anomalies were reported to be the cause of teasing and "general playground harassment" among children and are related with less social attractiveness (Hamdan, 2004).

In the specialty literature, there are no studies of the two questionnaires associated and applied on groups of subjects, but only separate studies on the COHIP and orthodontic questionnaires, associated or not with other questionnaires or clinical examinations.

Conclusions

In this study, the COHIP questionnaire demonstrated the ability to discriminate between groups of teenage students with and without orthodontic treatment and the level of psychological impact and self-reported OHRQoL, which was better at those who have aesthetic reasons in choosing the orthodontic treatment because of social attractiveness reasons. Similar studies can also be further extended to other childhood age ranges as well as to the adults who previously received orthodontic treatments.

CHAPTER 3
Clinical researches in the pathology of the permanent dentition development

Odontogenesis represents the embryologic formation and development of the teeth, which begins in the sixth intrauterine week and takes place in four stages (morphogenesis period): band stage, cup stage, bell stage and dental follicle (Boboc, 1995). Development of human dentition is a complex process, through cell proliferation from the epidermis and the subadjacent mezenchyma derived from the cells of the neural crest (Butler, 1995; Pispa & Thesleff, 2003; Caruntu *et.al.*, 2011). Odontogenesis is a mechanism regulated by sequential and reciprocal epithelio-mesenchymal interactions, controlled by activators and inhibitors. More than 300 genes were involved in tooth development (Thesleff, 2000; Kim *et al.*, 2006; Pawlowska *et al.*, 2010). Regulation of the dental pattern is generated by the combined expression of the HOX genes from the mezenchyma of the neural crest cells (Jernvall & Thesleff, 2000; Wang & Thesleff, 2005).

Interactions between genetic and environmental factors from the odontogenesis period can produce dental anomalies of number (anodontia/ supernumerary teeth), shape (fused/ geminated/ dwarfism teeth), size (macrodontia/ microdontia), eruption (impacted tooth) or structure (imperfect amelogenesis or dentinogenesis), clinically visible at the time of the dental eruption.

The clinical experience gained over time has allowed me to make an archive of patients with dental anomalies, who have asked for specialist advice. Later, I made statistical, clinical and radiological studies in this field, which I have published. The select topics depended on the increased prevalence of the cases encountered in the Romanian population, such as those with hypodontia and impaction tooth.

3.1. Clinical and therapeutic aspects of hypodontia

Permanent teeth hypodontia represents the congenital missing of a small number of teeth (1-6 teeth), excluding the third molar. Oligodontia is the congenital missing of more than 6 teeth (excluding the third molar). Anodontia is an extreme case that defines the total absence of teeth (Ionescu, 2005). The most common form of dental agenesis is hypodontia (Mattheeuws *et al.*, 2004). Population studies on permanent severe teeth hypodontia reported a prevalence of over 80% in the cases of 1-2 congenital missing teeth, less than 10% of cases with 4-5 missing teeth and less than 1% of cases with six or more missing teeth (Bergström, 1977; Larmour *et al*, 2005).

Hypodontia has a multifactor aetiology, a genetic predisposition and also environmental factors intervene in varying proportions. Two categories of authors support the etiopathogenic hypotheses of hypodontia (Galluccio *et al.*, 2012). The anatomical theories demonstrate that teeth development is produced under a strict genetic control, while hypodontia is the phenotypical expression of the mutation of the genes involved in the development process (Kapadia *et al.*, 2007; Kavitha *et al.*, 2010). The evolutionist theories explain agenesis as a numerical reduction of the last permanent teeth in the dental groups, as due to the reduction of the maxillary arches through diminution of mastication, as a result of food processing (Vastardis, 2000; Nieminen, 2009).

Hypodontia can be isolated (non-syndromic) or associated with other congenital anomalies (syndromic) (Arte, 2001). The most frequently reported phenotype of dental agenesis was the isolated hypodontia. In studies conducted on Caucasian populations, mandibular second premolars and maxillary lateral incisors were the most frequent congenitally missing teeth (Rose, 1966; Brook, 1974), and for the Asian population the mandibular central incisors were missing (Davis, 1987). Hypodontia is associated with around 150 syndromes (Baraitser & Winter, 1999), but the most common are the congenital anomalies associated (cleft lip and/or palate) (Ranta, 1986; Laatikainen & Ranta, 1994). Various genes were involved in tooth agenesis: homeobox genes MSX1 and MSX2, PAX9 or TGFA. MSX1 and MSX2 genes play an important role in mediating direct epithelial-mesenchymal interactions during tooth development. The mutation of genes that can stop the development of the teeth was previously suspected to be also associated with ovarian cancer (Iavazzo *et al.*, 2006; Fekonja *et al.*, 2015). Mutation of MSX1, MSX2 AND PAX9 genes was associated to dental agenesis, as well as to other congenital anomalies (Tribulo *et al.*, 2003;

Lammi *et al*., 2003). The MSX1 gene mutation was associated to clefts palate and to non-syndromic form of hypodontia, and the PAX9 gene mutation as well (Satokata & Maas, 1994; Vastardis *et al*., 1996; van den Boogaard *et al*., 2000; Das *et al*., 2003).

The orthodontic treatment might be a good option for many clinical cases with tooth agenesis, but the restoration of the integrity of dental arches requires a team of specialists to achieve the aesthetic result expected by the patient.

3.1.1. The prevalence of clinical forms of hypodontia and the correlations on genders

Numerous studies have reported the prevalence of permanent teeth hypodontia ranging between 2.6% and 11.3% in populations, among continents and races (Muller *et al*., 1970; Rolling, 1980; Davis, 1987; Arte, 2001; Mattheeuws *et al*., 2004; Larmour *et al*., 2005), with an insignificant predominance in women (Polder *et al*., 2004). In Europe, the prevalence of hypodontia differs in the various populations under investigation. Consequently, a prevalence of the permanent teeth hypodontia of 11.3% was registered for the population of Germany and Slovenia (Fekonja, 2005; Behr *et al*., 2011), of 7.25% for that of Spain (Tallón-Walton *et al*., 2010), of 4-5% for white population in the UK (Kirkham *et al*, 2005), of 4.5% for the population of Norway (Nordgarden *et al*., 2002), and of 4.3%, respectively, for that of Turkey (Celikoglu *et al*., 2010).

This research aims at elucidating the clinical aspects of permanent teeth hypodontia, with the exception of third molars, on a group of young patients from the North-Eastern region of Romania. *The study aimed* to establish the prevalence of hypodontia according to the number of congenitally missing teeth, the affected dental groups, the clinical forms, symmetry and the localization of hypodontia, and their correlations on genders.

Patients and methods

In this cross-sectional descriptive observational study, the sample consisted of 111 orthodontic patients, with ages between 5 and 28 years (mean age 11.09±4.289 years), 51 boys (mean age 10.41±2.954 years) and 60 girls (mean age 11.67±5.115 years), diagnosed with hypodontia (third molars excepted) and treated in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, between 1990 and 2012. The patients with genetic syndromes, oligodontia and clefts lip and/or palate were excluded. All patients/ children's guardians included in the study gave their informed consent. The diagnosis of hypodontia was established by the interview (absence of eruption of certain permanent teeth after exfoliation of the deciduous teeth), clinical (absence of some permanent teeth from the dental arches) and panoramic radiographic examinations (agenesis of some permanent dental buds).

Statistical analysis was performed in SPSS 20.0 (Chicago, Illinois, SUA) for Windows, the frequency distributions for the variables included in the study and the parameters of descriptive statistics being calculated. For establishing the statistical correlations among the parameters, the Chi-square (χ^2) Pearson test was used, with a level of standard signification of 0.05.

Results

According to the number of congenitally missing teeth, 4 categories of hypodontia were found, as follows: 54 (48.7%) patients with one tooth-hypodontia; 47 (42.3%) patients with two teeth-hypodontia; 2 (1.8%) patients with three teeth-hypodontia; and 8 (7.2%) patients with four teeth-hypodontia. The hypodontia of the dental groups registered a prevalence of 37.8% (42 patients) for the mandibular premolar group, of 36.0% (40 patients) for the maxillary incisor group, of 7.2% (8 patients) for the mandibular incisor group, of 6.3% (7 patients) for the maxillary premolar group and of 4.5% (5 patients), respectively, for the mandibular molar group; 9 (8.1%) patients presented hypodontia in several dental groups. According to the clinical forms of

hypodontia, three main patterns were found, as follows: 54 (48.7%) patients with monodental hypodontia, 50 (45.0%) patients with unidental hypodontia and 7 (6.3%) patients with bidental hypodontia. The unilateral hypodontia was observed in 55 (49.6%) patients, the bilateral one in 51 (45.9%) patients, and the combination of unilateral hypodontia with the bilateral one in 5 (4.5%) patients. There were no statistically significant differences between genders and variables of hypodontia ($p>0.05$).

Table 19. Correlations between clinical forms and other variables of hypodontia

Variables	Clinical forms of hypodontia						Total patients		χ^2	p value
	monodental		unidental		bidental					
	n	%	n	%	n	%	n	%		
Number of congenitally missing teeth									188.105	0.000*
one tooth-hypodontia	54	48.7	0	0.0	0	0.0	54	48.7		
two teeth-hypodontia	0	0.0	47	42.3	0	0.0	47	42.3		
three teeth-hypodontia	0	0.0	0	0.0	2	1.8	2	1.8		
four teeth-hypodontia	0	0.0	3	2.7	5	4.5	8	7.2		
Dental groups with hypodontia									115.564	0.000*
upper incisors	19	17.2	21	18.1	0	0.0	40	35.3		
lower incisors	4	3.6	4	3.6	0	0.0	8	7.2		
upper premolars	4	3.6	3	2.7	0	0.0	7	6.3		
lower premolars	23	20.7	19	17.2	0	0.0	42	37.9		
lower molars	4	3.6	1	0.9	0	0.0	5	4.5		
combined groups	0	0.0	2	4.5	7	6.3	9	10.8		
Symmetry of hypodontia									186.690	0.000*
unilateral	54	48.7	0	0.0	1	0.9	55	49.6		
bilateral	0	0.0	50	45.0	1	0.9	51	45.9		
unilateral with bilateral	0	0.0	0	0.0	5	4.5	5	4.5		
*statistically significant differences when $p<0.05$										

Hypodontia on 1st quadrant was found in 43 (38.73%) patients, on 2nd quadrant in 40 (36.03%) patients, on 3rd quadrant in a 56 (50.45%) patients, and on 4th quadrant in a 39 (35.13%) patients. According to the World Dental Federation numbering system, the most congenitally missing tooth was 35 (42.3% of patients), 12 (31.5% of patients), 22 (29.7% of patients) and 45 (26.1% of patients). Significant statistical differences between the clinical forms of hypodontia and the number of congenital missing teeth were found, and also between the affected dental groups and the symmetry of hypodontia ($p=0.000$) (tab. 19).

Discussions

In this study we established the prevalence of hypodontia according to the clinical situations of the Romanian patients studied, which defined the number of congenitally missing teeth, the affecting of dental groups, the clinical forms, the symmetry and the localization of the hypodontia and made correlations between these and the genders.

Several authors have tried to establish a diagnostic classification of hypodontia, according to severity, describing mild hypodontia as the congenital missing of 1-2 teeth, moderate hypodontia as the congenital missing of 3-5 teeth and severe hypodontia (or, as stated by other authors, oligodontia) as a congenital missing of 6 or more teeth (Goodman *et al.*, 1994; Dhanrajani, 2002). In studied sample, the most frequent was mild hypodontia, as related in previous studies, developed on other populations (Larmour *et al.*, 2005).

The present study mainly focused on the analysis of the dental groups affected by hypodontia and evidenced another order of the prevalence, as follows: the mandibular premolar group, the maxillary incisor group, the mandibular incisor group, the maxillary premolar group and the mandibular molar group. A novelty was the analysis of the prevalence of combination of the

affected dental groups with hypodontia, the most frequently occurring being the combination of the hypodontia of the mandibular premolar groups with the maxillary incisor one.

According to the clinical forms, hypodontia can be: unidental, when a bilateral tooth is congenitally missing from a dental group; bidental, when a bilateral tooth is congenitally missing from two dental groups of teeth (Haavikko, 1971); multidental, when a bilateral tooth is congenitally missing from several dental groups of teeth; and monodental, when one single tooth is congenitally missing from the dental arches. Analysis of the clinical forms of hypodontia in the study sample represented a new aspect of the investigation presented to the previously studies. Thus, the monodental and unidental clinical forms were more frequent than the bidental ones.

Previous studies to hypodontia mentioned no difference among unilateral and bilateral or maxillary and mandibular affectations (Silva Meza, 2003; Polder *et al.*, 2004; Altug-Atac & Erdem, 2007). In the present study, the number of patients with unilateral hypodontia was greater than those with bilateral hypodontia, and several patients had hypodontia on the mandible to maxilla. Hobkirk *et al.* (2011) reported the following prevalence of the congenitally missing teeth: 41.0% the mandibular second premolar, 22.9% the maxillary lateral incisor, 21.2% the maxillary second premolar, 3.5% the mandibular central incisor, 2.8% the maxillary first premolar and 2.5% the mandibular lateral incisor. In the analyzed sample, the order of prevalence of congenitally missing teeth was: 35, 12, 22 and 45.

In the present study, the clinical characteristics of hypodontia were not different by genders. But there were statistical differences between the clinical forms of hypodontia and the number of congenitally missing teeth, the dental groups and symmetry of hypodontia.

In terms of location, it is generally ascertained that hypodontia produces serious local, skeletal, functional and aesthetic complications, its treatment being an interdisciplinary one (Jones, 2009). The Index of Orthodontic Treatment Need (IOTN), using a 5-point scale, indicates the highest need of treatment in cases of mild or moderate hypodontia from any dental quadrant (category 5) (Waring & Jones, 2003; Ferguson, 2006), as it has a high impact upon the dental status (Shelton *et al.*, 2008). Even if the distribution of the congenitally missing teeth and the clinical forms were not severe in the group of population here under investigation, the therapeutically implications are major according to the IOTN index and will require the intervention of a team of specialists to solve late complications of hypodontia.

Conclusions

In the Romanian patients sample we found an increased prevalence of mild hypodontia in mandibular premolar and incisor maxillary groups. The patients presented frequently monodental and unidental clinical forms, without significant differences on gender. The clinical forms found in patients were different depending on the number of congenitally missing teeth, the dental groups and the symmetry of hypodontia. The prejudices of congenitally missing teeth upon the maxillary arches and also upon dental and facial aesthetics call for an early detected of hypodontia by practitioners, for achieving a therapeutically management for the benefit of patients.

3.1.2. The hypodontia patterns and the dental compensations in the remaining free space

Clinical and panoramic radiographic examinations of the teeth and the dental buds adjacent to the remaining free space on the dental arches that were created through the congenital missing of permanent teeth could increase the understanding of the natural phenomena of the dental compensations and could help to choose the orthodontic or prosthetic treatment.

The purpose of this study was to identify hypodontia patterns on each patient and genders and to establish the prevalence of temporary teeth persistence on the dental arcades and/or the decrease of the remaining free space on each hypodontia pattern, through compensatory movements of the permanent teeth/ permanent dental buds adjacent to hypodontia, on panoramic radiography.

Patients and methods

In this cross-sectional descriptive observational study, all patients were consulted in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, between January 1991 and October 2009. The informed consent was obtained from all patients/ children's guardians. The diagnostic of hypodontia was established clinical and panoramic radiographic examinations on 107 orthodontic patients (49 boys and 58 girls), with a mean age of 10.76±3.616 years (between 6 and 28 years). Patients with oligodontia, anodontia and the clefts lip and/or palate were excluded. The hypodontia of the three molar was not taken into account.

The panoramic radiographies made with the Orthopantomograph STRATO-X with magnification factor of 11.8%. Radiologic images were photographed with digital camera negatoscope Canon PC1012 (DC 4.3V, Japan) with a resolution of 1024x768 for fluorescent light. Two observers independently evaluated the images.

Statistical analysis was performed in the SPSS 17.0 (Chicago, Illinois, SUA) for Windows, through techniques of analysis of variables with descriptive statistics.

Results

The most frequently *hypodontia patterns* found on studied patients are: 35 hypodontia la 21 (19.6%) patients; 12-22 hypodontia in 20 (18.7%) patients; 35-45 hypodontia in 19 (17.8%) patients; 12 hypodontia in 11 (10.3%) patients; and 22 hypodontia in 8 (7.5%) patients (fig. 5a). The most frequent hypodontia pattern found on males are 12-22, 35-45 and 35 hypodontia, and on female, 35, 35-45, 12-22, 12 and 22 hypodontia (fig. 5b).

<table>
<tr><td align="center">a) on patients</td><td align="center">b) on sexes</td></tr>
</table>
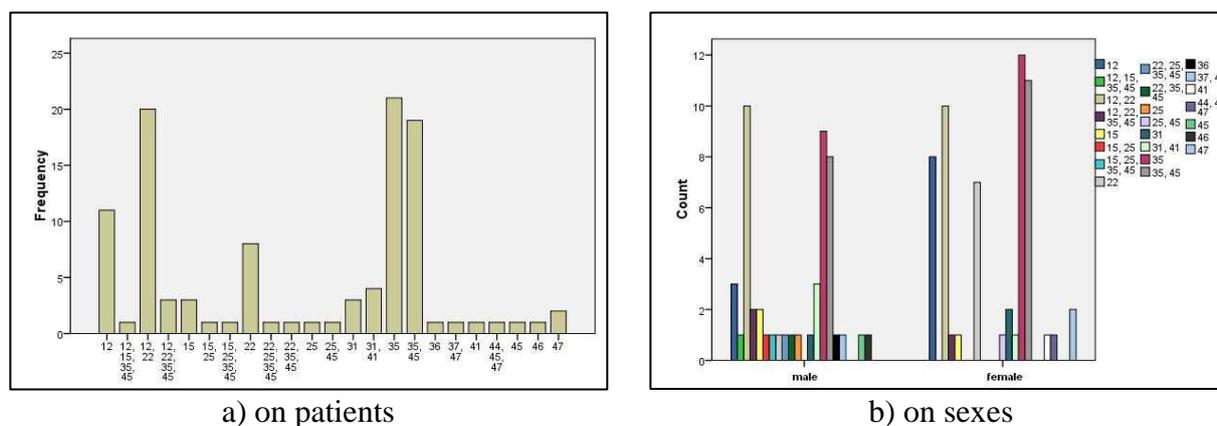
Fig. 5. The distribution of hypodontia patterns

In *the hypodontia of mandibular second premolars* (35 hypodontia pattern = 47 congenital missing teeth; 45 hypodontia pattern = 27 congenital missing teeth), 50 temporary mandibular second molars were present on the dental arch and 24 were absent and decreased the remaining free space. 17 permanent mandibular first molars made mesial movements and 14 mandibular first premolar made distal movements to remaining space, through tipping (24 teeth), translation (6 teeth) or rotation (1 tooth) movements. The panoramic radiography of a 9-year-old female patient in figure 6a shows the presence of 75 on the dental arch, the agenesis of the 35 bud and the distal tipping movement of the 34 in eruption; the absence of 85 on the dental arch and the agenesis of the 45 bud, distal tipping movement of 44 and the mesial translation movement of 46.

In *the hypodontia of permanent maxillary lateral incisors* (12 hypodontia pattern = 35 congenital missing teeth; 22 hypodontia pattern = 33 congenital missing teeth), 27 temporary maxillary lateral incisors were present on the dental arch and 41 were absent and decreased the

remaining free space. 60 permanent maxillary canines/ its buds made mesial movement and 8 permanent maxillary central incisors made distal movement to remaining space, through translation movements (20 teeth). The panoramic radiography of an 8-year-old male patient in figure 6b shows the presence of 52 and 62 on the dental arch, the agenesis of the 12 and 22 buds and the mesial translation movements of the 13 and 23 buds. The panoramic radiography of an 8-year-old female patient in figure 6c shows the absence of 52 and 62 on the dental arch, the agenesis of 12 and 22 buds and with the emergence of a maxillary midline diastema, the distal translation movement of 11 and 21 and the mesial tipping movement of the 13 and 23 buds.

In *the hypodontia of the permanent mandibular central incisors* (31 hypodontia pattern = 7 congenital missing teeth; 41 hypodontia pattern = 5 congenital missing teeth), 6 temporary mandibular central incisors were present on the dental arch and 6 were absent and decreased the remaining free space. 7 permanent mandibular lateral incisors made mesial movement to remaining space, through tipping (4 teeth) or translation (3 teeth) movements. The panoramic radiography of a 7-year-old male patient in figure 6d shows the agenesis of 31 and 41 on the dental arch and the mesial translation movement of 32 and 42.

| a) 35-45 agenesis | b) 12-22 agenesis | c) 12-22 agenesis |
| d) 31-41 agenesis | e) 25 and 45 agenesis | f) 46 agenesis |
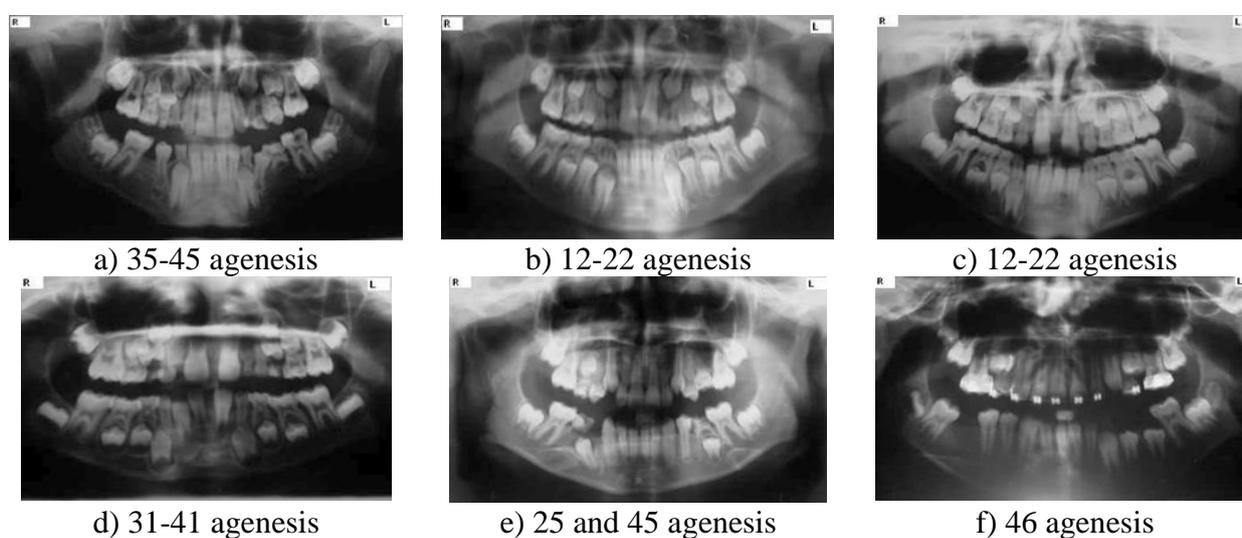
Fig. 6. Dental compensation in hypodontia

In *the hypodontia of the maxillary second premolars* (15 hypodontia pattern = 6 congenital missing teeth; 25 hypodontia pattern = 5 congenital missing teeth), 8 temporary maxillary second molars were present on the dental arch and 3 were absent and decreased the remaining free space. 3 permanent maxillary first molars made mesial movements and 3 maxillary first premolars made distal movement to remaining space, through tipping (2 teeth), translation (3 teeth) or rotation (1 tooth) movements. The panoramic radiography of a 9-year-old female patient in figure 6e shows the presence of 65 on the dental arch and the agenesis of the 25 bud; the presence of 85 on the dental arch, the agenesis of 45 bud and the distal tipping of the 44 bud.

In *the hypodontia of the permanent mandibular second* (5 congenital missing teeth) or *first molars* (2 congenital missing teeth), the free space was decreased by the permanent distal tooth's mesial movement (7 teeth), through tipping (1 tooth) or translation (1 tooth) movements. The panoramic radiography of a 10-year-old male patient in figure 6f shows the agenesis of 46 on the dental arch and the mesial tipping of 47 in eruption.

Discussions

In this study the hypodontia patterns were detected on a group of Romanian patients: the persistence of the temporary teeth on the dental arcades and the movements of the permanent/ temporary teeth and the dental buds that compensated the remaining excess space on the dental arcades, on each hypodontia pattern were observed, using clinical and panoramic radiographic examinations.

In the studies by Haavikko (1971), Thompson and Popovich (1974) the mandibular second premolar is the most frequent tooth that is congenitally missing, followed by the permanent maxillary lateral incisor or the maxillary second premolar. The congenitally missing of the permanent mandibular central incisors, mandibular first molars and canines is very rare (Arte, 2001). Present study confirmed this data from literature, and presented a new aspect, namely the prevalence of hypodontia patterns in patients and the difference of these patterns on genders, data which was not found reported in previous studies. Kirkham *et al.* (2005) stated that the hypodontia is similar to the two sexes, but in present study the most frequent pattern found on males was the permanent maxillary lateral incisors hypodontia followed by the mandibular secondary premolars hypodontia, and on females the mandibular secondary premolars hypodontia followed by the permanent maxillary lateral incisors hypodontia.

Generally, the anterior permanent teeth have a tendency to the distal drift, and the posterior permanent teeth have a tendency to the mesial drift, when there are free spaces on the dental arches. The temporary teeth do not have time to make dental movements in free spaces, because they have a short time on the dental arches.

In the cases of the hypodontia patterns of the mandibular/ maxillary secondary premolars, the presented results showed an increased prevalence of temporary molar persistence on the dental arcades, which will keep the space for a longer time, until it will be occupied by a prosthetic device/ implant in order to restore the dental arcade continuity. In the absence of temporary molars on the dental arcades, there is a tendency to close the remaining free space through the tipping movement of the adjacent teeth, as a natural dental compensation similar to the ones from single dental edentulous. In this case, an orthodontic correction with the rebound teeth and the reopening of the space is required, followed by the prosthetic restoration of the mandibular dental arch continuity.

In the cases of the hypodontia pattern of the permanent maxillary lateral incisors, the presented results showed an increased prevalence of absence of temporary lateral incisors on the dental arcades, causing the decrease of space, through distal movements of the permanent central incisors, with the occurrence of midline diastema, which many patients want to close orthodontic. During distal movement of permanent central incisors, they make an egression, aggravating the dental aesthetics and complicating the orthodontic treatment. Becker and Dunitz (1998) state that the in case of the congenitally missing of the maxillary lateral incisor, the canine comes directly in palatal position on the dental arch and will frequently erupt in the place of the congenital missing lateral incisor. The maxillary canine will most often erupt in an obliquely mesial position, because the congenitally missing of the maxillary lateral incisor can't provide guidance for the canine's bud, even if the distance between the canines is wide enough to allow the normal eruption of the canines.

These aspects observed in this study of the natural compensations of the teeth/ buds adjacent to the hypodontia for closing the remaining space, in excess on the dental arcades, which are not found in the literature, are important for choosing the orthodontic and/ or implant-prosthetic treatment.

Conclusions
This study reported for the first time the natural phenomena of compensatory movements of adjacent teeth/ dental buds of hypodontia. The study showed that there are different patterns of hypodontia for the two sexes and the movements of adjacent teeth/ dental buds were specific to the hypodontia patterns. These favourable or unfavourable clinical situations regarding the free space closure must be detected early on the panoramic radiography, because they will dictate the future therapeutic approach.

3.1.3. Diagnostic and therapeutic assessments of familial hypodontia
Genetic studies attempted to explain the mutation of the genes involved in dental agenesis (Suarez & Spence, 1974; Thesleff, 2000; Nanci, 2018), yet no unanimously accepted conclusion could be reached. Family and twin studies were relevant for emphasizing the strong genetic influence (Brook, 1984; Svinhufvud *et al.*, 1988; Liu *et al.*, 1998). Molecular genetic studies have

attempted to identify the familial genes involved in the craniofacial and dental development (Arte *et al*., 1996; Thesleff, 1998; 2000).

Not being clarified, the mechanisms of congenital missing of teeth have attracted the attention of specialists on the phenotypic and genotypic variation of hypodontia. Previous family studies have focused more on phenotypic and genotypic aspects of tooth agenesis, on the association with other dental anomalies, on finding the causal mutation gene and less on clinical aspects caused by tooth agenesis. Considering these, I have studied two families from the North-Eastern region of Romania, to identify the hypodontia phenotype and to analyse the dental, occlusal and skeletal aspects consecutive to the inheritance of tooth agenesis.

Zegan G, Braha E, Sodor A, Golovcencu L, Anistoroaei D. Phenotypic aspects of familial hypodontia in maxillary lateral incisors. *International Journal of Medical Dentistry,* 2018 Apr-Jun; 22(2): 69-76.

Zegan G, Mavru RB, Braha E. Craniofacial morphological changes of familial bilateral hypodontia of maxillary premolars. *Romanian Journal of Morphology and Embryology* 2014; 55(2): 443-448.

The first family was investigated to identify the phenotype of permanent maxillary lateral incisors hypodontia, with possible environmental risk factors, in order to reveal the dental and occlusal aspects consecutive to the inheritance of tooth agenesis.

Patients, material and methods

These observations were made on five selected members of the same Caucasian family in Neamt County, who participated as volunteers that gave informed consent. The family selection criterion was the proband, with permanent maxillary lateral incisors hypodontia, which addressed orthodontic treatment. The data on the occurrence of permanent maxillary lateral incisors hypodontia and the pathological history of this family was obtained by interviewing the proband and one of her sisters. The diagnosis of permanent maxillary lateral incisors hypodontia was established considering the dental history and by clinical and panoramic radiographic examinations.

Results

Family features. The family survey was conducted on five generations (90 members) at the Medical Genetics Office in Iasi, Romania and identified seven persons affected by permanent maxillary lateral incisors hypodontia (II.9, II.11, III.20, III.25, III.27, III.29 and III.32 - the proband) (fig. 7). The prevalence of permanent maxillary lateral incisors hypodontia was 41.66% in first degree relatives. It could not be determined whether the paternal grandparents (I.1 and I.2) in Vrancea County were affected by the permanent maxillary lateral incisors hypodontia, because the grandfather (I.1) was totally edentulous and the grandmother (I.2) was deceased. Also, it could not be determined if the grandchildren (IV.17 of 2 years old and IV.18 of 0.1 year) are affected, because hypodontia cannot be diagnosed in children under 6 years of age.

Permanent maxillary lateral incisors hypodontia has autosomal dominant inheritance with incomplete penetrance and variable expressivity. No ectodermal abnormalities of nails, hair, skin, or sweat glands were present in any of the examined family members or other associated congenital anomalies, indicating mild non-syndrome tooth agenesis. Two members of this family presented other dental and occlusal anomalies (III.22 and III.33).

Dental and occlusal features

Case III.32. The proband, female, 18-year-old from Neamt County addressed treatment, in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, for the absence of some maxillary anterior teeth. The dental history of the patient revealed that the deciduous maxillary incisors were not replaced by the permanent ones. Also, the patient previously performed odontal treatments and therapeutically extractions in some permanent posterior teeth due to the complications subsequent to decay. On the maxillary arch was observed

permanent young dentition, the absence of the lateral incisors (12 and 22), the decrease of the remaining free spaces, 5 mm of midline diastema and a remained root (2.6) (fig. 8a). On the mandibular arch, the permanent young dentition was interrupted by post-extraction spaces (36 and 46) (fig. 8b). In the examination of the dental occlusion, anterior (11 and 21) and posterior (16) crossbites were found (fig. 8c and d). The panoramic radiography examination confirmed the congenital missing of upper lateral incisors buds (12 and 22) and identified the agenesis of 18, impaction of 28, and pronounced mesial tipping of the buds of 38 and 48, as well as the decrease of the left temporomandibular inter-joint space (fig. 8e).
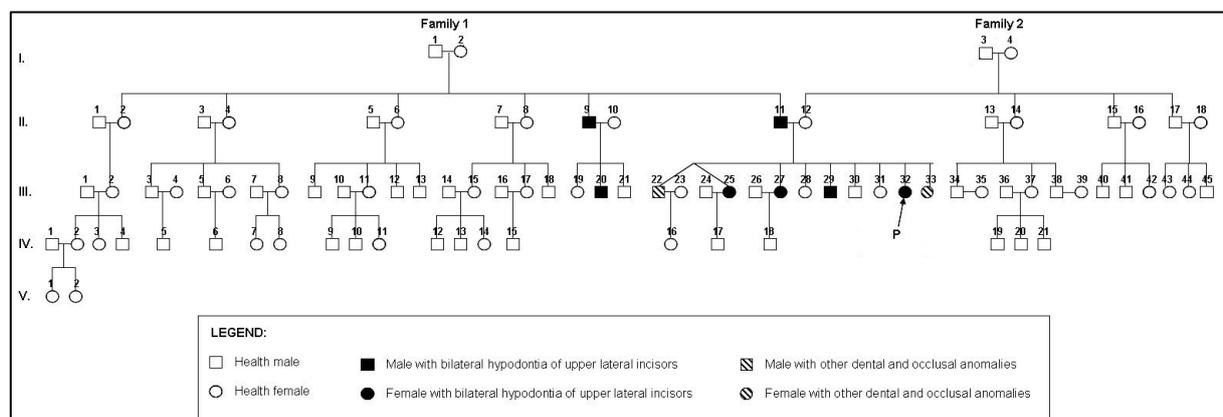
Fig. 7. Pedigrees of family with autosomal dominant inheritance of permanent maxillary lateral incisors hypodontia

a) discontinuity of the maxillary arch

b) discontinuity of the mandibular arch

c) anterior abnormal occlusal relations

d) posterior abnormal occlusal relations

e) permanent maxillary lateral incisors agenesis
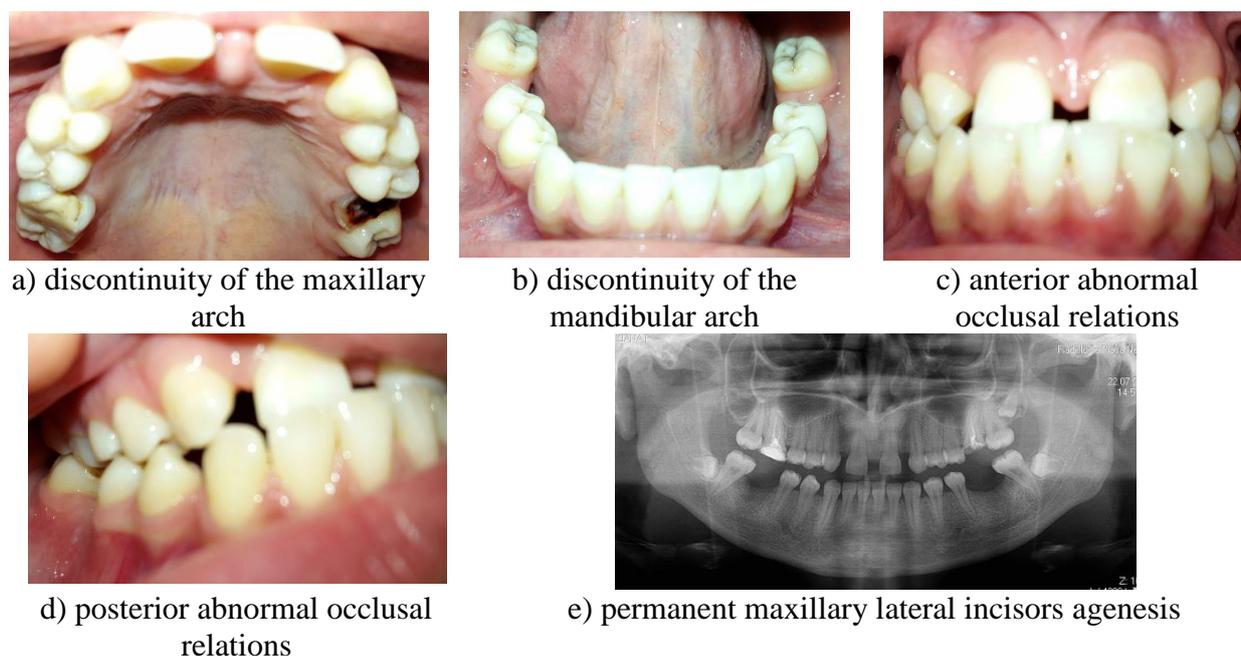
Fig. 8. Dental and occlusal features of proband

Case III.27. The 27-year-old proband's sister reported the same lack of eruption of the permanent maxillary lateral incisors after the exfoliation of the deciduous teeth. The patient was treated at the age of 24 years for midline diastema, anterior crossbites (11 and 21) and the mandibular left lateral deviation, followed by implant-prosthetic treatment, both performed in private offices in Iasi, Romania. The panoramic radiography examination prior to the treatment confirmed the congenital missing of upper lateral incisors buds (12 and 22) with the decrease of the remaining free spaces, the presence of midline diastema, as well as the decrease of the right temporomandibular inter-joint space (fig. 9a). The orthodontic treatment aimed to close the midline

diastema and to open the remaining free spaces for the insertion of the dental implants (fig. 9b). Implant-prosthetic treatment restored the integrity of the dental upper arch (fig. 9c).
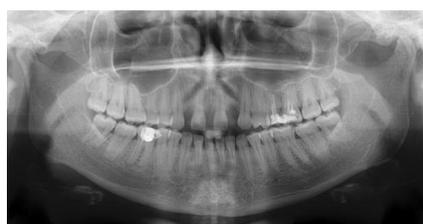
a) permanent maxillary lateral incisors agenesis

b) results of the orthodontic treatment

c) results of the implant-prosthetic treatment

Fig. 9. Dental and occlusal features of proband's sister

Case III.29. The 24-year-old proband's brother remarked the presence of a deciduous tooth in the upper arch. The maxillary arch presented permanent dentition, the absence of lateral incisors (12 and 22), the persistence of the deciduous canine (63), complete transposition of permanent canines (13 and 23) on the position of the absent lateral incisors (12 and 22) and root remains (14, 16 and 26) (fig. 10a). On the mandibular arch was observed permanent young dentition, mandibular anterior mild dental crowding and root remains (36 and 46). In the examination of dental occlusion a mild mandibular left lateral deviation was found (fig. 10b). The congenital missing of upper lateral incisors buds (12 and 22) was confirmed by panoramic radiography examination, and a decrease in left temporomandibular inter-joint space was observed (fig. 10c).

a) complete transposition of teeth

b) mild abnormal occlusal relations

c) the pathology of the dental status

Fig. 10. Dental and occlusal features of proband's brother

Fig. 11. Angle Class III malocclusion

Fig. 12. Dental and occlusal anomalies

Case III.22. Another 28-year-old brother of the proband did not present the permanent maxillary lateral incisors hypodontia as opposed to his twin sister (III.25), but he was diagnosed with Angle Class III malocclusion with complete lingual crossbite and mandibular right lateral deviation (Fig. 11).

Case III.33. Another 15-year-old sister of the proband did not have permanent maxillary lateral incisors hypodontia, but isolated teeth position anomalies and mandibular left lateral deviation were observed (Fig. 12).

Discussions

We selected this family in which the hypodontia presented dominant autosomal inheritance, to define the family's dental and occlusal characteristics consecutive permanent maxillary lateral incisors hypodontia.

Population studies of permanent maxillary lateral incisors hypodontia showed a prevalence of 0.79-2.6% (Coelho *et al.*, 2012; Mani *et al.*, 2014; Pagan-Collazo *et al.*, 2014; Kabbani *et al.*, 2017; Gracco *et al.*, 2017;)). Structured studies of families reported a prevalence of hypodontia that exceeds by far the values in the general population (Wang *et al.*, 2000; Arte *et al.*, 2001; Gabrikova *et al.*, 2016). The non-syndromic form of permanent teeth hypodontia is occasionally caused by environmental factors, most frequently monogenic, of dominant autosomal inheritance (De Coster *et al.*, 2009; Gabrikova *et al.*, 2016), with incomplete penetrance and variable expressivity in most families (Vastardis, 2000; Arte, 2001; Fekonja, 2005; Shimizu & Maeda, 2009). The autosomal dominant hypodontia form could be correlated with MSX1 or PAX9 mutation (Shimizu & Maeda, 2009). Women are usually more affected (Polder *et al.*, 2004) and the male/female ratio is about 2/3 (Medina, 2012). The higher prevalence observed in females could be associated with biological differences (smaller jaws which interacts with environmental factors) (Rakhshan, 2015). The PAX9, EDA, SPRY2, SPRY4 and WNT10A genes were identified as genetic risk factors for permanent maxillary lateral incisors agenesis in many previous family studies (Qin *et al.*, 2013; Alves-Ferreira *et al.*, 2014; Abdalla *et al.*, 2014).

In the presented family, the high prevalence of permanent maxillary lateral incisors hypodontia in first degree relatives and the father-son inheritance of the condition indicated autosomal dominant inheritance. The penetration of hypodontia was incomplete and variable in expressivity. The recurrence risk is theoretically 50% for a couple formed from an affected person and a healthy one. Permanent maxillary lateral incisors hypodontia was categorized according to its severity in mild hypodontia (Dhanrajani, 2002), because two bilateral teeth in the incisor group are congenitally missing and non-syndromic hypodontia, because of the lack of association with other congenital abnormalities.

The role of the etiological factors of family hypodontia has been studied by Brook (1984), who proposed a single multifactorial model, polygenic and environmental. Starting from this model, Parkin *et al.* (2009) have come to the conclusion that family hypodontia is not only determined by genetic factors, but also by epigenetic and environmental factors that are equally important.

In this family, paternal grandparents were born in Vrancea County, and the proband and her brothers in Neamt County, both of which are in the North-Eastern mountain region of Romania, with rich flora and cold waters, but also with many pollutants, which cannot be excluded as possible mutagens. The sources of pollution in the Vrancea area came from industrial toxic substances and agro-zoo-technical substances, from industrial combustion of fuels with sulphur and from dust and black smoke, which produced acid rain (APM Vrancea, 2013). The Neamt area was polluted with heavy industrial metals and uranium (ISU Neamt, 2017).

In the studied family, three members affected by permanent maxillary lateral incisors hypodontia presented common and individual dental and occlusal features. The absence of the replacement of deciduous maxillary lateral incisors with the permanent ones was related to the first two cases and in the third case only the persistence of a deciduous canine was observed. The permanent maxillary lateral incisors agenesis has been radiologically confirmed. In the first two cases the decrease of remaining free spaces and the presence of midline diastema, with parallel crowns and roots as an effect of distal drifts of central incisors were observed. In the third case, the remaining free spaces were closed by complete transposition of permanent canines in the absent lateral incisors area and by the persistence of a deciduous canine. The anterior crossbite of the maxillary central incisors in the first two cases were due to the numerical deficit of the maxillary teeth compared to the mandibular teeth. The mild mandibular anterior dental crowding observed in the third case was due to a smaller maxillo-mandibular dental disharmony. The three cases presented radiological and morphological unilateral changes of the temporomandibular joint.

The last two cases unaffected by permanent maxillary lateral incisors hypodontia were presented in this context, because their anomalies were common to the other cases from the family, namely anterior crossbite and mandibular lateral deviation. Of all the family members, only one received complex multidisciplinary orthodontic and implant-prosthetic treatment, for the rehabilitation of maxillary dental arch and occlusion.

Generally, there are two orthodontic treatment options for the morphological correction of the maxillary arch with permanent maxillary lateral incisors hypodontia: closure of the remaining spaces or opening of the free space and applying a bridge/ implant prosthetic (Sabri, 1999; Rosa & Zachrisson, 2007). The decision is taken depending by the clinical condition, type of malocclusion, available space, teeth inclination, aesthetic demands, duration of treatment and patient's choice (Cioloca-Holban *et al*., 2016; Rosu *et al*., 2017).

Previous studies were not conclusive for the most frequent option regarding the type of the orthodontic treatment (Andrade *et al*., 2013). The study of Rosa *et al*. (2016) showed that the orthodontic space closure by first premolar intrusion and canine extrusion in patients with permanent maxillary lateral incisors agenesis did not cause long term risks for periodontal tissue deterioration or for the temporomandibular joint. The study of Uribe *et al*. (2013) performed with come-beam computed tomography showed that after opening the space for the dental implant in patients with permanent maxillary lateral incisors agenesis, the alveolar bone width significantly decreased by 2-10 mm and the labial concavity increased after implantation. Generally, the alveolar bone of the permanent maxillary lateral incisors agenesis is labial-orally less developed and cannot be influenced by orthodontic treatment, compared to the alveolar bone resulting after dental extractions. Therefore, bone grafting is required to obtain a proper implant placement.

This work is of interest to both family members and practitioners. Early detection of permanent maxillary lateral incisors agenesis by performing a panoramic radiography at the age of 6-7 years, may be followed by closure of the remaining free spaces by the mesial drifts of all maxillary teeth (orthodontic movement in consensus with the natural movement of the posterior teeth), which will favor the three-dimensional alveolar bone formation. At a late age, the prognosis of the case depends on the severity of dental and occlusal imbalances consecutive permanent maxillary lateral incisors hypodontia and the orthodontic and restorative treatment involves more specialists and high costs (Mingardi *et al*., 2012; De Avila *et al*., 2012; Pereira *et al*., 2015). Although both therapeutic options are effective, previous studies have shown that, when possible, the orthodontic closure of the remaining free space is more advantageous than prosthetic rehabilitation (Kiliaridis *et al*., 2016).

Conclusions

We have described a typical phenotype of permanent maxillary lateral incisors hypodontia, which has been categorized as non-syndromic mild hypodontia with dominant autosomal inheritance and an increased risk of recurrence in the presented family. Additional environmental factors have been suggested as possible mutagens. We have identified the dental and occlusal characteristics, common and individual, of related persons. Only one case received complex orthodontic and implant-prosthetic treatment. We suggest early detection of the permanent maxillary lateral incisors agenesis, for the establishment of an interceptive orthodontic treatment, with great benefits for the patient.

The second family was investigated to identify the phenotype of maxillary first and secondary premolars hypodontia, which represents a rare case with a clinical significance on the changes in the dento-maxillary and craniofacial morphology with multidisciplinary diagnostic implications.

Patients, material and methods

The study was carried out on two white members of the same family, as volunteer participants, who gave their informed consent. The family selection criterion was the proband (Case III.13), who presented herself with bilateral upper premolars hypodontia for orthodontic treatment.

Results

Case III.13

Dental and maxillary assessment. A female patient, white, 25-year-old, presented herself at the Orthodontics Clinic at the Ambulatory of the „St. Spiridon" University Emergency Hospital, Iasi, Romania, for the absence of certain maxillary teeth. Her dental history included loss of maxillary deciduous molars without being replaced with premolars and therapeutic extractions of permanent posterior mandibular teeth due to complications subsequent to decay. The maxillary arch presented a mixed dentition, the absence of the premolar group (14, 15, 24 and 25), the persistence of temporary tooth 53, the transposition of 13-53 and closure of remnant free areas (fig. 13a). On the mandibular arch, a permanent dentition interrupted by the post-extraction spaces of 36, 45 and 46 was observed. Examining the dental occlusion, mesial canine sagittal relations and distal molar relations, crossbites (13 and 23) and right mandibular lateral deviation (fig. 13b) were found. When the temporomandibular joint was examined a jaw cracking was heard on its left side. The panoramic radiography examination confirmed the congenital absence of all upper premolars dental buds and the agenesis of third molars 28, 38 and 48 and the root remains of 36 and 46 (fig. 13c). Linear and angular measurements made on the lateral cephalogram (Steiner and Tweed computer analysis) detected the following abnormal values: S–L=53 mm, S–E=24 mm, ANB=5^0, SND=73^0, SN–OcP=23^0, SN–GnGo=34^0, 1U–NA=19^0, 1L–NB=27^0, Holdaway ratio=3 mm and Z to Merrified=91^0 (fig. 13d).

Medical general assessment. The proband was evaluated at the Office of Medical Genetics, Iasi, and it was observed that she presented normal morphometry (weight, height, and cranial perimeter), declarative precocious puberty, discrete exophthalmia, hypodontia, hypoplasic right thumb inserted proximally (fig. 13e), left preauricular operated hemangioma and kyphoscoliotic deformities. A series of additional tests were conducted. The thyroid ultrasound revealed a discrete hypoechogenic thyroid, with a normal thyroid volume. The pelvic ultrasound revealed four follicles with the diameter of 2/6.3 mm on the left ovary at three follicles with a 3/7.1 mm diameter on the right ovary, the anteversion and right lateral deviation of the uterus and a polycystic ovary was put under observation. The phosphocalcic metabolism was normal, and the right wrist radiography showed that the right thumb is inserted proximally (fig. 13f).

a) continuity of the maxillary arch

b) anterior abnormal occlusal relations

c) maxillary premolars agenesis

d) angular and linear measurements

e) discrete brachydactyly and right thumb hypoplasia

f) right thumb anomaly
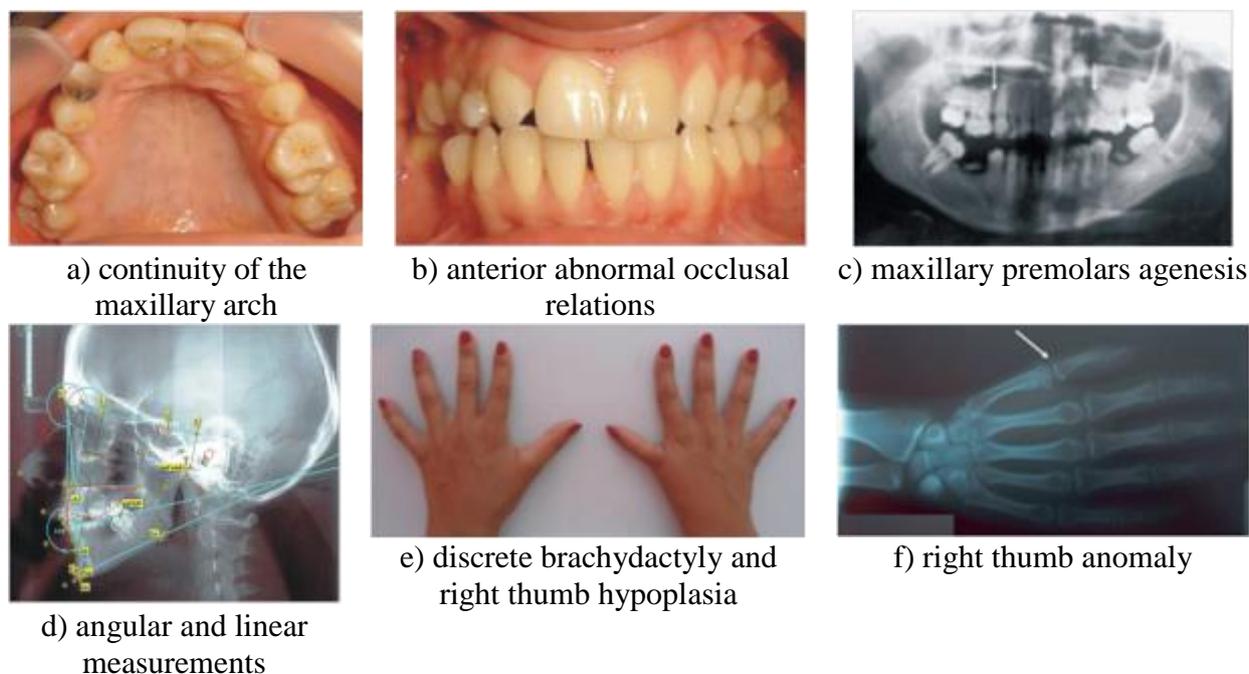
Fig. 13. Dento-maxillary and general features of probant

Case III.12

Dental and maxillary assessment. The 27-year-old proband's sister reported the same lack of maxillary premolars eruption after the exfoliation of deciduous molars. The maxillary arch

presented a mixed dentition, the absence of the premolar group (14, 15, 24 and 25), the persistence of temporary teeth 63 and the drifts of posterior teeth on the remaining free spaces (fig. 14a). The mandibular arch presented permanent dentition with frontal and lateral mild crowding. Examining the dental occlusion there were found mesial canine sagittal relations and distal molar relations, crossbites (13, 16, 23 and 26) and left mandibular lateral deviation (fig. 14b). Examining the temporomandibular joint, a jaw cracking on its right side was heard. The panoramic radiography examination confirmed the congenital absence of all dental buds of upper premolars, the agenesis of the third molars 18 and 28 and the root remains of 36 (fig. 14c). Linear and angular measurements made on the lateral cephalogram detected the following abnormal values: S–L=60 mm, S–E=27 mm, SNA=750, ANB=10, SN–OcP=210, SN–GnGo=350, 1U–NA=200, 1L–NB=140, Holdaway ratio=6 mm and Z to Merrified=620 (fig. 14d).

Medical general assessment. The proband's sister clinical genetic examination revealed a normal morphometry, discreet exophthalmia and kyphoscoliotic attitude.

| a) discontinuity of the maxillary arch | b) anterior abnormal occlusal relations | c) maxillary premolars agenesis |
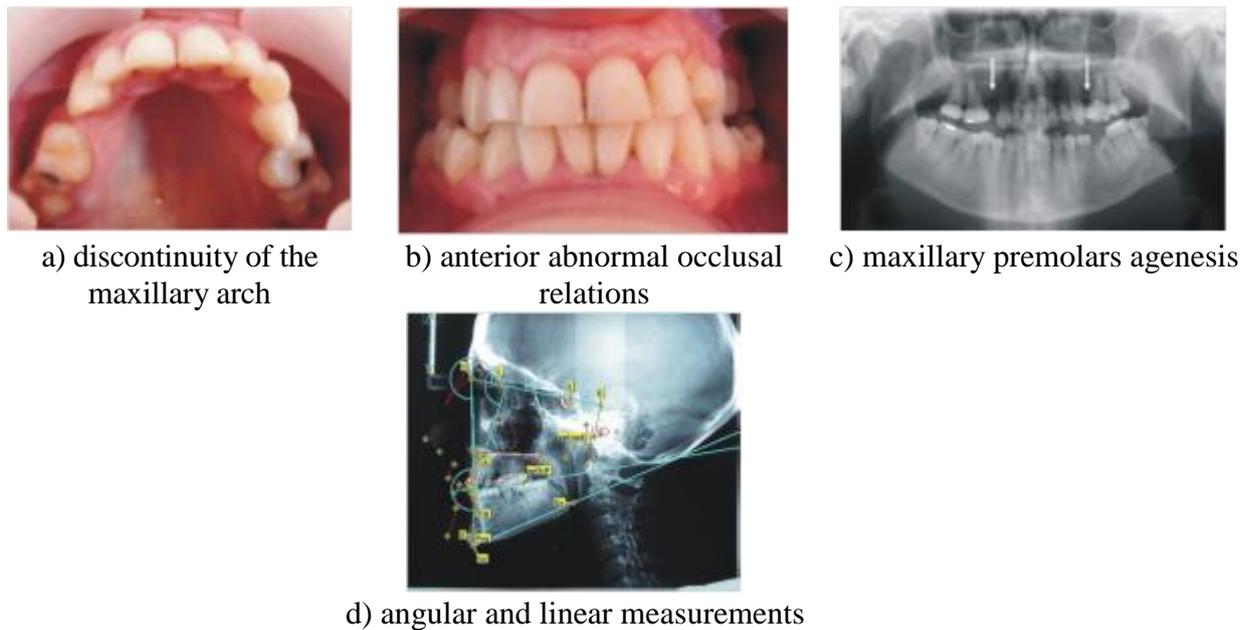
d) angular and linear measurements
Fig. 14. Dento-maxillary and general features of proband's sister
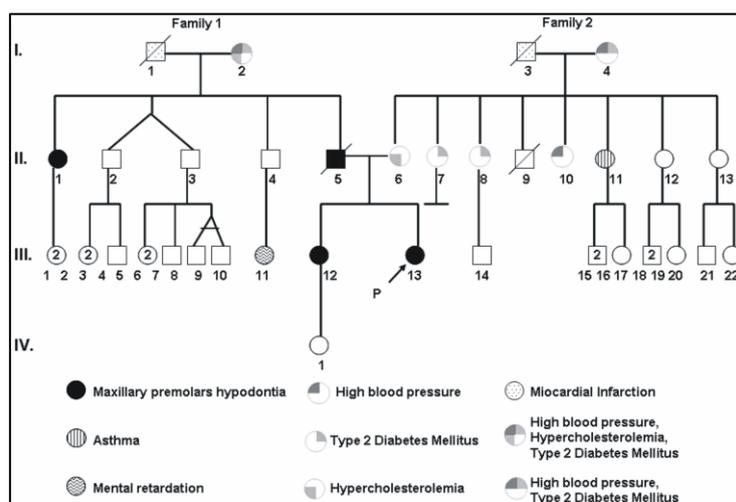
Fig. 15. Pedigrees of family with autosomal dominant inheritance of first and secondary maxillary premolars hypodontia

Family history. Following the completion of the family's pedigree investigation, the autosomal dominant inheritance was revealed, with high penetrance of bilateral hypodontia of first and second superior premolars at II.1, III.12, III.13 and probably II.5. We did not have reliable

clinical data or medical records about the II.5 person's hypodontia, because the person died at the age of 43 years from an accident. Declarative data was obtained from the proband (III.13) and her sister (III.12). There was no hand abnormalities found to other members of the family. It was also noted the family's concentration of multifactor diseases with genetic predisposition (fig. 15).

Discussions

The normal human dentition morphology of permanent teeth consists of four groups (incisive, canine, premolar and molar) symmetrically arranged in four quadrants, bilateral and bimaxillary. Within a quadrant, the incisive group consists in one central incisor and one lateral incisor, the canine group consists in one canine, the premolar group consists of first and second premolars, and the molar group consists in a first, a second and a third molar. Usually, in the non-syndromic hypodontia of permanent teeth, one tooth is congenitally missing from a dental group, unilateral or bilateral, maxillary or bimaxillary (third molars, second premolars, upper lateral incisors or lower central incisors) (Arte, 2001). The first premolars, permanent first molars and the canines are rarely congenitally absent and only in association with severe hypodontia or oligodontia (Hobkirk & Brook, 1980).

In this family, both of the presented cases were diagnosed with bilateral hypodontia of first and second maxillary premolars based on interview, clinical examination and panoramic radiograph analysis. None of the clinical forms could classify the hypodontia, because the two teeth were congenitally missing from a dental group, namely the premolar maxillary group. The hypodontia's severity was classified as mild to moderate (Dhanrajani, 2002), because of the congenitally absence of four teeth, except the third molars.

The natural drifts of maxillary posterior teeth into the remaining hypodontia spaces produced the shortening of the maxillary arch and a series of changes in teeth positions, occlusal and joint relations. The persistence of temporary teeth on the maxillary arch and the dental transposition were signs associated with the hypodontia. The canine and molar abnormal sagittal occlusal relations were considered false, inconclusive for classifying the diagnostic of malocclusion, because the maxillary teeth did not maintain their positions on the dental arches and could not be considered references in relation to the mandibular teeth. Crossbites formed due to changes of the teeth axis. Crossbites produced the mandibular lateral deviation and caused temporomandibular joint dysfunction.

By analysing the two sisters' side lateral cephalograms, an increased length of the skull base (linear dimensions S–E and S–L) was observed, which was not reported previously in the study of hypodontia. Consecutively to the teeth migration and the number of maxillary teeth reduction, the upper incisors retroclined in both cases (1U–NA angle). The latter is more relevant than the former, because the mandibular arch was integer and presented maxillary retrognathia (SNA angle). In a study conducted on 189 side lateral cephalograms of Caucasian patients with hypodontia and oligodontia, aged up to 16 years, Créton *et al.* (2010) reported retrusive maxillary position in 33 cases, retroclined upper and lower incisors in 15 cases and proclination of the lower incisors with retrusive mandible in 61 cases.

In both of the presented cases, the sagittal discrepancy between the skeletal bases (ANB angle) amended the maxillary relations with facial soft tissues (Z to Merrified angle) in an individualized manner, depending on the mandibular dental clinical situation. On the first case, the absence of posterior mandibular teeth due to extraction was beneficial to the compensation of the maxillary dental discrepancy and a Class II skeletal pattern was observed, while the proclination of the lower incisors (1L–NB angle) has formed as a dental compensation due to the mandibular dental arch shortening. Consecutively to this skeletal pattern, the soft tissue profile changed, the upper lip being tangent to the Z to Merrifield line. The second case presented a Class III skeletal pattern, while the lower incisors retroclined as a dental compensation to the maxillary shortened bone. Following this skeletal pattern, the soft tissue profile has modified, the lower lip being tangent to the Z to Merrifield line.

Previous studies conducted on side lateral cephalograms whose purpose was to detect skeletal effects associated to hypodontia, reported the predominance of Class I skeletal pattern, but also of Class II or III, these tendencies varying depending on the severity of hypodontia (Wisth *et al.*, 1974; Yüksel & Uçem, 1997). According to the ANB angle, the first case was classified as a Class II skeletal pattern, and the second case as a Class III skeletal pattern. These skeletal patterns present sagittal severe discrepancies with changes in the facial aesthetics profile.

Previous studies have concluded that the non-syndromic form of permanent teeth hypodontia have an autosomal dominant inheritance (Goldenberg *et al.*, 2000; Vastardis, 2000; Arte, 2001; Das *et al.*, 2002; 2003). Some authors have reported autosomal recessive (Ahmad *et al.*, 1998; Pirinen *et al.*, 2001; Arte, 2001), X-linked (Erpenstein & Pfeiffer, 1967; Burzynski & Escobar, 1983) and polygenic transmission patterns (Chosack *et al.*, 1975).

The family's pedigrees analysis revealed that maxillary premolars hypodontia of the studied family had an autosomal dominant inheritance with complete penetrance and variable expressivity. The upper limbs abnormalities were not observed in another family members and the association between hypodontia and thumb hypoplasia was considered random. The risk of hypodontia recurrence in this family depends on the consulting couples. Thus, for a couple composed of a sick and a healthy person, the risk of recurrence is 50%.

Table 20. Differential diagnosis of the proband III.13

Syndromes	Present abnormalities	Missing abnormalities	Inheritance
McCune-Albright syndrome	scoliosis possible endocrinopathies precocious puberty	fibrous dysplasia of bone café-au-lait skin spots	somatic mosaicism
Book syndrome	premolar aplasia	hyperhidrosis premature graying of hair	autosomal dominant
Cleidocranial dysplasia	delayed eruption of teeth scoliosis	delayed fontanels closure hypertelorism hypoplastic clavicles	autosomal dominant
Lachrymo-auriculo-dento-digital	hypodontia digitalized thumb	obstruction of nasal lachrymal ducts sensorineural hearing loss dry mouth	autosomal dominant

In the presented cases, the association of hypodontia with other congenital anomalies led to the hypothesis of a syndromic hypodontia. Thus, there were suspected the syndromes that presented the association with autosomal dominant inheritance and hypodontia. In Table 20, there are displayed the McCune-Albright syndrome, the Book syndrome, cleidocranial dysplasia and the lachrymo-auriculo-dento-digital syndrome (Salinas *et al.*, 1992; De Sanctis *et al.*, 1999; Cooper *et al.*, 2001; Rohmann *et al.*, 2006), which have been identified to support the diagnosis of the proband. The syndromes with autosomal recessive transmission, the X-linked and those associated with mental retardation and other birth defects that were not present in the proband's family were excluded.

The proband's general abnormalities were not identified with any syndrome that was taken into consideration, which disproves the hypothesis of a syndromic hypodontia. Nevertheless, there were not performed molecular genetic evaluations. Both of the presented cases were diagnosed with non-syndromic hypodontia of maxillary premolar group, one of them associated with proximally inserted hypoplasic thumb, a previously unreported clinical manifestation. This phenotype is atypical to permanent teeth isolated hypodontia and difficult to classify into a pre-existing diagnostic classification.

Preferably, patients and direct family members should be counselled regarding such implications when a diagnosis of rare hypodontia is given, to allow the family to prepare for these matters and reduce their potential impact.

Conclusions

We have described this as a rare phenotype of bilateral maxillary first and second premolars hypodontia, which we classified as mild-to-moderate, non-syndromic hypodontia which has an autosomal dominant inheritance and an increased risk of recurrence in the studied family. The hypodontia was associated with an abnormality of the thumb in one of the presented case. The absence of four maxillary posterior teeth caused severe harm to the dental status, with serious consequences on the craniofacial morphology. The diagnosis and the late, multidisciplinary and complex treatment of this hypodontia phenotype, represent challenges for practitioners at the expense of the economic benefits of the patient and the public health system. The early detection of these cases would be appropriate, in order to establish a therapeutic interceptive management to prevent further disorders in the skeletal growth, and maintaining a facial balance.

3.2. Clinical and radiological aspects of impacted teeth and risk factors and predictors of treatment

Tooth impaction represents the intra-osseous or intra-mucosal retention of a permanent tooth with a fully developed root, after the normal eruption period, without any potential of eruption (Daskalogiannakis, 2000; Torres-Lagares *et al.*, 2006). Most of the studies devoted to such topics reported an incidence of tooth impaction between 6.9-76.6%, depending on the tooth involved and on the studied population (Fardi *et al.*, 2011; Topkara & Sari, 2012; Kamiloglu & Kelahmet, 2014: Hijawi *et al.*, 2016). As to prevalence, the third molars, cuspids, second premolars, central incisors and the supernumerary teeth were most commonly impacted (Aydin *et al.*, 2011; Husain *et al.*, 2012). Impaction is rarely encountered in deciduous teeth (Mutluay, 2017).

During the formation of the dental buds in the cup stage, the middle layer of the stellate cells of the adamantine organ can have an excess fluid secretion, and the tooth will not erupt and will remain in a cystic form causing dental impaction with follicular cyst (Boboc, 1995). Previous studies have shown that the most frequent causes of teeth impaction were related to the lack of space in the dental arch, chenge of tooth eruption sequence, supernumerary teeth, odontomas, persistent deciduous teeth and trauma in the area of the alveolar process. General factors include heredity, vitamin deficiency, endocrine disorders, febrile diseases and bone degeneration (Becker *et al.*, 1991; Warford *et al.*, 2003; Kawala *et al.*, 2011; Manne *et al.*, 2012).

Radiological exams offer necessary information about the number, position, localization and axis of impacted teeth, for accurate diagnosis and therapeutic procedures. Radiographic analysis represents the main tool of the oral surgeon for determining the difficulty of the pathway to the impacted tooth (Ericson & Kurol, 1987; Mupparapu, 2002; Manne *et al.*, 2012) for surgical exposure or extraction of the impacted tooth (Nezar *et al.*, 2015). Numerous cases reported in orthodontic literature have described various conservative individualized therapeutic (usually, surgical exposure of the impacted tooth followed by orthodontic traction for dental arch guiding and alignment) or radical (usually, extraction of the impacted tooth followed by implant restoration) procedures (Gebert *et al.*, 2014; Gujar & Rani, 2016; Singh *et al.*, 2016; Falcón, 2017).

This study aimed to investigate the clinical and radiological aspects of the permanent teeth impaction, third molars excepted, on a sample of young patients from the North-Eastern region of Romania. The study focused on determining the prevalence of clinical forms and treatment types, as well as establishing the risk factors and predictors for treatment options of the impacted tooth.

Patients and methods

This cross-sectional analytic observational study was conducted on 67 orthodontic patients with impacted teeth, 29 (43.30%) boys and 38 (56.70%) girls, aged 6-37 years, from the North-Eastern region of Romania. Patients with syndromes, cleft palate, impacted third molar and

impacted supernumerary teeth were excluded. The diagnosis of impacted tooth was established both clinically (permanent tooth absence and/or persistence of deciduous tooth on the dental arch) and radiographically (panoramic, periapical and anterior occlusal radiographies). All patients were treated in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, during 1991-2010. The informed consent was obtained from all patients or from their parents.

Studied parameters. The data collected from orthodontic records and radiographies was uniformly evaluated according to socio-demographic parameters (gender, age and environment area), types of impacted teeth, Angle Classes of malocclusions, patterns of impacted tooth, number and distribution of impacted teeth in quadrants, etiological causes (reduced space, existence of possible obstructions and anatomic peculiarities), radiological features (according to intra-osseous position, severity, axis direction and localization of the impacted tooth inside the alveolar bone arch) and treatment options (conservative or radical).

The classification of impacted teeth was established radiographically according to: (1) intra-osseous position (total/ complete/ high impaction, when the tooth was entirely intra-osseous; partial/ incomplete/ low impaction, when the crown of the impacted tooth was not fully covered by the bone, yet separated from the oral cavity by the pericoronal sac and gingival fibromucosa); (2) severity or distance of the impacted tooth from the occlusal plane (mild impaction, when the distance was lower than 12 mm; moderate impaction, when the distance was between 12-15 mm; severe impaction, when the distance exceeded 15 mm); (3) direction of impacted tooth axis (vertical impaction; horizontal impaction, with the crown positioned between the roots of the erupted teeth and the distally oriented root; mesially oblique impaction, with the mesially oblique crown inclined; distally oblique impaction, with the distal oblique crown inclined); (4) localization of the impacted tooth inside the alveolar arch (buccal impaction, with the buccally situated crown and the palatally oriented root; palatal/ lingual impaction, with the orally situated crown and the buccally oriented root; medium impaction, with the crown situated in the middle of the alveolar arch).

Statistical analysis was performed using the SPSS 20.0 package (Chicago, Illinois, SUA) for Windows. The data was characterized through descriptive statistics and contingency tables, applying the Chi-square (χ^2) Pearson test, to compare the types of impacted teeth and to identify the significant differences ($p<0.05$). The risk factors for treatment options were calculated with the contingency tables, according to the Odds Ratio (OR) values: protective factors (OR<1), low risk factors (OR=1-1.5), moderate risk factors (OR=1.5-2), severe risk factors (OR=2-5) and very severe risk factors (OR>5); the Forward LR binary logistic regression model was used to classify these risk factors and to analyse their correlated predictive potential. The reliability of the binary logistic regression model was evaluated using the Hosmer-Lemeshow goodness-of-fit test (HL test), at a 5% level of significance and $p<0.05$.

Results

Sample characteristics. The study includes 67 orthodontic patients with mean age of 13.97±5.346 years (males with mean age 12.79±5.703 years and females with mean age 14.87±4.944 years), 41 (61.20%) patients from the urban area (mean age 14.37±6.115 years) and 26 (38.80%) patients from the rural area (mean age 13.35±3.8 years), respectively.

The prevalence of the clinical forms and treatment types

The types of impacted teeth studied were: cuspids in 36 (53.70%) patients, incisors in 25 (37.30%) patients and bicuspids in 6 (9.00%) patients, from the Angle Class I malocclusions in 51 (76.10%) patients, Class II in 10 (14.90%) patients and Class III in 6 (9.00%) patients. Statistically significant differences were found between environment area and impacted teeth types on genders ($p<0.05$).

The patterns of impacted teeth in the studied sample are as follows: 11 impaction in 15 (22.40%) patients, 13 impaction in 14 (20.90%) patients, 23 impaction in 11 (16.40%) patients, 13-23 impaction in 7 (10.40%) patients, 21 impaction in 7 (10.40%) patients, 33 impaction in 3 (4.50%) patients and 11-21, 14-24, 15, 15-25, 21-22, 22, 24, 25, 43, 45 impactions, respectively, in

1 (1.50%) patient (fig. 16). Depending on the number of impacted teeth, 56 (83.60%) patients had only 1 impacted tooth and 11 (16.40%) patients had 2 impacted teeth. 40 (59.7%) patients had impacted teeth in the 1st quadrant, 32 (47.80%) patients in the 2nd quadrant, 3 (4.50%) patients in the 3rd quadrant and 2 (3.00%) patients in the 4th quadrant, respectively.

The causes of impacted teeth are: decreased space on the dental arches in 33 (49.30%) patients (narrow alveolar arch in 46.30% of patients, mesial drifts of the posterior teeth in 1.50% of patients, or macrodontia in 1.50% of patients); the obstacles encountered by the erupting tooth in 27 (40.30%) patients (previa formations in 28.30% of patients or persistence of deciduous teeth after the normal exfoliation period in 11.90% of patients); or anatomic particularity of the impacted tooth in 7 (10.40%) patients (deviation of eruption tooth's axis in 9.00% of patients or angulation of the crown-root in 1.50% of patients) (fig. 17).
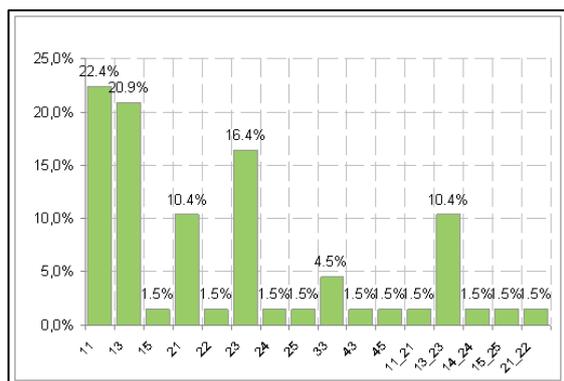
Fig. 16. Patterns of impacted teeth
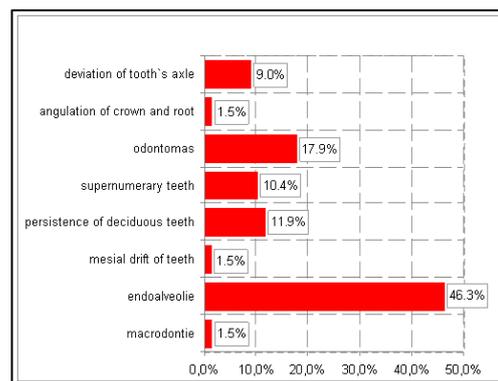
Fig. 17. Causes of impacted teeth

According to the intra-osseous position of the impacted tooth, total impaction in 51 (76.10%) patients and partial impaction in 16 (23.90%) patients were found. According to the distance of impacted tooth to the occlusal plane, mild impaction in 6 (9.00%) patients, moderate impaction in 10 (14.90%) patients and severe impaction in 51 (76.10%) patients were found. According to the direction of impacted tooth axis, vertical impaction (favorable position for conservative treatment) in 28 (41.80%) patients, horizontal impaction (favorable position for extraction of the impacted tooth) in 15 (22.40%) patients, mesial oblique impaction in 23 (34.30%) patients and distal oblique impaction in 1 (1.50%) patient were found. According to the localization of the impacted tooth inside the alveolar arch, buccal impaction in 25 (37.30%) patients, palatal impaction in 25 (37.30%) patients and medium impaction in 17 (25.40%) patients were found. The conservative surgical-orthodontic treatment (surgical exposure and tooth traction in favorable position on the dental arch) was applied to 54 (80.60%) patients, and radical surgical treatment (extraction of impacted tooth in unfavorable position) to 13 patients 19.40%) patients. Statistically significant correlations were found between the types of impacted teeth and gender (*p*=0.018), age intervals (*p*=0.024), direction of impacted tooth axis (*p*=0.002) and impacted tooth localization inside the alveolar arch (*p*=0.007).

The risk factors and predictors for treatment options

The risk factors calculated for the extraction of impacted tooth, according to the OR values, gave the following results: low risks in male patients (OR=1.155) and from urban areas (OR=1.018); moderate risks in patients with Angle Class I malocclusion (OR=1.925) and buccally or lingually impacted (OR=1.579); severe risks in patients under the age of 18 years (OR=2.400), with Angle Class III malocclusion (OR=2.273), impacted cuspids (OR=2.250), total impaction (OR=4.615), from the 4th quadrant (OR=4.417), anatomical causes of impacted tooth (OR=3.750). The values OR<1.00 correspond to protective factors: impacted incisors (OR=1.925) or bicuspid (OR=0.817); impaction's localization in the 1st (OR=0.504) or 2nd (OR=0.259) quadrant; impaction caused by reduced space (OR=0.580) or obstacles (OR=0.909); medium impaction (OR=0.198) (tab. 21).

In order to analyse their correlated action and to establish their predictive potential, the previously identified risk factors were included in a model of binary logistic regression. The

generated model was reliable (HL test, p=0.000), having a precision of 98.50%, identifying two predictive statistically significant factors for the treatment through extraction of the impacted teeth: Angle Class II division 1 malocclusion and horizontal direction of the impacted tooth axis (tab. 22).

Table 21. Analysis of risk factors for extraction of the impacted tooth

Parameters	χ^2	p value	OR	95% Confidence interval for OR	
				Lower Bound	Upper bound
Gender	0.054	0.816	1.155	0.342	3.900
Age intervals	0.665	0.415	2.400	0.276	20.850
Environment	0.001	0.977	1.018	0.293	3.533
Types of impacted teeth					
impacted cuspids	1.559	0.212	2.250	0.618	8.198
impacted incisors	1.398	0.237	0.436	0.108	1.769
impacted bicuspids	0.032	0.859	0.817	0.087	7.655
Angle Class malocclusions					
Class I	0.640	0.424	1.925	0.379	9.775
Class II division 1	0.496	0.481	0	0	0
Class II division 2	2.187	0.139	0	0	0
Class III	0.818	0.366	2.273	0.369	14.007
Number of impacted teeth	3.168	0.075	0	0	0
Localization of impacted teeth					
1st quadrant	1.230	0.267	0.504	0.149	1.712
2nd quadrant	3.939	0.047	0.259	0.064	1.045
3rd quadrant	13.046	0.000	0	0	0
4th quadrant	1.234	0.267	4.417	0.258	75.727
Causes of impacted teeth					
reduced space	0.752	0.386	0.580	0.168	2.002
obstacles	0.023	0.880	0.909	0.262	3.148
anatomic particularity	2.750	0.097	3.750	0.725	19.404
Intra-osseous position of teeth	2.325	0.127	4.615	0.551	38.649
Severity of impaction	2.325	0.127	4.615	0.551	38.649
Direction of tooth axis					
vertical impaction	11.580	0.001	0	0	0
horizontal impaction	55.916	0.000	0	0	0
mesially oblique impaction	8.431	0.004	0	0	0
Distally oblique impaction	0.244	0.621	0	0	0
Location inside the alveolar arch					
buccal impaction	0.539	0.463	1.579	0.464	5.376
palatal impaction	0.539	0.463	1.579	0.464	5.376
medium impaction	2.663	0.103	0.198	0.024	1.652

Table 22. Binary logistic regression analysis of predictor factors for extraction of impacted tooth

Parameters	B	S.E.	Wald Statistics	p value
Angle Class malocclusions				
Class II division 1	22.609	22521.417	0.000	0.999
Direction of impacted tooth axis				
horizontal impaction	-23.730	5523.732	0.000	0.997

Discussions

Permanent teeth impaction is a commonly found anomaly in any type of tooth in the general population. Most authors studied the clinical and radiological features of some of impacted teeth, most commonly the cuspid and the upper incisor, focusing on the prevalence, distribution, patterns, causes and therapeutic procedures. Thus, an incidence between 0.80-3.60% and 5.00% of the maxillary impacted cuspids (Mason *et al.*, 2003; Aydin *et al.*, 2004; Walker *et al.*, 2005; Szarmach *et al.*, 2006; Kamiloglu & Kelahmet, 2014; Dalessandri *et al.*, 2017), and an incidence of the maxillary impacted central incisors between 0.006-2.00% and 2.60% (MacPhee, 1935; DiBiase, 1969; Grover & Lorton, 1985) was reported.

In the orthodontic literature, few studies of dental impaction are available, conducted on different populations and ethnicities, which highlight the specific features of the population, are available (Ezoddini *et al.*, 2007). Topkara and Sari (2012), who investigated clinically and radiographically prevalence and distribution of permanent teeth impaction in a Turkish population, reported a global prevalence of 9.10% without statistical differences between gender, and a prevalence of maxillary cuspids of 5.24%, mandibular second bicuspids of 2.23%, maxillary second bicuspids of 1.11%, mandibular cuspids of 0.92%, molars of 0.72% and incisors impactions of 0.65%, respectively. The decreased maxillary arch length has been reported in 14.00% of the patients and the mandibular length in 10.00% of them. Kamiloglu and Kelahme (2014) studied the incidence of cuspids impaction on panoramic radiographs in a Cypriot population and detected no statistical differences between genders, location and malocclusion. However, the incidence of the impacted maxillary cuspids was significantly more frequent than that of the impacted mandibular cuspids. Gashi *et al.* (2014), who investigated clinically and radiographically the incidence of impacted maxillary cuspids in the population of Kosovo, reported an incidence of 1.62%, with statistical differences between genders, and a more frequent unilateral and palatal statistical location. Nezar *et al.* (2015)*,* studying patients in the Arabian community of Israel, reported a prevalence of 3.70% impacted maxillary cuspid, significantly higher in women than in men, with more frequent unilateral, maxillary and left side localization.

The present study analyses the clinical, radiological and statistical aspects of permanent teeth impaction specific to a sample of young patients from the North-Eastern region of Romania, in order to establish the prevalence of the clinical forms and treatment options, as well the risk factors and predictors for the treatment options of the impacted teeth. Thus, the impacted cuspid was the most frequent, followed by impacted incisors and impacted bicuspid, the order of prevalence between incisors and cuspid impactions being reversed, compared to previous studies (Aitasalo *et al.*, 1972; Moyers, 1991), due to the structure of the sample. Cuspid impaction was more frequent in girls than in boys, in a 2.6:1 ratio, as showed by Ericson and Kurol (1988), and Richardson and Russell (2000). In the studied sample, the impacted central incisor was more frequent in boys than in girls, in a 2.1:1 ratio, while the impacted bicuspid had an equal percentage in the two genders (a 1:1 ratio). Most impactions were detected before 18 years, while those identified after the age of 18 did not exceed one third of the patients under study. The bicuspid and cuspid impactions were most frequent in patients from the urban area, and incisor impaction in patients from rural area. Statistically significant correlations were found between the types of impacted teeth in patients with social variables (sex and age), which is a particular feature of the studied sample. Most of the detected teeth impactions were classified in Angle Class I malocclusion.

In the sample study, single permanent tooth impaction had a higher percentage than two teeth impaction (5.1:1 ratio), the maxillary arch being more affected than the mandible (9.75:1 ratio). Single tooth impaction was studied and two teeth impaction from the view point of the etiological factors involved (usually obstacles in the path of eruption), comparatively with previous studies devoted to unilateral and bilateral impaction (usually reduced space in the arch) (Gashi *et al.*, 2014; Nezar *et al.*, 2015). The most common cause for cuspid and bicuspid impaction was the reduced space in the dental arch while, for impacted incisors, the main cause was the presence of obstacles; the anatomical particularities of the tooth registered a low percentage. Most patients had total impaction, the ratio to partial impaction being 3.18:1. For all types of identified teeth, severe

impaction was more frequent, whereas moderate impaction of bicuspids and incisors registered a low percentage.

Statistically significant correlations were found between the types of impacted teeth and the clinical features (direction and localization of the impacted tooth), which shows that each type of impacted tooth has different characteristics, as a function of these parameters. Thus, most frequently, impacted cuspids had a mesially oblique direction of tooth axis, with buccal or palatal arch localization, while the impacted incisors had most frequently a vertical direction of tooth axis, with buccal or palatal arch localization. In most of the cases, impacted bicuspids had a vertical direction of the tooth axis, with medium location in the alveolar arch.

Depending on localization, the permanent teeth impaction produces serious localized, functional and aesthetic complications, the treatment being interdisciplinary, surgical and orthodontic (Shapira & Kuftinec, 1981; Rizzatto *et al.*, 2004; Pavlidis *et al.*, 2011; Msagati *et al.*, 2013). The Index of Orthodontic Treatment Need (IOTN) using a five-point scale indicates a highest need for the treatment of tooth impaction treatment (Waring *et al.*, 2011; Ferguson, 2006). In present study, conservative treatment was the therapeutic option in most patients, through surgical exposure and orthodontic traction of the tooth included in the dental arch. The ratio between the conservative and radical treatment (extraction of impacted tooth) was 4.14:1. Both treatment options present some surgical risks. Thus, (low, moderate and severe) risk factors were identified in cases of impacted tooth extraction, as well as protective factors, depending on the peculiarities of the studied sample. Angle Class II malocclusion division 1 and the horizontal direction of the tooth axis were predictors recommending impacted teeth extraction. Such factors have not been investigated in previous studies.

The presented research brings a benefit to the population study of impacted tooth, because it assesses the peculiarities of several types of impacted teeth in a sample of young patients of Romania, while proposing new aspects for further studies that may be extended to other populations, for obtaining comparable data.

Conclusions

The sample of patients of the studied population evidenced a significantly increased prevalence of impacted cuspid in girls and of impacted central incisor in boys, detected before the age of 18 years. Maxillary single tooth impaction and severe clinical forms prevailed. The direction and localization of the tooth have been statistically correlated with the types of impacted teeth. A 4.14:1 ratio was found between the conservative and the radical treatment, and the risk factors and predictors for impacted teeth extraction were identified. The present study outlines the importance of an early detection of this common dental anomaly, in order to prevent dental migrations and occlusal disorders, as well as radical surgical treatments.

CHAPTER 4
Clinical and cephalometric researches in the diagnostic and treatment of the dento-maxillary anomalies

Dental crowding is characterized by the negative difference between the required space and the existing space of the teeth on the dental arches and may be a symptom of skeletal dento-maxillary anomalies. Clinical forms of dental crowding may be: mild, moderate or severe, depending on their severity; anterior, intermediary, lateral or posterior, depending on their location; primary, secondary, tertiary, combined or transient, depending on their etiology. Dental crowding may have general (crossed heredity or disendocrinopathies), functional or local causes (Howe, *et al*., 1983; Moyers, 1988; Proffit *et al*., 2007; Ting *et al*., 2011; Hafez *et al*., 2012). The frequency of this anomaly is increased in populations, varying between 5-80% (Radnzic, 1988; Mugonzibwa *et al*., 2008; Rose & Roblee, 2009; Normando *et al*., 2013), being the most frequent cause for which patients address to the specialist. Orthodontic therapy makes use of a large range of appliances for solving problems like arcade space, dental alignment, occlusion and facial aesthetics. The therapeutic ways used vary depending on the causes and severity of the dental crowding and are a necessity for the patients' dental aesthetics (Yoshihara *et al*., 1999; Souames *et al*., 2006; Keim *et al*., 2008).

Introduction of the cephalogram, in 1934, by Hofrath in Germany and by Broadbent, respectively, in the USA, permitted the study of malocclusions by evidencing skeletal discrepancies. Several authors made known the cephalometric analyses that they had performed for the diagnosis of skeletal malocclusions, including various angular, linear measurements and ratios. The Angle Class II of malocclusions is characterized by a skeletal discrepancy between the maxillary bases and the skull basis, produced through maxillary protrusion and/or mandibular retrusion. The molar and canine sagittal relation is distalized, and has two clinical entities, according to the classification of Angle: division 1, with proclination of the upper incisors and increased overjet; division 2, with retroclination of the upper incisors and minimum overjet. (Angle, 1899). The Angle Class III of malocclusions is characterized by a mesial relation of the mandible toward the maxillary, the cause being skeletal and its origin is usually hereditary (Jacobson *et al*., 1974). This anomaly has a decreased prevalence in the white population (1-2%) and an increased frequency in Asian countries, like Japan (4-13%) (Ishii *et al*., 1987). Bourdet was the first researcher who described the 1737 skeletal Class III pattern in children with mandibular prognathia. In such cases, lateral cephalogram is essential in establishing the skeletal, dental and facial diagnosis in order to appropriately apply orthodontic or surgical treatment. Sagittal discrepancies are mainly due to excessive growth of the mandible with hyperdivergence growth pattern, resulting mandibular prognathia.

Crossbite is the only malocclusion that requires emergency treatment as soon as possible, prior to the occurrence of discrepancies in the maxillary bases. The maxillary teeth positions are lingual to the mandibular teeth in centric occlusion. It can vary in clinical forms from one single tooth to all, and can be anterior or/and posterior and unilateral or bilateral (Moyers, 1988; Proffit *et al*., 2007). Abnormal labiolingual relationship between upper and lower incisors is called anterior crossbite and the one between maxillary and mandibular canines, bicuspids or molars is defined as posterior crossbite. The prevalence found in population varies between 2.2-23.0%, depending on the age, the racial group and types of crossbite studied (Kennedy & Osepchook, 2005; DaSilva *et al*., 2007; Borrie & Beam, 2011). Crossbite can be a feature of some genetic and endocrine syndromes, but, usually it appears isolated (non-syndromic). Crossbite etiopathogenesis is still unknown. Several forms of crossbite were associated with muscle and mandibular functional changes (Andrade *et al*., 2009; Piancino *et al*., 2012), with delayed teeth development (Uysal *et al*., 2009), with temporomandibular joint disorders (Tecco & Festa, 2010; Thilander & Bjerklin, 2012), with cranio-mandibular asymmetry (Anistoroaei, 2009; Ferro *et al*., 2011; Talapaneni & Nuvvula, 2012), or with leg length inequality (Michelotti *et al*., 2007).

Clefts lip and palate, also referred as orofacial clefts, are coalescence defects of the facial processes occurring during embryogenesis, and are present at birth, localized in the lip, palate or both, in isolated forms or in chromosomal syndromes (Timosca & Burlibasa, 1985; Boboc, 1995). The causes of these congenital malformations are not elucidated, the causal factors being the combination of genetic factors with other factors (environment, diet, smoking, diabetes and drugs) (Honein *et al*., 2007; Correa, *et al*, 2008; Werler *et al*, 2011; Margulis *et al*, 2012; CDC, 2019). Treatment is complex, long-lasting, depending on the severity and association of genetic syndromes or other defects (ACPA, 2009). The treatment is necessary from the beginning due to the feeding difficulties of the infant, after which it is necessary to restore functionality and maxillo-facial aesthetics. In the first months of life, defect repair is performed surgically, and then children need additional surgery, but especially orthodontic therapy. The monstrous facial appearance from birth produces psychological traumas over their families, and children and teenagers have critical psychological problems (Pope & Ward, 1997). Specialists in some countries consider orofacial clefts to be a serious handicap, and after the advent of early foetal diagnosis methods (Costello *et al*., 2008), they allowed abortion. Individuals with orofacial clefts have multiple problems, such as early feeding difficulties, nutritional issues, developmental delays, abnormal speech and/or resonance, dento-facial anomalies, hearing loss and possibly psychosocial problems. A coordinated approach of the team from childhood to adulthood is recommended for optimal outcomes (CDC, 2019).

The perfect bilateral symmetry of the face and body is rare due to biological variation, being a theoretical concept. Right-left differences are due to functional and morphological asymmetries (Cheong & Lo, 2011; Thiesen *et al*., 2015). Slight, invisible, subclinical facial asymmetry is relatively common, being defined as the difference in size between the right and left sides of the face, observed only by the method of composite face photo (Bishara *et al*, 1994: Proffit *et al*., 2007). Severe facial asymmetry occurs in cranio-facial syndromes (Haraguchi *et al*., 2008). Asymmetry affects the lower face (lips, chin) more frequently than the middle (nasal pyramid, orbits) and upper face (Proffit *et al*., 2007; Haraguchi *et al*., 2008). According to transverse discrepancies, facial asymmetry is classified as dental, skeletal, functional and muscular (Thundukattil *et al*., 2018). Clinical features are polymorphic, depending on etiopathogenesis. Clinical and radiological diagnostic procedures aim to identify the causal factor, location and affected tissue. However, clinical examination remains the most important diagnostic tool for facial asymmetry (Patel *et al*., 2015). Aesthetic criteria require that certain landmarks of the face (the bisector of the pupil, Nasion, tip of the nose, tip of the philtrum and chin) should be placed in the same axis, forming facial midline (Peng & Cocke, 1999; Bidra *et al*., 2009). Also, for face symmetry the facial midline must be in accordance with the maxillary and mandibular dental midline (Eskelsen *et. al*., 2009; Farhan *et al*., 2014).

Scoliosis may be a pathogenic cause of facial asymmetry and it is a three-dimensional deformation of the spinal column, defined as a lateral deviation greater than 10° in frontal plane, associated with axial rotation, located at the cervical, thoracic and/or lumbar level (Trobisch *et al*., 2010; Miller, 2012). There are several types of scoliosis: congenital, neuromuscular and idiopathic. Idiopathic scoliosis is the most common type of scoliosis and has a multifactorial aetiology (genetic predisposition, metabolic, hormonal and biomechanical factors). It can appear at any age, it is more common in teenage girls, and in 90% of cases presenting a right-sided thoracic curve. The prevalence of adolescent idiopathic scoliosis is between 0.47% and 5.2% (Soucacos *et al*., 1997; Lee *et al*., 2007; Choudhry *et al*., 2016). The diagnosis of idiopathic scoliosis is made by history, physical and radiographic examination. Postero-anterior X-ray view of the spinal column determines the degree of severity of spinal curvature by the Cobb method (Kim *et al*., 2014). The interest in finding the relationship between idiopathic scoliosis and facial asymmetry is 50 year-old, being based on the hypothesis of the anatomical and functional relation between the dento-maxillo-facial complex and the spinal column (Saccucci *et al*., 2011).

The clinical experience and the orthodontic profile of the patient from this geographic area have led to the establishment of some databases for the evaluation and analysis of the most frequent

dento-maxillary anomalies in the studied population, identifying the prevalence, clinical and therapeutic aspects of some common anomalies, as well as some severe pathologies, difficult for the practitioner to establish the diagnosis and the therapeutic approach.

4.1. Clinical aspects of dental crowding and necessity factors and predictors of treatment

In the literature, there are numerous studies that address dental crowding from all aspects Bernard *et al.*, 1981; Minervini G, Posillico, 1990; Johal & Battagel, 1997; Janson *et al.*, 2011), on different populations, however the predictive factors of the dental crowding treatment are almost inexistent.

This study starts from the hypothesis that dental crowding is caused by certain specific causal factors and that their early removal may prevent serious subsequent complications. *The objective of the study* was to investigate the clinical and therapeutical aspects of the dental crowding, on a sample of young patients from the North-Eastern region of Romania. The study focused on evaluating the mean age of detection, establishing the prevalence of clinical forms and types of treatment, as well as establishing the necessity factors and predictors for treatment options of dental crowding.

> **Zegan G**, Dascalu GC, Mavru RB, Anistoroaei D. Necessity factors and predictors of dental crowding treatment. *International Journal of Medical Dentistry (Journal of Romanian Medical Dentistry),* Jul-Sep 2015; 5(3): 200-206.

Patients and methods

This cross-sectional analytical observational study was performed on 422 orthodontic patients, 165 (39.1%) boys and 257 (60.9%) girls, with ages between 6-24 years old, from the North-Eastern region of Romania, 311 (73.7%) patients in urban and 111 (26.3%) patients in rural areas. The criterion of patient selection was dental crowding associated with the Angle Classes of malocclusions. The subjects with genetic or endocrine syndromes and with clefts palatine were excluded. The orthodontic diagnosis was established by clinical and complementary exams (plaster cast, panoramic radiography and lateral cephalogram). All cases received orthodontic treatment in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, between 1991 and 2010. The informed consent was obtained from all patients or from their parents.

Studied parameters. The data was collected from orthodontic records and evaluated according to socio-demographic parameters (sex, age and environment area), dentition type (mixed or permanent), clinical forms, Angle Classes of malocclusions, other associated malocclusions (crossbite, overbite and mandibular lateral deviation), etiological causes, treatment types and applied orthodontic appliances.

Statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL), calculating the distributions of frequencies, the descriptive statistical parameters and the Spearman correlation coefficient between the categories of variables. The correlation between the clinical forms and the therapeutically solutions was used with cross-tabulation method, applying the Chi-square (χ^2) Pearson test. The significant predictors were identified with a model of binary logistic regression (B) and the validity of the model was determined with the Hosmer-Lemeshow goodness-of-fit test (HL test), at a significance level $p>0.05$. To eliminate the multicollinearity problems, the multiple correlations among factors were analysed. Statistical analysis was conducted at a significance level of 5% and $p<0.05$.

Results

Sample characteristics. The study included 422 orthodontic patients with mean age 11.05±3.175 years, boys with mean age 10.44±2.739 years and girls with mean age 11.44±3.373 years. The patients studied were divided into three age intervals, specific for the development of dentition and of dental occlusion: 96 (22.7%) patients below 9 years of age; 199 (47.2%) patients

aged 9-12 years; 127 (30.1%) patients over 12 years of age. 97 (23.0%) patients presented early mixed dentition, 154 (36.5%) late mixed dentition, 158 (37.4%) patients permanent young dentition, and 13 (3.1%) patients permanent complete dentition.

Table 23. Necessity factors for dental crowding treatment

n=422			Interceptive treatment	Corrective Treatment	Maxillary Expansion	Extractions teeth
Age intervals	>9 years	n	39	72	62	0
		%	58.2	18.3	33.0	0.0
	9-12 years	n	28	195	120	48
		%	41.8	49.6	63.8	32.9
	<12 years	n	0	126	6	98
		%	0.0	32.1	3.2	67.1
	Chi-square		68.478	63.993	171.675	171.675
	p value		0.000*	0.000*	0.000*	0.000*
Dentition types	early mixed	n	39	73	62	1
		%	58.2	18.6	33.0	0.7
	late mixed	n	28	150	118	8
		%	41.8	38.2	62.8	5.5
	permanent	n	0	170	8	137
		%	0.0	43.2	4.3	93.8
	Chi-square		75.875	63.369	268.949	268.949
	p value		0.000*	0.000*	0.000*	0.000*
Dental crowding	mild	n	25	98	102	0
		%	37.3	24.9	54.3	0.0
	moderate	n	3	225	84	116
		%	4.5	57.3	44.7	79.5
	severe	n	39	70	2	30
		%	58.2	17.8	1.1	20.5
	Chi-square		110.151	29.846	128.368	128.368
	p value		0.000*	0.000*	0.000*	0.000*
Angle Classes	Class I	n	46	263	107	108
		%	68.7	66.9	56.9	74.0
	Class II	n	12	108	63	35
		%	17.9	27.5	33.5	24.0
	Class III	n	9	22	18	3
		%	13.4	5.6	9.6	2.0
	Chi-square		16.546	47.035	13.740	13.740
	p value		0.001*	0.000*	0.000*	0.003*
*statistically significant differences when p<0.05						

The prevalence of the clinical forms and treatment types. According to the severity of dental crowding, were found 118 (28%) patients with mild dental crowding, 228 (54%) patients with moderate dental crowding and 76 (18%) patients with severe dental crowding, from the Angle Class I malocclusions in 286 (67.8%) patients, Class II in 112 (26.5%) patients, and Class III in 24 (5.7%) patients. The patients presented other associated anomalies: crossbite in 247 (58.5) patients, deep bite in 184 (43.4%) patients, and mandibular lateral deviation in 150 (35.5%) patients. The causes of dental crowding were local in 402 (95.3%) patients and general in 20 (4.7%) patients. 15.9% interceptive orthodontic and 93.1% corrective (13.5% precocious, 38.6% normal and 41.0% late) treatments have been applied. Space on the arches was obtained by maxillary expansion in 44.5% of patients, by extractions of permanent teeth in 34.6% of patients, and by serial extractions of

temporary teeth in 10.7% of patients, on using removable biomechanical appliances in 29.9% of patients, fixed biomechanical appliances in 27.5% of patients and functional appliances, respectively, in 1.8% of patients. We found statistically significant correlations between dental crowding and all those presented variables ($p<0.05$).

The necessity factors and predictors for treatment options. The interceptive treatment applied was specific to 58.2% of patients below 9 years of age, early mixed dentition, severe dental crowding ($p=0.000$) from the Angle Class I ($p=0.001$). The corrective treatment was specific to 49.6% of patients aged 9-12 years, late mixed dentition and permanent dentition, moderate dental crowding from the Angle Class I ($p=0.001$). Maxillary expansion was specific to 63.8% of patients aged 9-12 years, late mixed dentition, mild and moderate dental crowding from the Angle Class I and II ($p=0.000$). The orthodontic extraction of permanent teeth was specific to 67.1% of patients over 12 years of age, permanent dentition, moderate and severe dental crowding ($p=0.000$) from the Angle Class I and II ($p=0.003$) (tab. 23).

Table 24. Binary logistic regression analysis of the significant predictors

Parameters	B	Wald statistic	*p* value	OR	95% Confidence Interval for OR	
					Lower	Upper
Interceptive orthodontic treatment						
mild crowding	-1.366	18.052	0.000	0.255	0.136	0.479
moderate crowding	-4.370	48.913	0.000	0.013	0.004	0.043
maxillary expansion	2.643	23.438	0.000	14.060	4.822	40.995
Corrective orthodontic treatment						
local causes	-2.125	10.504	0.001	0.119	0.033	0.432
maxillary expansion	-1.053	5.070	0.024	0.349	0.140	0.873
orthodontic extraction	-3.702	16.842	0.000	0.025	0.004	0.145
Surgical-orthodontic treatment						
mild crowding	-2.699	11.668	0.001	0.067	0.014	0.317
moderate crowding	-1.036	4.073	0.044	0.355	0.130	0.971
orthodontic extraction	-5.112	152.738	0.000	0.006	0.003	0.014

The model of binary logistic regression permitted identification of the significant predictors for the interceptive orthodontic treatment (mild and moderate crowding and maxillary expansion), with a precision of 92.4% (test HL, $p=0.410$); for the corrective orthodontic treatment (local factors, maxillary expansion and orthodontic extraction), with a precision of 95.3% (test HL, $p=0.618$); for the surgical-orthodontic treatment (mild and moderate crowding and orthodontic extraction), with a precision of 91.5% (test HL, $p=0.306$) (tab. 24).

Discussion

This research was focused on a group of patients with various clinical forms of dental crowding, treated orthodontic, by evaluating their age intervals and type of dentition in the moment of its detection, the Angle Classes of diagnosis, the associated anomalies, the etiological causes and the therapeutically needs, in order to find the statistically significant necessity and predictive factors of the treatment.

For avoiding any possible statistical error, patients with the same characteristics have been selected. We used multiple variables resulting from the characteristics of the experimental group. The effects that we pursued were based on the statistically significant relations between sample characteristics, clinical forms, etiological cause and therapy of dental crowding. Considering the mean age of the patients, detection of dental crowding occurred during late mixed dentition and the permanent young one, for both sexes. If identified in early mixed dentition, dental crowding may be solved by means of conservative techniques and with minimum dental costs (Sandikcioglu & Hazar, 1007 Brennan & Gianelly, 2001); however, when identified in adolescent patients, the type of

intervention is different and more complex, both orthodontically and surgically (Bernabé & Flores-Mir, 2006 Akkaya *et al*., 2008).

In the present study, most frequent cases were represented by moderate dental crowding, included in Angle Class I malocclusion, associated with crossbite, induced by local causes.

The Pearson test identified the necessity factors for the interceptive orthodontic, corresponding to the age under 9 years, early mixed dentition, severe dental crowding from Angle Class I; corrective treatment corresponding to the age between 9-12 years, late mixed dentition, permanent dentition, moderate dental crowding from Angle Class I; and for maxillary expansion, corresponding to the age between 9-12 years, late mixed dentition, mild and moderate dental crowding from Angle Classes I and II; and for extraction of permanent teeth for orthodontic reasons, corresponding to the age over 12 years, permanent dentition, moderate and severe dental crowding from Angle Classes I and II.

The model of binary logistic regression applied identified the predictive factors for the types of orthodontic treatment, according to the nature of the intervention. The recommendation for mild and moderate dental crowding was maxillary expansion, as predictive factors of the interceptive orthodontic treatment. For dental crowding with local causes, maxillary expansion and orthodontic extraction - as predictive factors of the corrective orthodontic treatment - were indicated. The mild and moderate crowding had the orthodontic extraction as indication, being a predictive factor for the surgical-orthodontic treatment. All these predictive factors had not been identified up to now, the existing studies having only discussed some unilateral aspects of this anomaly (Toyama *et al*., 2019; McNamara *et al*., 2019).

Conclusions

In this study, the mean age at which dental crowding was detected was an advanced one, the highest prevalence being registered for moderate crowding of Angle Class I, with local causes, associated with crossbite. The necessity and predictive factors of treatment were adapted to age, dentition, severity of dental crowding and Angle Class of malocclusion. The study suggests the need to involve dentists in the early interception of this common dental anomaly, for preventing subsequent complications. Similar further studies should be extended to other types of malocclusions, known as affecting both the health condition and dental aesthetics of our population.

4.2. Cephalometric aspects of the skeletal anomalies

The literature of the field provides numerous cephalometric studies, developed comparatively on skeletal Classes I and II, on gender, age, clinical divisions, dentitions and different populations (Pancherz *et al*., 1997; Ishii *et al*., 2001; Antonini *et al*., 2005; Sayin & Turkkaraman, 2005; Isik *et al*., 2006; Al-Khateeb, 2009; Hassan, 2011). The results are debatable, if considering the size and selection criteria of the experimental groups, ethnic heterogeneity, races and diversity of the investigation methods applied (Rosenblum, 1995; Brezniak *et al*., 2002; Saltaji *et al*., 2012). Most studies of skeletal Class III malocclusion were performed on Eastern Asian populations (Chang *et al*., 1992; Jeong & Hwang, 1999; Chen & Terada, 2006; Chung & Jung, 2008; Choi & Kim, 2010). Diversity of opinion on parameters values of cephalometric characteristics of skeletal Class III are supposed to be the expression of the ethnic composition of the studied groups (Mouakeh, 2001; Reyes *et al*., 2005; Proff & Will, 2008).

These researches were conducted in order to investigate the cephalometric characteristics of the skeletal anomalies Classes II and III on a sample of patients without orthodontic treatment from the North-Eastern region of Romania. *The aim of the study* was to compare the cephalometric parameters of skeletal Classes II and III with the skeletal Class I, to identify quantitative and relational differences of bone, dental and soft tissues structures, by gender and age range.

Material and methods

The analytical observational study was performed on 120 conventional lateral cephalograms, from the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, between January 2005 and December 2014. The cephalograms' selection criterion was their good technical quality. All X-rays were performed with the same X-ray device, the STRATO-X Orthopantomograph with magnification factor of 11.8%.

The lateral cephalograms of patients without previous orthodontic treatment were divided in three groups, by skeletal Classes (ANB angle): 60 patients (22 boys and 38 girls) with skeletal Class II (ANB>4°); 36 patients (17 boys and 19 girls) with skeletal Class III (ANB<2°); 24 patients (11 boys and 13 girls) with skeletal Class I (ANB≤4°). The divisions of Class II have been clinically diagnosed, on considering the incisive sagittal relation: 24 patients with division 1 (9 boys and 15 girls with increased overjet (>2mm); and 36 patients with division 2 (13 boys and 23 girls) with minimum overjet (<2mm). The patients with ages between 7-26 years (mean age 14.33±5.758 years) were divided in two age intervals, below and over 14 years. The patients with craniofacial dysostosis, craniofacial synostosis, endocrine and genetic syndromes were excluded.

Techniques and measurements. The anatomical outlines of the conventional lateral cephalograms were drafted on tracing paper with a 0.5mm diameter pencil. The tracing paper was scanned (MFD Canon Pixma MP280) in digital format (JPG File), after which it was stored on a computer (Asus Eee PC 1015BX) (Chen *et al*., 2004). The digital cephalometric analysis was performed with Onyx Ceph^{TM} (Onyx CEPH 2.7.18 (174) Image Instruments GmbH, Chemnitz, Germany). The landmarks were located with the mouse pointer directly on the digital paper image on the computer display. For each imagines there were measured 28 angles (°), 21 linear dimensions (mm) and 4 ratios (%) of jaw, dental and soft tissues (n=53 measurements), using Steiner (1953), Tweed (1954) and Jarabak (1973) analyses. Every single scanned paper was digitally measured under identical calibration conditions for the size of the cephalometric images. All data obtained was extracted and stored in the electronic format (Microsoft Office Excel 97-2003 Worksheet). The protocol included a quantitative evaluation of the skull basis, maxillary, mandible, and the relations between them, as well as with the dental and soft tissues.

Statistical analysis was made with SPSS 16.0 program (SPSS Inc., Chicago, IL), for Windows. The numerical values were calculated according to the parameters of descriptive statistics. To compare the measured values of the two skeletal Classes II and III with skeletal Class I, on gender and age intervals, the Kolmogorov-Smirnov test, *t*-Student test and Levene test for the equality of variances used. The $p ≤ 0.05$ value, corresponding to the 95% confidence interval, was considered statistically significant.

Results

The measurements mean values of the skeletal Classes II and III lateral cephalograms were compared with those from skeletal Class I.

Comparison of cephalometric measurements between skeletal Classes II and I

Multiple statistically significant differences were found between the two skeletal Classes for the mean values of the angular measurements (SNA, SN-GoGn, FMA, MeGo-OcP, NSAr, ArGoMe, NGoMe, SN-GoMe, Sum, NSGn, NAPog, Max1-NA, Mandl-NB, FMIA, Z-angle), linear dimensions (S-L, Go-Me, Wits, 1u-NA, 1l-NB, Holdaway, 1u-NPog, 1l-NPog, Ls-PnPog', Li-PnPog'), and ratios (AFH:PFH, GoMe:NS, SGo:NMe) of the bone, dental and soft tissue structures ($p<0.05$).

Depending on the two divisions of Angle Class II, statistically significant differences were found for the mean values of the angular measurements (Ii, Max1-NA, Max1-SN, IMPA, Mand1-MeGo), linear dimensions (N-S, Wits, 1u-NA, 1u-NPog), and ratios (GoMe:NS) of bone and dental

structures; on gender for the mean values of the dental angular measurements (IMPA, Mand1-MeGo), and linear dimensions of bone structures (S-Ar, Go-Me, N-Go, S-Go); on intervals age for the mean values of the angular measurements (SN-GoGn, FMA, MeGo-OcP, ArGoMe, NGoAr, NGoMe, SN-GoMe, Sum, NAPog, Ii, Max1-NA, Max1-SN, Mand1-NB), linear dimensions (Ar-Go, PFH, AFH, N-Go, S-Go, N-Me, Wits, Pog-NB, 1l-NPog, Ls-NsPog', Li-NsPog'), and ratios (AFH:PFH, SAr: ArGo, SGo:NMe) of the bone, dental and soft tissues structures ($p<0.05$). Other differences of angular, linear and ratios measured mean values were not significant ($p>0.05$).

Comparison of cephalometric measurements between skeletal Classes III and I

For the Steiner analysis were found statistically significant differences between skeletal Classes for the sagittal position of the mandible to the anterior plane of skull basis (SNB angle, $p=0.032$), sagittal position of symphysis mentalis to the anterior plane of skull basis (SND angle, $p=0.046$) and for Holdaway ratio ($p=0.018$).

For the Tweed analysis were found statistically significant differences between skeletal Classes for the mandibles' growth direction (FMA angle, $p=0.009$), sagittal position of the lower incisor to the mandibular plane (IMPA angle, $p=0.010$), sagittal relation of maxillary-mandible (Wits, $p=0.000$) and for facial anterior height (AFH, $p=0.033$).

For the Jarabak analysis were found statistically significant differences between skeletal Classes for inclination of the occlusal plane to the mandibular basis (MeGoOcP angle, $p=0.001$), sagittal position of the lower incisor to the mandibular basis (Mand 1-MeGo angle, $p=0.010$), sagittal position of the upper incisor to the Downs facial plane (distance 1u-NPog, $p=0.006$), temporomandibular joint and Glenoid Fossae position (NSAr angle, $p=0.004$), type of condylar growth (ArGoMe angle, $p=0.001$), type of mandibular growth (NGoMe angle, $p=0.002$) and sagittal position of mandibular basis to the anterior plane of skull basis (SNPog angle, $p=0.050$). Other differences of angular, linear and ratios measured mean values were not significant ($p>0.05$).

The mean values of the digital measurements of skeletal Class III were compared between the two sexes and no statistically significant differences were found. The mean values of the measurements of skeletal Class III were compared for two intervals of age below 14 years and over 14 years and were found statistically significant differences for the type of mandibular growth (NGoAr, $p=0.007$), sagittal position of the upper lip (Ls-NsPog', $p=0.015$) and for the skull basis length and mandibular ramus height ratio (S-Ar:Ar-Go, $p=0.008$).

Discussions

This research was focused on comparing skeletal Classes II and III cephalometric measurements with skeletal Class I, for identifying the quantitative features and relations of the bone, dental and soft tissues structures on sexes and age intervals. The skeletal class was identified by angle ANB, accepted in literature as an index of skeletal discrepancies (Panagiotidis & Witt, 1977; Iwasaki *et al.*, 2002).

Cephalometric measurements of skeletal Class II

Class II divisions were clinically identified, according to the overjet, in agreement with some other studies (McIntyre & Millett, 2006; Al-Khateeb, 2009) yet no additional variables have been introduced, as in the case of other works (Sayin & Turkkaraman, 2005), for not complicating too much the statistical analyses.

The dento-maxillary anomalies skeletal Class II was characterized by 28 measurements (15 angular, 10 linear dimensions and 3 ratios): (a) the skeletal sagital angle of the maxillary and of the temporomandibular joint to the skull basis, the growth pattern and the convexity angle; (b) the skeletal vertical angle of the mandibular growth pattern and of the occlusal plane to the mandibular plane; (c) the dento-skeletal sagittal angle of the upper and lower incisors; (d) the angle of the soft tissue profile; (e) the skeletal linear dimension of skull anterior basis, of the mandible and of the A-O distance to the occlusal plane; (f) the dento-skeletal linear dimension of the upper and lower incisors; (g) the linear dimension of the lips; (h) the ratio of the anterior and posterior facial height and the ratio of the mandible and skull anterior basis sizes. These results were extensive in measurements than previous cephalometric studies, which reported some sagittal

dento-skeletal features of the skull basis length, position of the maxillary, mandible and upper and lower incisors, and of the growth patterns (Pancherz *et al.*, 1997; Antonini *et al.*, 2005; Isik *et al.*, 2006; Franchi *et al.*, 2007; Vasquez *et al.*, 2009).

The present study, the two divisions of Class II were different by 10 cephalometric measurements (5 angular, 4 linear dimensions and 1 ratio): (a) the dento-skeletal sagittal angle of the upper and lower incisors; (b) the skeletal linear dimension of the skull anterior basis and of the A-O distance to the occlusal plane; (c) the dento-skeletal linear dimension of the upper incisor; (d) the ratio of the mandible and skull anterior basis sizes. These results showed several differences of cephalometric measurements compared to previous studies, which reported especially, vertical facial dento-skeletal characteristics (Isik *et al.*, 2006; Stahl *et al.*, 2008; Al-Khateeb, 2009; Saltaji *et al.*, 2012).

The results obtained showed differences of cephalometric measurements for Class II divisions on gender (6 measurements: 2 dental angular and 4 mandibular linear dimensions), and the age intervals (28 measurements: 14 angular, 11 linear dimensions and 3 ratios). These results are different from those of previous studies (Lau & Hagg, 1999; Rothstein & Yoon-Tarlie, 2000; Sayin & Turkkaraman, 2005; Hassan, 2011) and may be due to the ethnical characteristics and the intervals age of the studied samples.

Cephalometric measurements of skeletal Class III

We used three cephalometric analyses used by orthodontists, in order to identify three aspects (skeletal, dental and soft tissues) characteristic to the dento-maxillary anomalies Class III. Using statistical tests allowed us to find significant differences between skeletal Classes, which represented the defining parameters of skeletal Class III malocclusion for the studied population. Thus, we found 14 statistically significant measurements (10 angular, 2 linear dimensions and 2 ratios), that characterized the skeletal Class III: mandibular prognathia by the anterior sagittal position of the mandible to the anterior plane of skull basis (increased SNB angle and decreased Ao-Bo distance); progeny by the anterior sagittal position of symphysis mentalis to the anterior plane of skull basis (increased SND and SNPog angles and Holdaway ratio); mandible's hyperdivergent growth (increased FMA and NgoMe angles); lower dental retroclination (decreased IMPA and Mand 1-MeGo angles and 1u-Npog distance); inclination of the occlusal plane to the mandible basis (increased MeGo-OcP angle); anterior position of temporomandibular joint (decreased NSAr angle); posterior condylar growth, with the opening of the mandibular angle (increased ArGoMe angle); increase of the facial anterior height (increased AFH ratio).

Previous comparative studies (beetween Classes III and I) reported similar parameters as prognathia, mandibular posterior rotation, forward position of the mandible and lower incisor retroclination (Guyer *et al.*, 1986; Mitani, 1986; Mitani *et al.*, 1993; Miyajima *et al.*, 1997; Jeong &, Hwang, 1999; Chen & Terada, 2006; Chung & Jung, 2008; Choi & Kim, 2010; Sara & Wolfe, 2011). The different parameters as skull basis shortening, underdeveloped maxillary, upper incisor proclination and facial deficiency of the zygomatic region were reported (Miyajima *et al.*, 1997; Mouakeh, 2001; Proff, 2008). Results are still controversial and some authors argue that the cranial morphology in subjects with Class III does not differ from those with Class I (Anderson & Popovich, 1983; Chung & Jung, 2008). Contradictions in existing studies results are due to the heterogeneity of ethnic, race and age diversity of studied groups and research methods.

In the present study, the measurement values of skeletal Class III were compared between genders and we did not find statistically significant differences. The literature reports significant differences between the sexes regarding the position of the upper lip (Anderson & Popovich, 1983).

In the present study, the measurements differences between the two age ranges (prepubertal and pubertal-postpubertar) of skeletal Class III showed emphasis of several characteristics together with the dento-maxillo-facial complex growth, at the studied population. Thus, we found 3 parameters that showed the emphasis of the vertical growth of the mandible (decreased NGoAr angle), mandible prognathia (decreased S-Ar:Ar-Go ratio) retrusion of the upper lip (decreased Ls-NsPog' distance). Previous studies show that specific Class III parameters are established from an early age and are maintained throughout the skeletal growth (Graber & Vanarsdall, 1994 Chen &

Terada, 2006), and puberty is of significant importance on the mandibular length growth, the maximum pubertal being extended in subjects with Class III (Reyes *et al*., 2005; Kuc-Michalska &; Baccetti, 2010).

This study presents anthropometric importance in the studied population and has clinical-diagnostic value for a correct specialized orthodontic or surgical therapy.

Conclusions

This study evidenced the large variety of cephalometric features of the dento-maxillary anomalies Class II and of its divisions on genders and age intervals in the sample studied, which calls for a specific and individualized orthodontic therapy of each patients suffering from it. Also, the present study showed that the dento-maxillary anomalies Class III presents a distinct pathology, characterized by a series of specific cephalometric parameters which set the diagnosis and indicate the morphological dento-maxillo-facial segment that should be orthodontically corrected as early as possible. This study has particular importance for practitioners and suggests the necessity in their involvement in identifying at an early age these severe skeletal discrepancies.

4.3. Clinical aspects of crossbite and risk factors and predictors of treatment

Previous studies have not been reached a unanimous conclusion on the cause - effect relationship of crossbite. Many studies have focused on the crossbite treatment with various orthodontic appliances, but authors was not specified a causal therapy (Kennedy & Osepchook, 2005; Borrie & Beam, 2011). Also, predict factors crossbite' treatments are almost non-existent in previous studies.

This study is based on the hypothesis that crossbite may occur due to determinants cause factors. The detection and early removal can prevent future severe skeletal discrepancies. Correctives treatment options depend on the child's development stage at the moment of orthodontic intervention. *The aim of this study* was to compare two samples of patients with the same characteristics, with/without crossbite, with orthodontic treatment, to assess the mean age for detecting crossbite, etiological causes, oral status, types of treatment, and to establish the risk factors and predictors for treatment options.

> **Zegan G,** Dascalu GC, Mavru RB, Golovcencu L. Risk factors and predictors of crossbite at children. *The Medical-Surgical Journal (Rev Med Chir Soc Med Nat, Iasi),* Jun 2015; 119(2): 564-571.

Patients and methods

This analytical observational study type case-control was performed on 525 orthodontic patients, 217 (41.3%) males and 308 (58.7%) females, aged between 6-18, at the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, between January 2009 and April 2013. 270 (51.4%) patients with crossbite were included as cases sample and were divided in three groups, to the anomaly's location: 175 (33.3%) patients with anterior crossbite, 46 (8.8%) patients with posterior crossbite, and 49 (9.3%) patients with antero-posterior crossbites. Patients with endocrine and genetic syndromes or clefts palate were excluded. The diagnosis was established by clinical and complementary exams (plaster cast, panoramic radiography and lateral cephalogram). All cases received orthodontic treatment. The control sample consisted in 255 (48.6%) patients without crossbite, being diagnosed with other malocclusions throughout the visit at the orthodontic office, in order to be compared with the cases sample. The informed consent was acquired from all of the patients' parents.

Studied parameters. The data was collected from orthodontic records for both samples that were uniformly evaluated according to social parameters (gender and age) and oral status (dentition types and Angle Classes of malocclusion). The groups of crossbite cases were compared to each other, introducing etiological factors (dysfunctional and dental), other associated anomalies (dental

crowding, overbite, mandibular lateral deviation, jaw discrepancies and temporomandibular joint disorders), as well as by needs and types of treatment.

The skeletal discrepancies were established on lateral cephalogram by measuring the angles between the points A Down, Nasion, B Down (ANB) in: skeletal Class I ANB=2-4°, skeletal Class II ANB>4° and skeletal Class III ANB<2° (Steiner, 1960). The treatment needed was defined by the discrepancy of the teeth between retruded contact position and intercuspal position, using guidelines of Dental Health Components of the Index of Orthodontic Treatment Need (DHC-IOTN) classifying them in moderate need (≤1 mm discrepancy), urgent need (with >1 mm but ≤ 2 mm discrepancy) and very urgent need (>2 mm discrepancy) (Brook & Shaw, 1989).

Statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL) for Windows. The data was characterized through descriptive statistics and cross tables; we used the Chi-square (χ^2) Pearson test to compare the variables between cases-controls samples and between the three groups of crossbite cases, in order to find the significant differences. The risk and predictive factors that were significant on binary analysis were brought into a forward stepwise binary logistic regression model (B) and the independent factors were determined. In order to select the most suitable model, the Hosmer-Lemeshow goodness-of-fit test (HL test) was used and a *p*-value >0.05 was considered as good fit. Any association between the factors was tested using correlation, to eliminate the multicollinearity problems. The entire statistical analysis was conducted at 5% level of significance and *p* value <0.05 was regarded as significant.

Results

Samples characteristics. The study included 525 orthodontic patients, 270 (51.4%) patients with crossbite with mean age of 10.96±3.225 years (males with mean age 10.82±3.079 years and females with mean age 11.06±3.331 years) and 255 (48.6%) patients without crossbite with mean age of 10.25±2.770 years (males with mean age 10.08±2.818 years and females with mean age 10.36±2.739 years). The baseline characters were similar for both samples, no significant differences (p>0.05) (tab. 25).

Table 25. Baseline characteristics of cases and controls samples

Characteristics	Cases (n=270)		Controls (n=255)		χ^2	p value
	n	%	n	%		
Gender					0.005	0.943
males	112	41.5	105	41.2		
females	158	58.5	150	58.8		
Dentition stages					3.744	0.154
early mixed dentition	66	24.4	78	30.6		
late mixed dentition	107	39.6	103	40.4		
young dentition	97	36.0	74	29.0		
Angle Class malocclusions					0.262	0.877
Class I	190	70.4	184	72.2		
Class II	39	14.4	36	14.1		
Class III	41	15.2	35	13.7		
*statistically significant differences when *p*<0.05						

The group of anterior crossbite cases consisted of 175 (64.8%) patients with mean age of 10.71±2.926 years (males with mean age 10.53±2.864 years and females with mean age 10.85±2.984 years), the group of posterior crossbite cases of 46 (17.0%) patients with mean age of 10.91±3.693 years (males with mean age 11.15±3.760 years and females with mean age 10.73±3.705 years) furthermore, the group of antero-posterior crossbite cases of 49 (18.1%) patients with mean age of 11.90±3.653 years (males with mean age 12.08±3.095 years and females with mean age 11.83±3.873 years). The groups of crossbite cases were compared using multiple studied parameters and statistically significant differences were found (*p*<0.05).

The risk factors and predictors for treatment options. Through the binary logistic regression model, we found statistically significant etiological risk factors, with an internal precision accuracy of 66.3% for all crossbites (HL test, *p*=0.890), of 66.5% for anterior crossbite (HL test, *p*=0.811), of 85.7% for posterior crossbite (HL test, *p*=0.994) and of 83.2% (HL test, *p*=0.449) for antero-posterior crossbite. Value Odds Ratio (OR) <1.00 corresponds to protective factors (tab. 26).

Table 26. Binary logistic regression analysis of risk factors

Parameters	B	Wald statistic	*p* value	OR	95% Confidence Interval for OR	
					Lower bound	Upper bound
All crossbites						
tooth decay	-2.691	12.698	0.000	0.068	0.015	0.298
eruption anomalies	-1.183	18.674	0.000	0.306	0.179	0.524
non-attrition	1.002	4.175	0.041	2.725	1.042	7.128
Anterior crossbite						
tongue-thrust swallowing	-2.821	7.305	0.007	0.060	0.008	0.461
tooth decay	-2.460	10.644	0.001	0.085	0.019	0.374
eruption anomalies	-1.365	17.866	0.000	0.256	0.136	0.481
Posterior crossbite						
Angle Class II	1.146	8.920	0.003	3.147	1.483	6.678
non-attrition	1.847	7.912	0.005	6.341	1.751	22.968
Antero-posterior crossbite						
non-attrition	1.866	8.150	0.004	6.461	1.795	23.259
permanent teeth extraction	2.997	5.110	0.024	20.033	1.490	269.371

Table 27. Binary logistic regression analysis of predictor factors

Parameters	B	Wald statistic	*p* value	OR	95% Confidence Interval for OR	
					Lower bound	Upper bound
Precocious treatment						
sucking habit	2.342	3.723	0.054	10.397	0.964	112.168
non-attrition	1.689	10.259	0.001	5.416	1.926	15.228
buds crowding	0.904	5.671	0.017	2.469	1.173	5.193
Normal treatment						
mouth breathing	0.881	4.451	0.035	2.413	1.065	5.469
buds crowding	2.069	32.417	0.000	7.918	3.884	16.143
Late treatment						
Angle Class III	1.998	16.779	0.000	7.376	2.835	19.191
dental crowding	1.572	11.922	0.001	4.818	1.974	11.764
TMJ disorders	2.288	30.224	0.000	9.855	4.359	22.280
Maxillary expansion						
eruption anomalies	1.337	4.867	0.027	3.809	1.161	12.497
buds crowding	2.335	52.212	0.000	10.329	5.483	19.459
Oral surgical associate						
macrodontia	2.870	30.782	0.000	17.639	6.399	48.618
TMJ disorders	1.374	18.962	0.000	3.951	2.129	7.332

We found significant predictors for the precocious corrective treatment with a precision of 78.5% (HL test, *p*=0.708), for normal corrective treatment with a precision of 71.5% (HL test,

p=0.821) and for late corrective treatment with a precision of 83.0% (HL test, p=0.485), as well as for alternative methods of correction (overall percentage 77.0%, HL test, p=0.992 for maxillary expansion and 75.9%, HL test, p=0.102 for oral surgical) (tab. 27).

Discussions

Early detection of malocclusion and finding the cause-treatment relationship represents a necessity for practitioners, especially in emergency situations.

Complex analysis of types of crossbite in children compared with other malocclusions, in order to establish the risk factors and predictors of orthodontic treatment were investigated in this study. There were selected samples with same characteristics to avoid statistical errors. Patients without syndromes and clefts palate were selected, in order to avoid introducing additional variables, such as growth deficiencies, general skeletal and jaw development. The crossbite cases were divided into three groups and studied by comparisons. Types of crossbite presented highly significant differences, which explain the separate study of the clinical forms of crossbite by other authors (Kennedy & Osepchook, 2005; Borrie & Beam, 2011). We used multiple variables from clinical and complementary exams, which is an enhanced method against previous studies (Volk *et al*., 2010; Primožič *et al*., 2013).

This study was focused on the evaluation of relationship between age detection of malocclusions, etiological cause and therapy of crossbite. According to the average age of the studied patients, the detection of crossbite was done in the period of late mixed and permanent dentitions, for both sexes. Identified at the early mixed dentition, the crossbite benefit from a simple, non-invasive technique, that is also quick (3-6 months), and with minimal cost (Binder, 2004; Ge *et al*., 2011; Petrén *et al*., 2013) Detected at the adolescent patient, the nature of intervention must be different, as a complex orthodontic and surgical treatment, for a longer time (2-3 years) and with a maximal cost (DaCosta & Utomi, 2011; Zuccati *et al*., 2013).

The binary logistic regression model found only dental causes as risk factors for the crossbite occurrence (non-attrition of deciduous teeth, Angle Class II malocclusion and permanent teeth extraction), which may be easily intercepted by practitioners. In addition, protective factors of the crossbite were identified (the tooth decay of deciduous teeth, eruption anomalies and tongue-thrust swallowing), which were not investigated by previous studies.

Predictors for corrective treatment options of crossbite were different according to the nature of the intervention. The buds teeth crowding and eruption anomalies were indicated for maxillary expansion in precocious and normal treatment. Macrodontia and temporomandibular joint disorders were indicated for oral surgical associated procedures in late treatments. In addition, sucking habit and mouth breathing were predictors for treatment in mixed dentition as well as dental crowding and Angle Class III malocclusion for treatment in young permanent dentition. These predictors were not identified in previous studies, dealing with predict factors for future mandibular growth of children with anterior crossbite (Yano *et al*., 2009).

Conclusions

This study revealed the following results: the mean age of malocclusions' detection was late; the groups of crossbite cases were highly significant different; etiological risk factors were only dental; protective factors of crossbite were identified; predictors of treatment were adequate to the dentition, aetiology and disorders. The study suggests the necessity of dental practitioners' involvement in intercepting this occlusal disorder. Similar further studies can be extended on other malocclusions that affect the young population's dental and facial aesthetics.

4.4. The prevalence of orofacial clefts and correlations with social parameters

Center for Disease Control and Prevention in the U.S. (2019) recorded an incidence of live birth of new-borns with clefts lip with/without palate of 10.63‰ and clefts palate of 6.35‰, the isolated cleft forms representing 70% (Parker *et al*., 2010).

In this respect, *the aim of the study* is to establish the prevalence of the orofacial clefts in a community of institutionalized children, to determine the correlations with gender, the birth weight of the new-borns, the environment area, the maternal age and associated affections of the children with clefts lip with/without palate and to compare these data with the results of previous studies.

Material and methods

In this longitudinal analytical observational study, we carried out a review of the medical records of the children with orofacial clefts institutionalized in the placement centers in Iasi "("St. Parascheva" no. 1 and 2, "St. Andrei" and Bucium) in the period 1987-1997. A total of 37 live births of new-borns with clefts lip and palate, 15 boys and 22 girls were identified. Depending on the mother's domicile, 16 new-borns with orofacial clefts came from Iasi County, 9 new-borns from Botosani County, 5 new-borns from Neamt County, 3 new-borns from Vaslui county, 2 new-borns from the county Bacau and 2 new-borns from Slobozia County, 59% from the urban and 41% from the rural areas. Children have been diagnosed since birth and survived. The study group received surgical treatments at "St. Maria" Hospital in Iasi were medically monitored but not treated orthodontic.

Studied parameters. Data from medical records was collected and analysed, as the following: socio-demographic characteristics, medical history, localization of orofacial cleft, birth weight, associated affections, laboratory analysis, and applied treatments.

Statistical analysis was performed using EpiInfo 3.4.3 software of Center for Disease Control and Prevention in the U.S. (CDC). The data was characterized by descriptive statistics and Fisher test ($F_{calculated} > F_{Fisher}$) and linear and multiple regression for statistical correlations between variable were used.

Results

The incidence of children with orofacial clefts. In 1987, of the 2150 institutionalized children, 160 (7.42%) children had orofacial clefts and represented a ratio of 1:13.44 children compared to 1997, when out of 1146 institutionalized children, 25 (2.18%) children had orofacial clefts and represented a ratio of 1:45.80 children.

In 1987, 65.30% of children had clefts lip (50.84% of boys and 49.16% of girls), and in 1997, 42.50% of children had clefts lip (41.07% of boys and 58.93% of girls) and 57.50% of children had clefts lip and palate (33.65% of boys and 66.35% of girls). The distribution of clefts palate by gender was 61.21% for boys and 38.79% for girls. In the period 1987-1997, the annual average rate of prevalence of live birth of new-borns with orofacial clefts was 3.7/year: 1.4/year new-borns with clefts lip, 2.0/year new-borns with clefts lip and palate and 0.3/year new-borns with clefts palate.

The prevalence of the anatomo-clinical forms of the orofacial clefts. The prevalence of orofacial clefts was 54.06% of children, 37.84% of children with clefts lip, and 8.10% of children with clefts palate. Clefts lip and palate/ only clefts palate were more common in girls and cleft lip was more common in boys. Unilateral orofacial clefts were more frequent on the left side.

Social parameters. The birth weight of live birth of new-borns with orofacial clefts was normal in 54.06% of children, 18.91% of children suffered from dystrophy, and 27.03% of children were overweight. The age of mothers who gave birth to new-borns with orofacial clefts was less than 20 years old at 5.40% of mothers, between 21 and 34 years old to 91.89% of mothers, and over 34 years old to 2.71% of mothers.

Statistical correlations. Using the Fisher test, a positive linear regression slope was found for the statistically significant correlation of the anatomo-clinical forms of the orofacial clefts with maternal age, and a negative linear regression slope for the correlation of the anatomo-clinical forms of the orofacial clefts with gender, the weight of live births of new-borns and the urban/ rural areas of mothers (tab. 28).

Through multiple regression, the correlations of the anatomical-clinical forms of orofacial clefts with the associated conditions of the surviving children and some laboratory analyses were calculated and statistically significant values directly proportional with facial muscular hypodevelopment, facial asymmetries, tachycardia, extrasistles, bleeding time and inversely proportional with facial and trigeminal nerves disorders and facial bone hypoplasia were found (tab. 29).

Table 28. Correlations between the anatomo-clinical forms of the orofacial clefts with the socio-demographic variables

Variables	r (correlation coefficient)	β (slope of the regression line)	F (Fisher test)
Gender	- 0.31	- 0.8090	3.5902
Weight at birth	- 0.04	- 0.0009	0.0487
Environment area	- 0.20	- 0.5363	1.4920
Maternal age	0.22	0.0764	1.8615

Table 29. Correlations between the anatomical-clinical forms of the orofacial clefts and the associated diseases of the children

Variables	β (slope of the regression line)	F (Fisher test)
Muscle hypodevelopment	0.6449	$1.04 < F < 1.3999$
Facial asymmetries	0.8601	$5.62 < F < 18.3784$
Bone system	- 0.7593	$3.96 < F < 6.1258$
Cardiovascular system	0.4717	$1.04 < F < 1.6832$
Nervous system	- 0.4020	$1.04 < F < 12.6176$
Bleeding time	0.4447	$0.22 < F < 0.6401$

Discussions

An epidemiological study in 34 countries and 30 countries between 2002 and 2006 showed an increase in the U.S. and an international decrease in the prevalence of live birth of new-borns with orofacial clefts or other congenital malformations (Tanaka *et al*., 2012). In the studied group, the decrease in the incidence of orofacial clefts children over the years is in line with international data, but is also explained by the decrease in the number of children institutionalized in the Iasi placement centers. If in 1987 gender distribution was approximately equal, in 1997 girls had a high frequency of clefts lip with/without palate and boys had a high frequency of clefts palate.

Epidemiological studies estimate that every year 2.651 children with clefts palate are born in the U.S. and 4.437 children with clefts lip with/without palate (Parker *et al*., 2010). The incidence rates of live birth of new-borns with orofacial clefts were different for racial groups: American natives 3.74‰; Japanese 0.82‰ and 3.36‰; Chinese 1.45‰ and 4.04‰; Caucasians 1.43‰ and 1.86‰; American Latin 1.04‰; American Africans 0.18‰ and 1.67‰. The prevalence of palatine uvula split varied between 0.02% and 18.8%, the highest being in the Indians and the lowest in American Africans (Dudas *et al*., 2012). In the studied group, the annual average rate of prevalence of live births with orofacial clefts was similar to the American natives, being the highest rate in the racial groups of previous studies.

Orofacial clefts have various anatomo-clinical forms, so Tessier (1976) had described 16 orofacial cleft lines, but their occurrence frequency is much more uncommon. Generally, three distinct entities are defined: clefts lip, clefts palate, and clefts lip and palate. The clefts lip occurs more frequently on the left side or on both sides. The clefts palate are more common in females and affect all human races. The clefts lip and palate, in which there is communication between the mouth and the nose, are also called velo-pharyngeal insufficiency and are more common in male, on the left-side, bilateral, in Asian and in American Africans (Watkins *et al*., 2014). In present

study, the clefts lip and palate/ only palate were most frequent in the girls, and the clefts lip in the boys. Also, unilateral orofacial clefts were more frequent on the left side, as literature data shows.

Causes of orofacial clefts are multifactorial, including genetic, environmental, chemical or drug factors. Some authors have mentioned risk factors as maternal hypoxia due to smoking, alcoholism, diabetes and maternal hypertension (Little *et al.*, 2004; Honein *et al.*, 2007; Correa, *et al.*, 2008; Werler *et al.*, 2011; Margulis *et al.*, 2012). The present study examines the birth weight of live birth of new-borns and maternal age at birth in order to find a causality with orofacial clefts. The high percentage of cases with normal birth weight showed that the intrauterine development of the foetus was not affected by the existence of an embryopathy. There is a small risk of clefts that doesn't allow conclusions to be drawn regarding the small number of children with orofacial clefts belonging to extreme age mothers. We did not have data about the age of fathers and the possible consanguinity among the parents.

The Fisher test showed a statistically significant correlation between clefts palate and girls, the small birth weight of new-borns and the maternal rural area. Also, the Fisher test showed a statistically significant correlation between orofacial clefts and the high birth weight of new-borns, maternal urban area, and maternal births below 20 years of age.

Complications that occur in children with orofacial clefts are multiple. The maxillo-facial development of children is almost normal in surgically uncorrected orofacial clefts, in which there is a three-dimensional developmental deficiency due to postoperative scars and bone hypoplasia, with severe consequences on the functions of the dento-maxillary complex (Phillips *et al.*, 2012).

Conclusions
The data obtained is in agreement with literature and respects the frequency of the orofacial clefts from the general population. This study showed the decreased incidence of children with orofacial clefts, the high prevalence of clefts lip and palate, the existence of orofacial cleft complications and the need for orthodontic treatment. Some higher values are probably the result of the structure of the population surveyed in the Iasi placement centers. The realization of such studies provides long-term data of the medical-surgical monitoring of the studied population. It is possible to extend studies in other areas of interest, for some correctional facilities.

4.5. The prevalence of facial, occlusal and skeletal asymmetries and correlations with socio-demographic parameters
The need to improve facial aesthetics is one of the main reasons why patients are addressing the orthodontist, facial symmetry having a determining importance in facial aesthetics (Tjan & Miller, 1984). If minor facial asymmetry can be the positional consequence of components of the dento-maxillary complex, severe facial asymmetry is due to a right-left transverse difference of these components, which occurs mainly in craniofacial syndromes (Proffit *et al.*, 2007; Haraguchi *et al.*, 2008). The aetiology of facial asymmetry is not entirely known, but has been classified into three main categories: congenital, developmental and acquired (Cheong & Lo, 2011). The facial asymmetry clinically or photographic identified (by composite face photo method) (Good *et al.*, 2006) is confirmed by a frontal cephalometric examination (Bishara *et al.*, 1994; Cohen *et al.*, 1995).

Some studies reported 5% upper face asymmetry, 36% middle facial asymmetry and 74% inferior face asymmetry (Severt & Proffit, 1997), due in most cases to Angle Class III malocclusion (Chew, 2005; 2006). The previous studies showed that occlusal asymmetries are found in all the types of dento-maxillary anomalies, being most frequent in Angle Class II malocclusions (Rose *et al.*, 1994; Harris & Bodford, 2007). Although the dental midline deviation is commonly found in orthodontic practice, few studies reported the prevalence of this anomaly, with an important role in facial aesthetics due to the dominant attribution of the anterior teeth while smiling (Jerrold & Lowenstein, 1990; Beyer & Lindauer, 1998; Johnston *et al.*, 1999; Khan *et al.*, 2014; Mavani *et al.*, 2017). The posterior crossbite is a frequent occlusal imbalance in population (Kutin & Hawes, 1969) and may exist even in primary dentition (13-25%) (Duncan *et al.*, 2008; Dimberg *et al.*,

2010). The unilateral posterior crossbite is the most common form and occurs in 80-97% of cases of posterior crossbite (Moyers, 1998; Thilander & Lennartsson, 2002).

These researches were conducted in order to investigate the facial, occlusal and skeletal asymmetries on a sample of patients without orthodontic treatment from the North-Eastern region of Romania, by clinical and cephalometric examinations. *The purpose of the study* was to determine the prevalence of facial, occlusal and skeletal asymmetries, and to find statistical correlations with socio-demographic parameters, dentition type, and Angle Classes of malocclusions.

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Material and methods
This cross-sectional descriptive observational study was performed on 384 orthodontic patients, 164 (42.70%) boys and 220 (57.30%) girls, with ages between 6-23 years old (mean age 10.11±2.97 years), 273 (71.10%) from urban and 111 (28.90%) from rural area, from the North-Eastern region of Romania, who asked for orthodontic treatment in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, during 1999-2010 period. The informed consent was acquired from all patients or their parents.

Studied parameters. The data obtained from the clinical and cephalometric examinations was analyzed in order to assess the facial asymmetry (Bishara *et al.,* 1994; Burstone, 1998), asymmetry of occlusion relationships (molar sagittal relationships, dental midline deviation and posterior crossbite) and skeletal asymmetry (Grummons, 1987). The data obtained was analyzed according to the socio-demographic parameters (gender, age and environment), type of dentition (mixed and permanent) and Angle Classes of malocclusion.

Statistical analysis was performed using the SPSS 16.0 package (Chicago, Illinois, SUA) for Windows. The statistical evaluation of obtained data was done by descriptive statistics and Chi-square Pearson test; any values less than *p*<0.05 were interpreted as statistically significant.

Results
The facial asymmetry (the absence of parallelism of the horizontal planes) was present in 16 (4.70%) patients, chin deviation in 22 (5.70%) patients and nasal pyramid in 14 (3.40%) patients, most frequently in girls from urban area (21 – 5.50% patients). The facial and chin asymmetry was most frequently in patients with Angle Class III malocclusion (10 – 2.60% patients) and nasal pyramid asymmetry in patients with Angle Class II division 1 malocclusion (11 – 2.90% patients).

The statistically significant differences between facial asymmetry and age ranges ($p=0.001$), dentition type ($p=0.016$) and Angle Classes of malocclusions ($p=0.000$) were found. There were no statistically significant differences between facial asymmetry and genders and environment area ($p>0.05$).

The asymmetry of the sagittal molar relations (left-right). The static differences at level molar varied from 0-9 mm (mean 0.58 mm), being statistically significant ($p<0.000$), asymmetry being most frequently on the right side. 37 (9.64%) patients had asymmetric molar relationships, distalized relationships (30 patients) or mesialized relationships (7 patients) in different quantitates on the left-right side, and 60 (15.63%) patients had different right-left side molar relationships. The asymmetry of sagittal molar relationships was most frequently in patients with Angle Class II division 2 malocclusions (35% of patients) and lower in patients with Angle Class III (16% of patients), Angle Class II division 1 (15% of patients) and Angle Class I (14% of patients) malocclusions. The statistically significant differences between the asymmetry of the sagittal molar relationships and the Angle Classes of malocclusions ($p=0.000$) were found. There were no statistically significant differences between asymmetry of the sagittal molar relationships and socio-demographic parameters and dentition type ($p>0.05$).

The dental midline deviation was present in 79 (20.70%) patients, in the maxillary arch in 47 (12.50%) patients and in the mandibular arch in 32 (8.30%) patients, the deviation being between 0-4 mm (mean 0.40 ± 0.86 mm), most frequently in girls (47 – 12.24%) than in boys (32 – 8.33%). The patients from urban area are the most affected (55 – 14.32%) than those in rural area (24 – 6.25%). Statistically significant differences between the dental midline deviation and the age ranges ($p=0.028$), dentition type ($p=0.001$) and Angle Classes of malocclusions ($p=0.006$) were found. There were no statistically significant differences between the dental midline and genders and environment area ($p>0.05$).

The posterior crossbite was present in 42 (10.94%) patients, unilateral form in 32 (8.34%) patients and bilateral form in 10 (2.60%) patients. Girls are most affected (25 – 6.52%) than boys (17 – 4.42%). At the age of 6-7 years, the ratio of the two clinical forms was equal (1:1), but over the age of 12 years, the ratio increased in favour of the unilateral form (4.66:1). The patients from urban area are more affected (27 – 7.55%) than those from rural area (13 – 3.38%). The posterior crossbite was most frequent in patients with mixed dentition (24 – 6.25%) than those with permanent dentition (18 – 4.69%). The unilateral crossbite was most frequent in patients with Angle Class I (19 – 4.95%), Angle Class II division 1 (12 – 3.12%) and Angle Class III (9 – 2.34%) malocclusion. In 24 (6.25%) patients with unilateral or bilateral crossbite was associated the midline deviation, indicating a mandibular lateral deviation. In 15 (3.91%) patients the unilateral crossbite was not associated with the midline deviation. The significant statistical differences between posterior crossbite and Angle Classes of malocclusions ($p=0.000$) were found. No statistically significant differences were found between posterior crossbite and socio-demographic parameters and dentition type ($p>0.05$).

The skeletal asymmetry. From the studied sample, in 53 (13.80%) patients (26 boys and 27 girls; 30 from urban and 23 rural areas) we performed postero-anterior cephalograms for confirmation of skeletal asymmetry, which were analyzed by the Grummons method: in 9 (17.10%) patients we noticed the absence of the parallelism of horizontal plans; 23 (42.90%) patients presented the inequality areas of mandibular triangles; 20 (34.30%) patients presented the inequality areas of maxillary-mandibular quadrilaterals; 30 (57.10%) patients presented the inequality areas of maxillo-mandibular triangles; 20 (37.10%) patients had linear asymmetry; 10 (20%) patients had asymmetry of the maxillary-mandibular relations. The statistically significant differences between skeletal symmetry-asymmetry and age ranges ($p=0.006$) and Angle Classes of malocclusions ($p=0.038$) were found. No statistically significant differences were found between skeletal symmetry-asymmetry and genders, environment area and dentition type ($p>0.05$).

Discussions

In this study we have evaluated a sample of Romanian orthodontic patients in order to determine the prevalence of facial, occlusal and skeletal asymmetries and to establish their differences according to genders, age ranges, environment area, dentition type, and Angle Classes of malocclusions, identifying some new aspects regarding the dental-maxillary-facial asymmetry.

In the present study, the facial asymmetry slightly observable by orthodontist between those two hemi-faces had a low prevalence, being most frequent the inferior face asymmetry associated with the Angle Class III malocclusion. The results of the study showed differences in facial asymmetry according to age ranges, the dentition type and the Angle Classes of malocclusions. These results are in consensus with those of previous studies which reported that facial asymmetries modify with age (Melnick, 1992), the prevalence of asymmetry varying from 8% in the child to 68% in adolescents (Jackson *et al.*, 2013). Some authors reported that the right side is more developed (Farkas & Cheung, 1981; Peck *et al.*, 1991), Vig and Hewitt (1975) that the left one and Melnik (1992) that the dominant part of the face gets modified with age. The facial asymmetry is a relative distortion of the various anatomical component parts (Van Keulen *et al.*, 2004), being a combination of several minor asymmetrical components (Jämsä & Rönning, 1985).

In the presented study, the prevalence of the sagittal molar relations asymmetry was lower, being most frequent associated with the Angle Class II division 2 of malocclusion. The results of the study showed differences of the sagittal molar relations asymmetry according the Angle Classes of malocclusions. These results are in consensus with those of previous studies (Alavi *et al.*, 1988; Sheats *et al.*, 1998; Harris & Bodford, 2007), and studies by Janson (2001) and Shroff (1998) reported that sagittal asymmetric relations at molar are produced by the dento-alveolar component.

In this study, the dental midline deviation had an increased prevalence. Differences according to the age ranges, dentition type and the Angle Classes of malocclusions were found. These results cannot be compared, because the data from previous studies is almost non-existent. Some authors showed that the dental midline deviation is a clinical expression of skeletal or dental asymmetries (asymmetric crowding, spacing, tooth rotation, or tooth size discrepancies) (Bishara *et al.*, 1994; Nanda & Margolis, 1996).

In the present study, the clinical forms of the posterior crossbite had a low prevalence, being most frequent a unilateral form associated with the Angle Class I malocclusion. The results of the study showed differences of posterior crossbite according the Angle Classes of malocclusions. These results are in consensus with those of previous studies, which reported a prevalence of posterior crossbite between 4-23% (Dimberg *et al.*, 2010; Gungor *et al.*, 2016).

From the studied sample, a small number of patients were suspected of skeletal asymmetry and made postero-anterior cephalograms to confirm the diagnosis and to establish the asymmetric components. Thus, the most frequent were inferior skeletal asymmetries. These results cannot be compared, because previous studies are controversial about precise localization and quantification of component impairment using frontal cephalogram (Athanasiou, 1995; Sicurezza *et al.*, 2012, Damstra *et al.*, 2013, Tai *et al.*, 2014).

Asymmetries represent complex anomalies which may involve all elements of dento-maxillary complex, in the three spatial directions, being sometimes cumulated or compensated (Lindauer, 1998). The evaluation of the right-left side symmetry at the level of bilateral structures represents one of the main objectives of orthodontic treatment (Burstone *et al.*, 2000).

Conclusions

The identification of dento-maxillary-facial asymmetries in orthodontic patients constitutes an important element for the practitioners. A correct evaluation of the asymmetric components should be made prior to the beginning of any orthodontic treatment; this may orient towards the option a surgical-orthodontic treatment.

4.6. The correlation between idiopathic scoliosis, facial asymmetry and dento-maxillary anomaly

Idiopathic scoliosis is a progressive disease with aesthetic (important asymmetry) and functional consequences (cardiac and respiratory dysfunctions). It requires clinical observation over time to assess the risk of curve progression. Orthopaedic treatment (physiotherapy, braces) does not correct scoliosis, but prevents its progression during the growth period of the patient. The goal of surgical treatment is to restore and maintain alignment, being indicated when the curve exceeds 40 degrees (Weiss *et al.*, 2003; Izatt *et al.*, 2010; Choudhry *et al.*, 2016). The results of observational and case-control previous studies and case reports were inconclusive and a cause-effect relationship between idiopathic scoliosis, facial asymmetry and dento-maxillary anomaly was not found. (Amat, 2008; Gault, 2008; Saccucci *et al.*, 2011). Some cephalometric studies investigating relationships with posture have mentioned a correlation between facial morphology and head inclination in relation to the spinal column and vertical plane (Solow & Tallgren, 1987; Solow & Siersbaek-Nielsen, 1992; Huggare, 1998).

This study aimed to investigate the relationship between idiopathic scoliosis and facial asymmetry in a sample of young patients treated in rehabilitation centers, to find the correlations between the characteristics of idiopathic scoliosis and facial asymmetry, and the coexistence of dento-maxillary anomalies in young orthopaedic patients.

> **Zegan G**, Cernei ER, Anistoroaei D, Toma V. Relationship between idiopathic scoliosis and facial asymmetry in young patients. *The Medical-Surgical Journal (Rev Med Chir Soc Med Nat, Iasi),* Jun 2019; 123(2): 353-358.

Patients and methods

In this cross-sectional descriptive observational study, we carried out a review of patients with idiopathic scoliosis admitted and treated in Medical Rehabilitation Centers in the Iasi and Bacau cities from the North-Eastern region of Romania, between January 2014 and April 2015. A total of 17 orthopedic patients, 1 (5.9%) male and 16 (94.1%) females, aged 15-28 years (mean age 20.06±4.575 years), with idiopathic scoliosis were identified. Informed consent was obtained from all patients. Only young patients (adolescents and young adults) who had clinical and radiological evidence of lateral spinal curvature were included and were diagnosed with idiopathic scoliosis. Patients with other spinal conditions, genetic or endocrine syndromes, as well as adults over 30 years of age or those who refused orthodontic examination were excluded from the study.

Studied parameters. The data from the medical records and spinal radiographs of the patients with idiopathic scoliosis included in the study was collected and analysed, as the following: medical history, clinical signs, scoliosis localization, convexity of the spine, Cobb angle, type of treatment and post-treatment outcomes. The patients were examined clinically and photostatically by the orthodontist in order to establish: facial asymmetry by facial examination and head posture; presence/absence of dento-maxillary anomalies (Angle Classes, anomaly and sagittal, transverse or vertical inter-arches asymmetry) by examining the dental arches and occlusion.

Statistical analysis was performed using SPSS 16.0 software (Chicago, Illinois, USA) for Windows. The data was characterized by descriptive statistics and contingency tables, using the Chi-square (χ^2) Pearson test. Any value less than $p<0.05$ was interpreted as statistically significant.

Results

Idiopathic scoliosis characteristics. According to their medical history, all patients were diagnosed with idiopathic scoliosis at puberty, at the age of 13 to 15 years. Patients had clinical signs of scoliosis: lateral deviation of the head, shoulder asymmetry, winged scapula (right/left), waist angle deformity (difference between right-left waist angles), hip asymmetry, lateral deviation in frontal plane of the spinal column when the trunk is bent forward with the occurrence of gibbosity on the side of the convexity of the curve. The lateral curve of the spinal column was unique ("C" shaped) at cervical level in 2 (11.8%) patients, thoracic level in 9 (52.9%) patients and

lumbar level in 1 (5.9%) patient or double ("S" shaped) at the cervicothoracic level in 1 (5.9%) patient and thoracolumbar level in 4 (23.5%) patients. The Cobb angle measured on the postero-anterior spine radiograph was ≤20° in 6 (35.3%) patients, between 20-40°(45°) in 3 (17.6%) patients and ≥40°(45°) in 8 (47.1%) patients. Convexity of the major spinal curve was on the right side in 15 (88.2%) patients and left side in 2 (11.8%) patients. Progression of scoliosis was stopped by orthopaedic treatments (physiotherapy, Milwaukee, Cheneau, Boston braces) in 10 (58.8%) patients and surgical treatments in 7 (41.2%) patients. Post-treatment outcomes were favourable.

Table 30. Statistically significant correlations between scoliosis and orthodontic variables

Orthodontic variables	Angle Cobb						Total		χ^2 test	p value
	≤20°		20-40°(45°)		≥40°(45°)					
	n	%	n	%	n	%	n	%		
Lateral deviations of head									8.743	0.013*
mild	6	35.3	0	0.00	4	23.5	10	58.8		
moderate	0	0.00	3	17.7	4	23.5	7	41.2		
Dento-maxillary anomaly									10.812	0.004*
absent	0	0.00	1	5.9	7	41.2	8	47.1		
present	6	35.3	2	11.7	1	5.9	9	52.9		
Angle Class malocclusions									17.797	0.007*
Class I	4	23.5	0	0.00	0	0.00	4	23.5		
Class II	1	5.9	0	0.00	0	0.00	1	5.9		
Class III	1	5.9	2	11.7	1	5.9	4	23.5		
Anomalies type									14.432	0.025*
crowding	3	17.7	0	0.00	0	0.00	3	17.7		
asymmetry	2	11.8	1	5.9	0	0.00	3	17.7		
both	1	5.9	1	5.9	1	5.9	3	17.7		
	Convexity of spinal column									
	right			left						
	n	%		n	%		n	%		
Facial asymmetry type									4.156	0.041*
overall face	11	64.7		0	0.00		11	64.7		
lower face	4	23.5		2	11.8		6	35.3		
	Treatment of scoliosis									
	physiotherapy		braces		surgical					
	n	%	n	%	n	%	n	%		
Facial asymmetry type									6.600	0.037*
overall face	3	17.6	1	5.9	7	41.2	11	64.7		
lower face	5	29.4	1	5.9	0	0.00	6	35.3		
Dento-maxillary anomaly									14.993	0.001*
absent	0	0.00	1	5.9	7	41.2	8	47.1		
present	8	47.0	1	5.9	0	0.00	9	52.9		
Angle Class malocclusions									16.469	0.011*
Class I	4	23.5	0	0.0	0	0.00	4	23.5		
Class II	1	5.9	0	0.0	0	0.00	1	5.9		
Class III	3	17.6	1	5.9	0	0.00	4	23.5		
Anomalies type									17.345	0.008*
crowding	3	17.7	0	0.0	0	0.00	3	17.7		
asymmetry	3	17.7	0	0.0	0	0.00	3	17.7		
both	2	11.8	1	5.9	0	0.00	3	17.7		
*statistically significant differences when $p<0.05$										

Facial asymmetry characteristics. Asymmetry of the two hemi-faces was established according to the facial midsagittal plane, right hemi-face being more developed in 9 (52.9%) patients and the left hemi-face in 8 (47.1%) patients. Also, 11 (64.7%) patients had overall facial asymmetry and 6 (35.3%) patients lower face asymmetry. Head inclination was to the right side in 10 (58.8%) patients and to the left side in 7 (41.2%) patients. Also, 10 (58.8%) patients had mild head deviation and 7 (41.2%) patients moderate head deviation. Only 9 (52.9%) patients had dento-maxillary anomalies: 4 (23.5%) patients with Angle Class I, 1 (5.9%) patient with Angle Class II and 4 (23.5%) patients with Angle Class III, associated with dental crowding in 3 (17.6%) patients, inter-arches asymmetry in 3 (17.6%) patients or both in 3 (17.6%) patients.

Statistical correlations. We found statistically significant differences between: the values of Cobb angle of the major spinal curvature and head deviation ($p=0.013$), dento-maxillary anomalies ($p=0.004$), Angle Classes ($p=0.007$) and types of anomaly ($p=0.025$); between the convexity of the major spinal curvature and the facial asymmetry types ($p=0.041$); between the scoliosis treatment types of and the facial asymmetry types ($p=0.037$), dento-maxillary anomalies ($p=0.001$), Angle Classes ($p=0.011$) and types of anomaly ($p=0.008$ (tab. 30). We did not find statistically significant results between the other variables studied: scoliosis localization, right/left facial asymmetry, and head inclination to posture the right/left ($p>0.05$).

Discussions

In this study we evaluated clinically and photostatically a group of Romanian patients diagnosed with idiopathic scoliosis to establish the relationships between the features of scoliosis (location, convexity of the major curve, Cobb angle and treatment) and the features of facial asymmetry (right/left, overall/lower face, head deviation) and the presence/absence of dento-maxillary anomalies (Angle Class, crowding and/or sagittal, transverse or vertical inter-archers asymmetry), presenting some new aspects regarding the inclusion and analysis of these statistical variables.

Most of the studied patients were women diagnosed with idiopathic scoliosis at puberty, as it was the case with other studies (D'Attilio *et al.*, 2004; Kouwenhoven & Castelein, 2008; Saccucci *et al.*, 2011). Patients were assessed orthodontically by clinical and photostatic examinations as they were admitted and treated for their orthopedic condition. Kim *et al.* (2014) conducted their study on frontal and lateral cephalograms, because patients with scoliosis have attended the orthodontic clinic. In present study, all our patients had facial asymmetry (right/left and overall/lower face), as other studies have reported (Saccucci *et al.,* 2011; Zhou *et al.,* 2013). Also, all patients presented right/left mild/moderate head inclination. In scoliosis, the head changes its inclination in relation to the spine, so that it can compensate for the spine deviation and maintain the orthostatic position (Slota *et al.*, 2008). Scoliotic curves in frontal plane and lateral head inclination in posture contribute to the development of various dento-facial asymmetries (Huggare *et al.*, 1991; Pedrotti *et al.*, 2007; Lippold *et al.*, 2007). Body posture and cervical muscles are closely associated with the function of the head and mandible support system (Ito & Yamazaki, 1995).

In present study, not all patients presented dento-maxillary anomalies. Most common were Angle Classes I and III anomalies, dental crowding and occlusal asymmetries (sagittal molar and cuspid, mandibular lateral deviation, unilateral crossbite, canted occlusal plane, and infraocclusions). From our observations, these anomalies were present only in the orthopedically treated patients and not in the surgically treated ones. In literature, there are no recent studies about the relationship between scoliosis and facial asymmetry (Segatto *et al.*, 2008; Saccucci *et al.*, 2011), and our data cannot be compared. The previous studies have reported only the relationships between scoliosis and Angle Class II anomaly (Lippold *et al.*, 2003; Ben-Bassat *et al.*, 2006), cranio-cervical posture and anterior dental crowding (Solow & Sonnesen, 1998; Solow & Sandham, 2002), scoliosis and mandibular lateral deviation (Zhou *et al.*, 2013), scoliosis and crossbite (Zepa *et al.*, 2003; Korbmacher *et al.*, 2007) and scoliosis and midline deviation of the dental arches ((Dußler *et al.*, 2002).

In present study, head deviation, the presence/absence of dento-maxillary anomalies, Angle Classes and types of anomalies were different depending on the severity of scoliosis. Also, the types of facial asymmetry correlated with the direction of convexity of the major spinal curve. Differences were also found between the types of facial asymmetries, presence/absence of dento-maxillary anomalies, Angle Classes and types of anomalies depending on the treatment of scoliosis. All these correlations are original results that are not found in previous literature studies.

The spinal column provides the main support for all segments of human body posture (head, mandible, shoulders, trunk, hips, legs and feet) (Amat, 2008 Gault, 2008). When the spinal column is misaligned, whole-body posture is influenced, and all body segments become misaligned as they are taking up unequal muscle loads. After surgical correction of spinal deviation, the adaptation of subjacent muscles is slow, with the persistence of functional asymmetries through neuromuscular mechanisms, thus explaining the presence of facial asymmetry and head inclination in posture even after the treatment of scoliosis.

This research may improve the study of young patients with idiopathic scoliosis, because unlike previous studies it identified a relationship with facial asymmetry and the presence/absence of dento-maxillary anomalies. Similar future studies may also find other ways to investigate these patients, so that our data is comparable.

Conclusions

In this study, all orthopedic patients presented facial asymmetry (right/left and overall/lower face) and lateral head deviation in posture (right/left and mild/moderate) and only those who received orthopedic treatments (physiotherapy and braces) presented dento-maxillary anomalies (more commonly Angle Classes I and III, dental crowding and inter-arches asymmetry). The value of Cobb angle correlated statistically with head deviation in posture, presence/absence of dento-maxillary anomalies, Angle Classes of diagnostic and types of anomalies; the direction of convexity of the major spinal curve was statistically correlated with the types of facial asymmetry; the orthopedic/surgical treatment of scoliosis correlated statistically with the types of facial asymmetry, presence/absence of dento-maxillary anomalies, Angle Classes and types of anomaly. These aspects suggest that the interdisciplinary concepts of young people diagnosed with idiopathic scoliosis should be extended, both for the complete and complex skeletal treatment, as well as for the neuromuscular rehabilitation body segment posture.

CHAPTER 5
Clinical and biological researches of the biocompatibility of the components of orthodontic appliances

Orthodontic appliances are temporarily applied in the oral cavity of patients, in order to correct malocclusions and dental malpositions. Generally, removable appliances consist of an acrylic baseplate and metallic elements of stainless steel (SS) alloy (screw, clasps and springs). Metallic brackets, bands, arches, springs and ligature wires of fixed appliances are made of SS alloy, and some archwires of Nickel-Titanium (NiTi) alloy (Thompson, 2000; Chaturvedi & Upadhayay, 2010). These active metallic elements are attached to brackets by elastic ligatures.

In the last decades, due to the high development of the biomaterials, their appropriate selection is a big challenge. Biomaterials used in orthodontics must be inert, enabling only the desired interactions, without changes in the original properties of the material used and in the environment of introduction (Brantley & Eliades, 2001).

During the orthodontic treatment, these biomaterials are in permanent contact with the oral fluids and subjected to the microorganism's action and to the effect of the complex factors of the oral cavity. Also, these biomaterials are subjected to the action of oral microbiosis and microorganism in the bacterial plaque and the entire arsenal of complex and ever changing conditions of the oral cavity. The complex actions of these factors can lead to changes in the proprieties of biomaterials, which in turn can be the cause of adverse reactions in the structures of the oral cavity.

The adverse reaction of the body as a result of contact with sub-toxic doses of some foreign substances is known as hypersensitivity. Hypersensitivities reactions are produced by three different mechanisms: allergy, intolerance and hyper-reactivity. Intolerance reactions are often the result of ingestion of substances or metabolic errors. Hyper-reactivity responses are due to irritation substances. Allergy is an acquired condition and is an exaggerated reaction as a result of contact with a foreign substance. Some oral clinical manifestations occur in orthodontic patients, such as gingival hyperplasia, labial desquamation, angular cheilitis, multiform erythema, and periodontitis. These manifestations might be associated with an inflammatory reaction inducted by corrosion of metal elements orthodontic appliances and subsequent release of nickel ions (Leite & Bell, 2004; Genelhu et al., 2005; Volkman et al., 2007; Cobourne et al., 2015).

The potentially harmful substances used in orthodontics are metals salts derived from metal devices, monomers, cross agents and chemical substances associated to polymerization, the latex from gloves and mixtures of substances (Eliades, 2007). Metallic allergy is demonstrated in patients and medical staff (Kanerva et al., 1992; Lindsten & Kurol, 1997). The most frequent metallic allergies are to nickel, chromium and cobalt. Nickel is the most frequent metal accused of causing contact allergies in orthodontics. The nickels present in alloys, such as SS or NiTi alloys are largely employed in orthodontics. The components appearing as acrylic resins contain a series of chemically-active elements. (Marcusson et al., 1999). Extra-oral orthodontic devices may be made of materials containing dyers or other chemical substances, and may cause allergies on contact with face and neck skin.

Numerous researchers have studied corrosion of the metallic elements of the orthodontic devices, which is responsible for the production of allergic reactions, as well as a decrease in the properties of the orthodontic devices (Kerosuo et al., 1996; Patrascu, 2002; Eliades et al. 2004; Lin et al., 2006; Rakosi et al., 2010). By acting simultaneously, the enzymatic, microbiological, ionic and thermal properties of the oral environment can cause metal biodegradation. Other authors have studied corrosion through the analysis of nickel content in saliva and bacterial plaque of patients carrying orthodontic appliances (Ilken et al., 2000; Günseli et al., 2001; Fors et al., 2006).

Human saliva is a clear, slightly acidic mucoserous exocrine secretion, consisting of 99% water and 1% dry residue composed of inorganic (chlorides, bicarbonates, phosphates of sodium, potassium and calcium) and organic substances (enzymes, proteins and immunoglobulins). The salivary function has to maintain oral health and has to create an appropriate ecological balance

(Humphrey & Williamson, 2001; Edgar *et al*., 2004; Murthykumar, 2014). The authors, who have studied the NiTi alloy cytotoxicity, have not yet reached a final and clear conclusion (Mikulewicz & Chojnacka, 2010; Menezes & Quintão, 2010; Sugisawa *et al*., 2018).

The cytotoxicity tests have the capacity to detect the ability of a material test to induce sub-lethal or lethal effects at cellular level. Latex has an allergenic component that can cause common allergic reactions (Santos *et al*., 2009). The orthodontic material companies have different technological approaches (e.g., injection molding and die stamping for processing the elastomeric modules), in order to obtain the polyurethane products. The biological answers to these biomaterials can be toxic, inflammatory, allergic or mutagenic reactions, and the cytotoxicity is one of the fundamental parameter, in order to assess the biologic response (Pithon *et al*., 2009).

A permanent concern of my research was the analysis of the tissue effects caused by the materials used in orthodontics and their interactions with the oral environment. In order to evaluate these biomaterial-induced reactions, it was necessary to use advanced techniques (atomic microscopy, X-ray energy dispersion spectroscopy, cytotoxicity tests) and the setting up of multidisciplinary teams.

5.1. The prevalence of allergic reactions to orthodontic appliances and their correlations

Nowadays, no consensus has been reached on the fact that the allergic reactions would be caused by the substances entering the composition of orthodontic devices, or upon the release level of the ions with harmful potential in the oral cavity. Some authors have studied nickel, chromium and cobalt (Eliades, 1997; 2007; Saglam *et al*., 2004; Kolokitha & Chatzistavrou, 2008; Tarvade & Ramkrishna *et al*., 2015), others stainless steel (Schierano *et al*., 2000), and others polymethylmethacrylate (Kanerva *et al*., 1992; Munksgaard, 1992) as possible allergens.

The purpose of the study was to assess the side effects on intra-oral and extra-oral tissues produced by the insertion of orthodontic appliances, to determine their prevalence and to compare them with socio-demographic parameters, dento-maxillary anomaly, orthodontic appliance type and oral hygiene of patients.

> Sodor A, Dorobat V, **Zegan G**. Orthodontic devices *vs*. allergic reactions in children and young adults. *International Journal of Medical Dentistry (Journal of Romanian Medical Dentistry),* Jul-Sep 2012; 2(3): 195-201.

Materials and method

This cross-sectional analytical observational study was performed on 621 orthodontic patients, 255 (41.1%) boys and 366 (58.9%) girls, with ages between 6-29 years (mean age: 17.69 ± 5.350 years), 352 (56.68%) patients from the urban area and 269 (43.32%) patients from the rural area, from the North-eastern region of Romania. All patients were treated in the Orthodontics Clinic of the Ambulatory of the „St. Spiridon" Universitary Emergency Hospital of Iasi, Romania, during 1990-2010. Patients with gingival inflammations, periodontal diseases, previous nickel allergy history and extra- and intra-oral allergic reactions to the metallic and acrylic components of the orthodontic devices where included in the study. The informed consent was obtained from all patients or from their parents.

Studied parameters. The data collected from orthodontic records was evaluated according to socio-demographic parameters (gender, age and environment area), Angle Classes of malocclusions, orthodontic appliances types, dental hygiene before and during treatment by Quigley and Hein colorimetric method, types of allergic reactions (gingivitis, bleeding, bone resorption and oral aphtous ulcerations) and types of allergens (metallic, acrylic, polymeric).

The statistical analysis was performed using SPSS 17.0 software (Chicago, Illinois, SUA) for Windows. The data was characterized by descriptive statistics and Chi-square Pearson test and Fisher test ($F_{calculated} > F_{Fisher}$) and linear regression for statistical correlations between variable were used.

Results

Sample characteristics. The Angle Classes of malocclusions: 260 (41.9%) patients with Angle Class I; 240 (38.6%) patients with Angle Class II; and 121 (19.5%) patients with Angle Class III. The types of orthodontic appliances: 201 (32.4%) patients with removable appliances and 420 (67.6%) patients with fixed appliances. Oral hygiene before orthodontic treatment: 187 (30.1%) patients with 0 score; 381 (61.4%) patients with 1 score; 46 (7.4%) patients with 2 score; and 7 (1.1%) patients with 3 score. Oral hygiene during orthodontic treatment: 155 (25.0%) patients with 0 score; 308 (49.6%) patients with 1 score; 120 (19.3%) patients with 2 score; and 38 (6.1%) patients with 3 score.

The prevalence of patients' adverse reactions following application of orthodontic devices: 147 (23.7%) patients with localized gingivitis; 32 (5.2%) patients with generalized gingivitis; 173 (27.9%) patients with gingival bleeding; 35 (5.6%) patients with gingival hyperplasia; 43 (6.9%) patients with oral aphtous ulcerations; 4 (0.6%) patients with bone resorption; 36 (5.8%) patients with history previous nickel allergy; 19 (3.1%) patients with intra-oral allergic reactions to metallic elements; 6 (1.0%) patients with extra-oral allergic reactions to metallic elements; 19 (3.1%) patients with allergic reactions to acrylic resins; and 16 (2.6%) patients with allergic reactions to other polymeric biomaterials.

The statistical correlations show significant differences between localized/ generalized gingivitis/ gingival bleeding and oral hygiene during orthodontic treatment (p=0.000), gingival hyperplasia and oral hygiene before/ during orthodontic treatment (p=0.000), bone resorption and genders (p=0.000), oral aphtous ulcerations and oral hygiene before/ during orthodontic treatment (p<0.05), history previous nickel allergy and age, orthodontic appliance and oral hygiene before/ during orthodontic treatment (p<0.05), intra-oral allergic reactions to metallic elements and oral hygiene before to orthodontic treatment (p=0.001), (p=0.001), allergic reactions to acrylic resins and age, environment area of patients and orthodontic appliance (p=0.000), allergic reactions to other polymeric biomaterials and orthodontic appliance (p=0.005).

Using the Fisher test, a positive linear regression slope was found for the statistically significant correlation between: the type of orthodontic appliance and history previous nickel allergy (β=0.0147 and t=0.013) and the allergic reactions to acrylic resins (β=0.213 and t=0.000); the oral hygiene of the patient before to orthodontic treatment and localized gingivitis (β=0.153 and t=0.000), gingival bleedings (β=0.183 and t=0.000), history previous nickel allergy (β=0.160 and t=0.000) and the allergic intra-oral reactions to the metallic elements from the orthodontic devices (β=0.131 and t=0.002).

A negative linear regression slope was found for the statistically significant correlation between: the gender and localized gingivitis (β=-0.073 and t=0.018), gingival bleeding (β=-0.066 and t=0.009) and oral aphtous ulcerations (β=-0.137 and t=0.001); age and the history previous nickel allergy (β=-0.313 and t=0.000); Angle Classes of malocclusion and localized gingivitis (β=-0.075 and t=0.016) and gingival bleeding (β=-0.057 and t=0.024); type of orthodontic device applied and the allergic reactions to polymeric biomaterials (β=-0.030 and t=0.034); the oral hygiene of the patient before to orthodontic treatment and the gingival hyperplasia (β=-0.179 and t=0.000) and bone resorption (β=-0.131 and t=0.001); the oral hygiene of the patient during the treatment and localized (β=-0.654 and t=0.000) and generalized gingivitis (β=-0.394 and t=0.000), gingival bleeding (β=-0.798 and t=0.000) and gingival hyperplasia (β=-0.242 and t=0.000).

Discussions

In this study the prevalence of adverse effects of the orthodontic appliances on intra-oral and extra-oral tissues was determined and the correlations with socio-demographic parameters, Angle Classes of dento-maxillary anomalies, types of orthodontic appliances and oral hygiene before and during orthodontic treatment were established.

The gingival bleeding and localized gingivitis were most frequent in the studied patients, the significant differences depending on dental hygiene status and correlatively related with age, gender and dental hygiene, and Angle Classes of dento-maxillary anomalies. The oral hygiene status of

patients before the orthodontic treatment is a decision-making factor for the application of orthodontic appliances. The insertion of the orthodontic appliance into the oral cavity determines increase of the retentive areas, hardly accessible to dental brushing, with decrease of the corresponding condition of oral hygiene. The greatest addressability of the patients was for 8 years and respectively, 14 years (puberty age). At the age of 8 years, the first signs of dental crowding appear due to the lack of space on dental arches, and are indicated the removable appliances, made of acrylic resins. At the age of 14, mainly in girls, aesthetic concerns are being manifested, and fixed appliances are indicated, made of archwires with shape-memory made from NiTi alloys. The statistical correlations showed a relevant connection between the gingival signs and the Angle Class III of malocclusion, where the anterior/ total crossbite exposes the periodontium to direct traumatisms during mastication.

A tentative evaluation of reactions' prevalence on orthodontic appliances showed that 1:100 of the patients develop allergic reactions, which can be irritating or they can develop hypersensitive responses, and the percent ratio by real allergies cannot be estimated (Greppi *et al*., 1989; Pantuzo *et al*., 2007). The correlation between the quality of oral hygiene and the manifestation of periodontal phenomena is largely discussed in the literature of the field (Newman *et al*., 2009). This serious decrease in the quality of oral hygiene during the orthodontic treatment is mentioned in other studies Caterjee & Kleinberg, 1979; Bishara *et al*., 1995).

In the present study, the prevalence of nickel allergy, known in antecedents was low compared to literature data (Bass *et al*., 1993; Chakravarthi *et al*., 2012; Golz *et al*., 2015), significantly different depending on age, dental hygiene and orthodontic appliances, but also correlatively related to these variables. This is justified by the fact that allergic phenomena occur after the contact with the allergen, entering the composition of clothes or other personal products with high nickel content, usually at older ages and allergy tests are requested especially after the age of 15 years.

Investigated patients in this study had very low prevalence of allergic reactions to metallic elements, acrylic resins and polymeric biomaterials from the composition of the orthodontic appliances, being in agreement with the previous literature data (Park & Shearer, 1983; Hensten-Pettersen *et al*., 1984; Schubert *et al*., 1987; Staerkjaer & Menne, 1990; Nielsen & Menne, 1993; Laffler & Effendy, 1999). The results of the present study showed a relevant relationship of these reactions with the types of appliances applied to patients.

The orthodontic appliances may cause allergic reactions to heavy metals or to polymers, while their effects should not be minimalized. This study provided conclusive information on the frequency of the symptoms characteristic to allergic reactions and their possible causal connections with the orthodontic appliances.

Conclusions

In this study, the prevalence of the localized gingivitis and gingival bleeding was increased, but reactions to possible allergens in the components orthodontic appliances had a very low prevalence. Thus, the question arises whether these gingival signs that occurred during the orthodontic treatment had no other causes. Future studies would be useful to solve the issue of the biocompatibility of orthodontic appliances.

5.2. Biodegradation of NiTi archwires with/without physiognomic coating in the oral environment

NiTi archwires gained popularity due to their elasticity of 20% higher than SS alloys and the biocompatible nature. However, due to the level of nickel (Ni) over 50% which, once released in the oral cavity can cause local and general side effects (Hensten-Pettersen, 1989; Greppi *et al*., 1989; Chaturvedi & Upadhayay, 2010), but also a decrease in mechanical properties as a result of the corrosion processes (Kim & Johnson, 1999; Iijima *et al*., 2001; Cai *et al*., 2010). To decrease the negative effects and to meet patients' aesthetic needs, the manufacturing companies were covered of the NiTi archwires with Teflon based materials, composite resins, hydrogenated carbon

or zirconium dioxide, which have restricted corrosion, confined the release of Ni by 80% (Husmann *et al.*, 2002; Ohgoe *et al.*, 2007; Elayyan *et al.*, 2008).

This study aimed to assess the morphology and elemental composition of the surface NiTi alloy orthodontic archwires with/without physiognomic coating, before and after 2-3 months of exposure oral cavity.

Material and methods

In this experiment two orthodontic archwires types, NiTi (DENTSPLY GAC International) and Titanol Cosmetic archwires (FORESTADENT® USA Inc.) were assessed, with cross-section dimensions of 0.014 inch and 0.016 x 0.022 inch, before and after they retrieved from oral cavity (after 2-3 months) of the patients with fixed treatment (Straight Wire technique, brackets of 0.022 inch slot size and elastic ligations), and very good oral hygiene, using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX). The retrieved samples were washed in distilled water and immersed in alcohol in an ultrasonic bath for 5 minutes at 46 KHz and then the specimens were dried.

Results

SEM analysis of the NiTi archwires morphology before insertion presented surface threads and internal blisters, resulted from the wiredrawing process. The surface flaws were scratches and micrometric sinkholes, sufficiently numerous to facilitate organic deposits (fig. 18a). Surfaces of the retrieved NiTi archwires after intra-oral exposure displayed retention of organic matter in the oral cavity, as regional accumulations of amorphous material (fig. 18b).
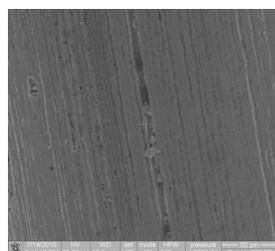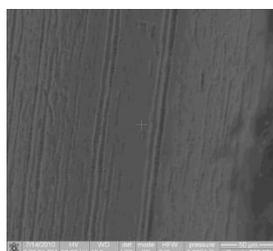
| a) before insertion archwire | b) after retrieved archwire | a) before insertion archwire | b) after retrieved archwire |

Fig. 18. SEM analyses of surface of NiTi archwire   **Fig. 19. SEM analyses of surface of Titanol Cosmetic archwire**
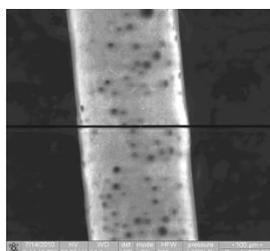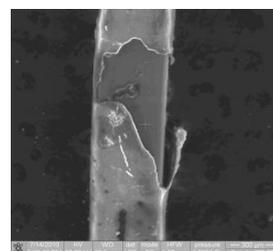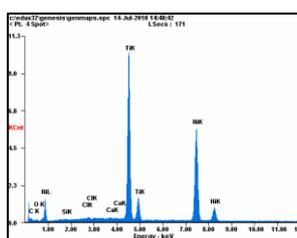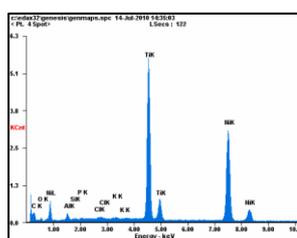
| a) before insertion archwire | b) after retrieved archwire | a) before insertion archwire | b) after retrieved archwire |

Fig. 20. EDX analyses of surface of NiTi archwire   **Fig. 21. EDX analyses of surface of Titanol Cosmetic archwire**
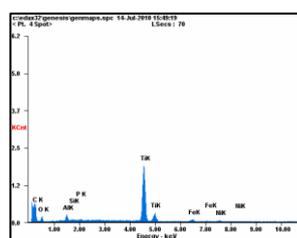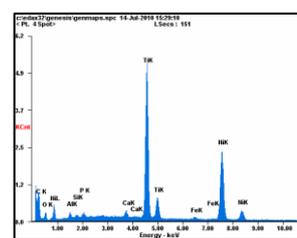
Titanol Cosmetic archwires before insertion presented the inhomogeneous protective coating that creates internal tensions between the layer and substrate, which is favorable for the exfoliation

process (fig. 19a). On the retrieved coated wires after intra-oral exposure there were observed two alternative protecting coating areas, depending on the archwire's wear. The coated areas that were not in contact with brackets did not suffer the exfoliation process. There were found areas with exfoliated coating on the oral side of the archwire that was in contact with slot brackets, that were destroyed due to it's friction with the brackets during dental movement, therefore losing it's initial properties (fig. 19b).

EDX analysis of a NiTi archwires before using revealed Ni and Ti as specific chemical elements in alloys with shape memory and uniform distribution throughout the analyzed surface, ensuring the same physical properties of the archwire at any point on its surface. Also, other chemicals were observed that are nonspecific like carbon (C), oxygen (O), silicon (Si), chlorine (Cl) and calcium (Ca), in smaller percentages (fig. 20a). On the retrieved NiTi archwires surface there were observed additional other chemicals like aluminium (Al), phosphorus (P) and potassium (K), confirming the presence of organic adhesions after intra-oral exposure (fig. 20b).

Titanol Cosmetic archwires before insertion presented the composition of chemical elements in substrate and the protective layer (fig. 21a). On the exfoliated area of retrieved coated archwires after intra-oral exposure there were found higher peaks of Ti and Ni in substrate and Ca, an additional element (fig. 21b).

There are changes of the elemental composition of the surface after intra-oral exposure of archwires. On NiTi archwires a decrease of Ni and Ti were observed, and on the Titanol Cosmetic archwires a decrease of Ti and an increase of Ni (tab. 31).

Table 31. Comparative outcomes of EDX analysis

Elements	NiTi archwire				Titanol Cosmetic archwire			
	before using		after using		before using		after using	
	% wt	% at	% wt	% at	% wt	% at	% wt	% at
Ni	53.27	41.02	44.38	24.81	01.29	00.39	28.28	11.09
Ti	40.32	38.05	33.49	22.95	30.24	11.10	27.40	13.17
C	04.04	15.20	14.85	40.59	45.50	66.61	31.82	61.00
O	01.71	04.82	03.65	07.49	17.05	18.74	08.01	11.53
Al	-	-	02.38	02.89	02.87	01.87	01.58	01.35
Si	00.28	00.45	00.29	00.34	00.67	00.42	00.66	00.54
P	-	-	00.39	00.42	00.51	00.29	00.78	00.58
Cl	00.16	00.20	00.31	00.29	-	-	-	-
Ca	00.23	00.26	-	-	-	-	00.82	00.47
K	-	-	00.25	00.21	-	-	-	-
Fe	-	-	-	-	01.87	00.59	00.65	00.27

Discussions

The complex analysis of the surface's archwires made from coated physiognomic NiTi alloy, compared with the uncoated ones represents a particular interest of the performance of *in vivo* dental biomaterials investigated in this study. Literature provides information about properties of orthodontic polymers (Eliades *et al*., 2000; 2004; Rejman, *et al*., 2008), of brackets (Zinelis *et al*., 2004; Gioka *et al*., 2004), of stainless steel archwires and NiTi uncoated alloys (Eliades *et al*., 2000; 2004) and the polymeric elastics (Eliades *et al*., 1999).

In this experiment patients with good oral hygiene were selected, to avoid introducing additional variables, such as plaque bacteria effect on the archwires. The desired effects were focused on the action of oral fluids and friction of the archwires with brackets during the orthodontic treatment.

To avoid measuring errors, the samples were prepared before the examination because the investigation methods used were sensitive to surface contamination. We used a combination of SEM and EDX, to have a full picture of the surface's state and the chemical composition of the analyzed archwires. The convergence of these methods and short working distance allows

sectioning "slice-and-view" with precision and a high resolution chemical analysis, which is an enhancement technique used in similar studies (Eliades *et al.*, 1999; 2000).

Following the analysis SEM of NiTi archwires we noticed flaws on the material's surface due to the manufacturing technology, which favoured bacteria plaque accumulation and calculus deposits, with undesirable effects on the periodontal structures. These irregularities of calculus deposits can cause limitations of the archwire's action, by affecting its physical properties (elasticity, memory capacity and mechanical strength). Eliades *et al.* (1999) reported surface composition alteration of NiTi archwires after intra-oral exposure for 1–6 months due to the occurrence of amorphous precipitates and microcrystalline particles in proteinaceous biofilm.

Materials used as coating for the orthodontic archwires must meet certain qualities: to be biocompatible, to provide pleasant aesthetics to have translucence similar to the aesthetic brackets, to be easily applied, to provide increased electrical resistance, higher toughness, lower friction and proper thermal conductivity (Mockers *et al.*, 2002; Piel *et al.*, 2011). Recent studies on the effect of slip resistance of physiognomic coated archwires have noticed superior surface properties and have reported the influence of friction on the effectiveness of teeth movement (Muguruma *et al.*, 2011; Doshi & Bhad-Patil, 2011).

We have observed some discontinuities of the protective layer in Titanol Cosmetic archwire before using, which cause the appearance of intern tensions and exfoliation of this archwire after using in the oral friction with the brackets zones. The layer's discontinuities can affect the properties and mechanic efficiency of the archwire, because of the growth in friction and uniformity of the information transferred to the brackets that can cause unwanted and uncontrolled dental movements. The accumulation of the layer's material from the tubes and the brackets' slots can limit or cancel the dental movement. The phenomena of friction between the metallic surface of the exfoliated archwire and the bracket's slots of composite material can cause shape modifications or the fracture of the brackets. The quality and difference of elasticity between the protective layer and substrate, high variations of temperature or acidity in the oral cavity and the undue tooth brushing can also cause the exfoliation of the protective layer, which can represent future research topics.

From the EDX analysis, we observed three categories of significant changes in the chemical composition of the studied archwire's surface. An ionic depletion, with the release of Ni and Ti ions in the oral cavity is followed by processes of corrosion in the oral sphere. The corrosion phenomenon was more striking at NiTi archwires. The appearance of other chemical elements in the initial composition of the surface was due to the organically adherences and dental calculus. The change in the percentages of the other chemical components was due to the ionical changes with saliva. For the retrieved NiTi archwires, the percentages have increased, but decreased for the retrieved Titanol Cosmetic archwires, with the exception of P, which are high.

The aesthetic coating protects the subjacent archwire against corrosion processes, but the archwire's corrosion is possible after a prolonged utilization in the oral cavity, due to the complex action of the oral fluids (Neumann *et al.*, 2002). Cai *et al.* (2010) studied the resistance to corrosion and to fracture of different types of archwires with protective coatings compared to conventional archwires and found three categories: archwires without modifications, archwires with crystallographic changes and archwires with distanced Teflon coating. We can assume that the processes of corrosion have negative consequences over biocomparability, aesthetics and frictional behavior at the brackets level and guidance of the dental archwire over the duration of the orthodontic treatments (Neumann *et al.*, 2002).

Conclusions

In this experiment, the NiTi archwires showed defects of fabrication on the surface, where calculus deposits appeared after being intra-orally exposed. The protective layer of the physiognomic archwires showed imperfections of depositing, and the exfoliation have taken place in the mechanical strain of the archwires, during the orthodontic treatment. The composition of the chemical elements of the orthodontic archwires' surface changed in the oral fluid. The microstructural and chemical changes in the described surface showed that the retrieved orthodontic

archwires alter during the treatment, with effects over the mechanical dental movements and the biocomparability in the oral sphere. The following research is necessary to solve the described implications of the exfoliation of the protective layer in the friction with the brackets zones, the adherence of organic materials, corrosion processes and ionical changes with oral fluids

5.3. Release of metallic ions in the oral environment

Previous studies have shown that, in saliva, the SS and NiTi alloys become corrosive (Lee *et al*., 2009; Varma *et al*., 2013; Sugisawa *et al*., 2018), reducing their biocompatibility and releasing metal ions into it (Menezes & Quintão, 2010; Mikulewicz & Chojnacka, 2011; Amini *et al*., 2012; Nayak *et al*., 2015). Other authors investigated the allergic, carcinogenic, mutagenic and cytotoxic effects of Ni, but the results obtained were controversial (Natarajan *et al*., 2011; Hafez *et al*., 2012; Cunha *et al*., 2018; Buczko *et al*., 2018).

This study aimed to assess the evaluation of crystallized unstimulated human saliva of subjects with/without orthodontic appliances, in order to identify the morphological aspect and surface elemental chemical composition of salivary crystals, by the SEM-EDS method.

> **Zegan G,** Anistoroaei D, Sodor A, Dascalu CG. Structural changes of the human saliva chemical composition at wearers of orthodontic appliances. *Book Series: E-Health and Bioengineering Conference IEEE*, Jun 2017; 17066107: 229-232.
>
> **Zegan G**, Anistoroaei D, Dascalu CG, Cernei ER, Toma V. Morphological aspect and surface chemical analysis of salivary crystals in young patients with/without orthodontic appliances. *International Journal of Medical Dentistry (Journal of Romania Dentistry),* Jan-Mar 2019; 23(1): 57-63.

Material and methods

Saliva was collected from 19 subjects (11 males and 8 females), aged 11-26 years (mean age 20.64±5.316 years) from the North-eastern region of Romania. The subjects were divided into three groups: 5 (26.32%) subjects without orthodontic appliances, 8 (73.68%) subjects with orthodontic appliances and 6 (31.58%) subjects with previously applied orthodontic appliances. The selection criteria were: no health problems, no medication and no other substances administered orally and without piercings. The informed consent was obtained before salivary collection from all subjects. Unstimulated saliva was collected from each patient, on a 0.5 mm glass slide, previously degreased and decontaminated. Collecting was done in the morning, between 10-11 am, after teeth brushing. The salivary samples were dried in a controlled environment, under the dome, in order to avoid atmospheric deposits.

The morphological and chemical characteristics of crystals of salivary samples were assessed through SEM and EDX analyses. 4-6 salivary crystals taken over from different regions of each salivary sample were examined and 110 SEM photomicrographs were obtained for the groups of subjects. To avoid measurement errors caused by the presence of organic material in the analyzed saliva, measurements were carried out without taking into account the chemical element carbon.

The statistical analysis was performed in SPSS 20.0 (SPSS Inc., Chicago, IL) for Windows. Data was characterized by descriptive statistics, frequency distributions and contingency tables. To compare the numerical variables between the groups of subjects, we used the *t*-Student test for independent samples (as confirmed by the Kolmogorov-Smirnov fitting test). To estimate the confidence intervals (CI) 95% of the calculated values we used the bootstrapping procedure with 1000 bootstrap samples. The 5% level of significance and the *p*-value<0.05 was regarded as significant.

Results

The SEM photomicrographs showed salivary crystals with various shapes and sizes, including rectangular, polyhedral, ovoid, rhomboid and rod-like forms, separated or congested in the organic matter of dried saliva. According to their morphological characteristics, three main salivary crystals

types were considered for analysis: (a) type I, big size crystals with polyhedral regulated (rectangular, polyhedral) shape, single or in cluster arrangements (92.72% salivary crystals) (fig. 22 and 23); (b) type II, big and medium size crystals with irregular shape (irregular polyhedron, ovoid, rod-like forms), in cluster arrangements (2.72% salivary crystals) (fig. 24); (c) type III, isolated, medium and small size crystals with irregular shape (irregular polyhedron, ovoid, rod-like forms), characterized by luminescence phenomena when analyzed by scanning electron microscopy (4.54% salivary crystals) (fig. 25).

EDX analysis. The surface elemental chemical composition of the three identified morphological types of salivary crystals was analyzed. Increased average values were recorded for: (a) Cl (36.07% wt and 32.52% at) and K (35.36% wt and 29.08 at) of the salivary crystals type I; (b) O (19.25% wt and 31.12% at), Na (19.19% wt and 23.42% at) and Si (22.86% wt and 24.23% at) of the salivary crystals type II; (c) O (30.42% wt and 47.80% at), P (19.22% wt and 15.54% at) and K (32.20% wt and 20.88% at) of the salivary crystals type III.

The surface elemental chemical composition of salivary crystals on the groups of subjects was analyzed, statistically significant differences were founded between the mean values of Ni and Ca on the groups of subjects without orthodontic appliances and with previously applied orthodontic appliances ($p<0.05$) (tab. 32). Comparison between the mean values of chemical composition of salivary crystals showed no statistically significant differences ($p>0.05$).
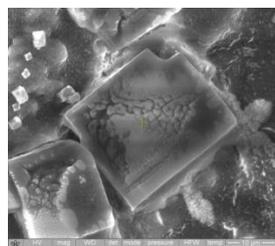
| Fig. 22. Rectangular single salivary crystals type I (5000x) | Fig. 23. Type I salivary crystals, polyhedral in cluster arrangements (5000x) | Fig. 24. Type II ovoid salivary crystals in cluster arrangements (5000x) | Fig. 25. Type III luminescence ovoid salivary crystals (5000x) |
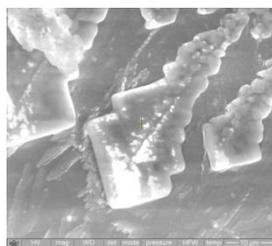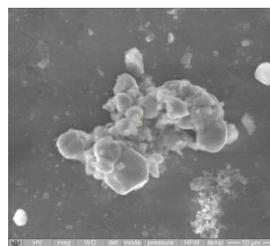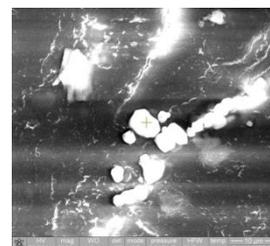
Table 32. The *t*-Student test for comparing the mean values of nickel and calcium of salivary crystals on groups of subjects with/without orthodontic appliances

Chemical elements		*t*-Student	*p* value	Mean difference	SE	95% CI	
						Lower	Upper
Ni	wt%	-2.363	0.021*	-0.61599	0.26062	-1.13649	-0.09548
	at%	-2.176	0.033*	-0.31591	0.14520	-0.60589	-0.02592
Ca	wt%	5.531	0.000**	1.26839	0.22933	0.80003	1.73675
	at%	5.448	0.000**	0.99226	0.18214	0.62029	1.36423
*statistically significant differences ($p<0.05$);							
**extreme statistically significant differences ($p<0.0001$).							

Discussions

The morphological aspect and surface elemental chemical composition of salivary crystals have been investigated by the SEM-EDS method, to appreciate the differences observed in the ionic composition of normal human saliva, for bringing up new data to the existing literature.

This study identified three morphological types of human salivary crystals, related to the surface elemental chemical composition. Type I of salivary crystal with regulated polyhedral aspect was formed of a complex chemical compound with a high percentage of K and Cl. Type II of salivary crystals with irregular ovoid aspect was formed of a complex chemical compound with a high percentage of Na, O and Si. Type III of salivary crystal with ovoid luminescent layout was

formed of a complex chemical compound with a high percentage of P, O and K. These aspects have not been presented in the literature.

Previous studies have shown that the cation K and the ion P are predominant in human saliva, compared to the Na cation. Na and Cl ion concentration increases with the salivary flow rate, while the cation K is independent on the salivary flow (Almståhl & Wikström, 2005; Picu, 2010).

Unlike other studies, the present investigation carried out surface elemental chemical analysis of salivary crystals for all chemical elements of the salivary components. Comparison of the mean values of salivary crystals' surface chemical elements on groups of subjects showed no statistical differences, with the exception of Ni and Ca.

Na, Mg, P, S, Cl and K presented in the salivary crystals derive from the ion content of human saliva. Ca comes from the ionic content of human saliva, its concentration depending on age, pH and salivary flow (Edgar *et al.*, 2003; Picu, 2010). Si may also come from dust particles in the atmosphere or from the glass composition of the samples used. Ni, Al and Zn do not derive from the ionic content of saliva. Ni was found in all groups of the studied subjects, regardless of the presence/absence of orthodontic appliances in the oral cavity. A statistically significant difference was found for the groups of subjects without present orthodontic appliances, which indicates that they were not the source of Ni ions.

Previous studies have investigated the release of metal ions in saliva of patients with orthodontic appliances at different time intervals, compared to a control group without orthodontic appliances (Petoumenou *et al.*, 2009; Sahoo *et al.*, 2011; Amini *et al.*, 2015; Khaneh Masjedi *et al.*, 2016; Lages *et al.*, 2017; Jurela *et al.*, 2018), but the results obtained were controversial. Most studies have reported an increased release of metal ions in saliva, caused by orthodontic appliances (Kuhta *et al.*, 2009; Milheiro *et al.*, 2012; Yassaei *et al.*, 2013; Soni *et al.*, 2018), due to the saliva corrosion phenomenon (Polychronis *et al.*, 2018; Malkiewicz *et al.*, 2019), fluoride mouthwash and toothpaste (Katic *et al.*, 2018; Yanisarapan *et al.*, 2018) or potentiated by the effect of radiofrequency electromagnetic fields from mobile phones (Mortazavi *et al.*, 2018). A group of authors have found a certain level of Ni in subjects with orthodontic appliances, caused by the ingested diet composition (Amini *et al.*, 2012).

This research should convince orthodontic patients that orthodontic appliances have no influence upon their health status. Similar future studies may find other ways to investigate these patients, to obtain comparable data with ours.

Conclusions

This research identified three morphological types of salivary crystals made of different percentages of complex chemical compounds. Some of the surface chemical elements of the salivary crystals were derived from the ionic composition of human saliva. Ni was not released from orthodontic appliances in the oral cavity. The source of the metallic elements Ni, Al and Zn from the surface of the salivary crystals must be investigated by further research of the environment.

5.4. The cytotoxic potential of latex-based orthodontic materials

The first latex allergy cases were reported at the early '80 in Europe (Everett & Hice, 1974). It's incidence is still unknown, although there were developed tests in order to assess it's frequency (Palosuo *et al.*, 2002). The orthodontic companies tried to minimize the allergen agents in latex products. The most common allergens in latex products are Hev b0.02 and Hev b5. Other two common allergens determined allergies on children with spina bifida, Hev b3 and Hev b1 (Tomazic *et al.*, 1992). The severity of the allergy varies from skin rash, oral lesions, and the most severe the anaphylactic reactions. The prevalence of severe reactions has increased in the last years (Hain *et al.*, 2007).

This experiment aimed to test the reaction of human fibroblasts cultures to different elastic chain and elastomeric ligatures from fixed orthodontic appliances components.

Material and methods

In this experiment we used human fibroblasts cultures (Normal Human Dermal Fibroblasts - NHDF), over which the following materials were placed: dental copper amalgam (Amalgam Alloys (Pty) Ltd), elastic chain (Orthoclassic) and elastomeric ligatures Orthoclassic with low and high elasticity and elastomeric ligatures GAC.

On three trays, with 24 wells each, were applied an equal number of cells: in the first wells of each tray only cells, on the second well dental copper amalgam fragments were added, on the other 22 wells there were applied the samples of chain and ligatures. The trays were incubated at 37°C and 5% CO_2. The morphological analysis of the cells situated in the proximity and at distance from the material and their density has been achieved with a Nikon phase-contrast microscope Eclipse TE300, at 3 and 6 days, by 4x, 10x and 20x magnification.

Results

The fibroblast population from the first well reveals a confluence of 70-80% at 3 days (fig. 26a), and the multiplication rate is increased by adding the suitable growth factors and confluence of 90-95% at 6 day (fig. 27a).

For *the dental copper amalgam* from the second well there are no vital cells observed at 3 days and not even 6 days.

For *the elastic chain* (Orthoclassic) normal fibroblasts are observed in the proximity and the inside eyelets of the elastic chain, with a normal multiplication rate, after 3 days (fig. 26b). Outside of the elastic chain, some cells are picnotic, and others with enlarge bogy (fig. 26c). At distance from the elastic chain, many cells appear with rounded cell body and reduced cell density (fig. 26d). Outside and proximity of the elastic chain, the number of cells with normal growth is smaller than the one of the inside chain. Under the elastic chain, picnosis and apoptosis of the cells are observed. At 6 days, the same aspects are observed. The cells density is superior to the one at 3 days, but lower then to the one of the first well (fig. 27c). The cell density from inside and at distance from the elastic chain is highly reduced (fig. 27b and d). Under the elastic chain there were no viable cells observed.
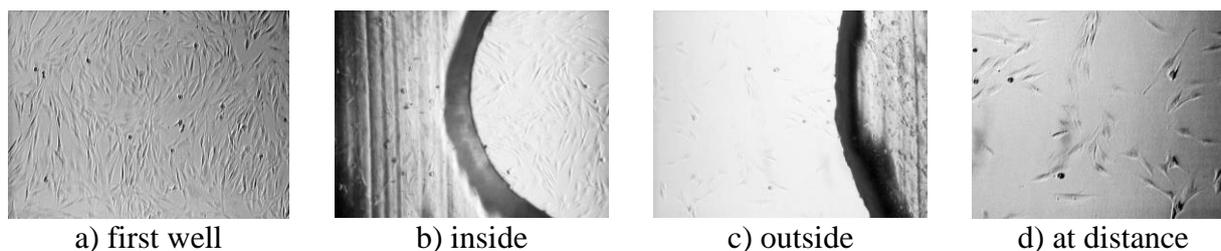
a) first well   b) inside   c) outside   d) at distance
Fig. 26. Fibroblasts in contact with the elastic chain at 3 days
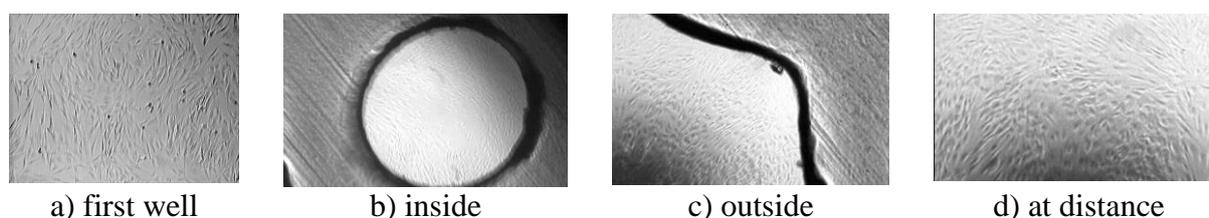
a) first well   b) inside   c) outside   d) at distance
Fig. 27. Fibroblasts in contact with the elastic chain at 6 days

For *the elastomeric ligatures Orthoclassic with low elasticity* normal fibroblasts were observed in the proximity and at a distance from the ligature, and the decreased cell density, after 3 days (fig. 28). At 6 days, the same aspects were observed, but the cell density is more reduced (fig. 29).
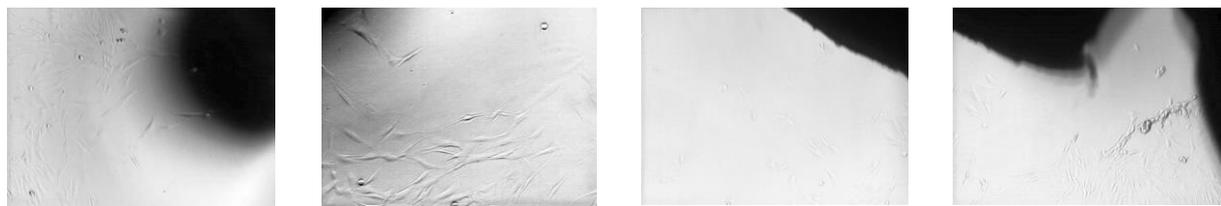
a) in proximity   b) at distance   c) cell density   d) to periphery

Fig. 28. Fibroblasts in contact with the elastomeric ligature Orthoclassic with low elasticity at 3 days
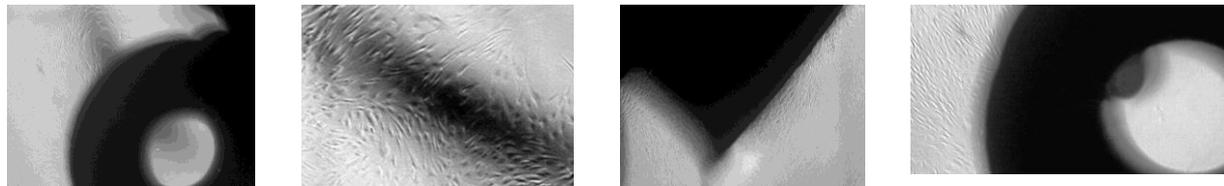
a) in proximity   b) at distance   c) cell density

Fig. 29. Fibroblasts in contact with the elastomeric ligature Orthoclassic with low elasticity at 6 days
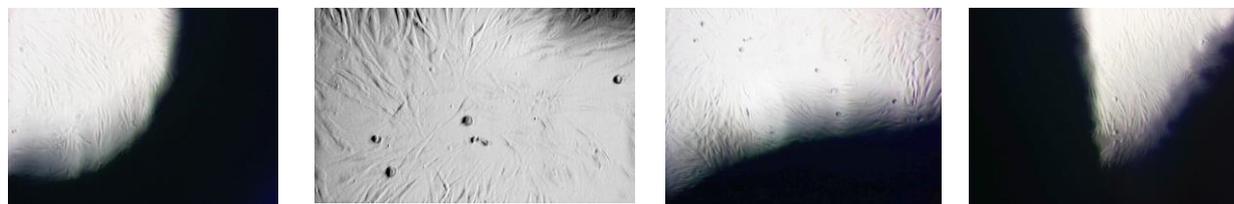
a) in proximity   b) at distance   c) cell density

Fig. 30. Fibroblasts in contact with the elastomeric ligatures Orthoclassic with high elasticity at 3 days
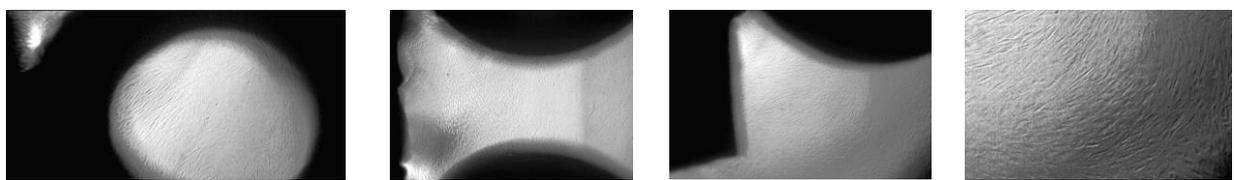
a) in proximity   b) at distance   c) cell density

Fig. 31. Fibroblasts in contact with the elastomeric ligatures Orthoclassic with high elasticity at 6 days
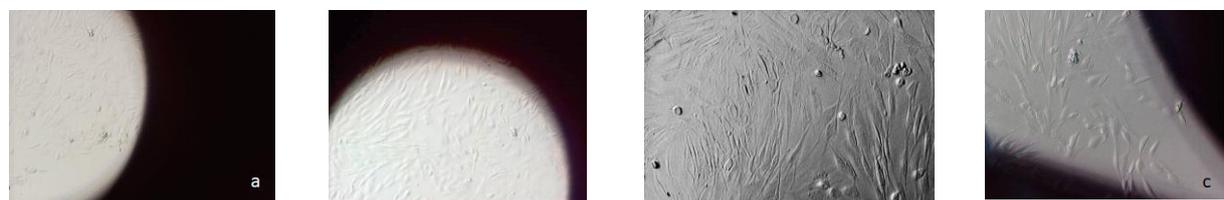
a) in proximity   b) inside   c) at distance   d) cell density

Fig. 32. Fibroblasts in contact with the elastomeric ligatures GAC at 3 days

a) in proximity   b) at distance   c) cell density

Fig. 33. Fibroblasts in contact with the elastomeric ligatures GAC at 6 days

For *the elastomeric ligatures Orthoclassic with high elasticity* normal fibroblasts are observed in the proximity and at distance of the ligature, after 3 days. The cell density is higher close to the ligature than at distance from it (fig. 30). At 6 days, the same aspects and cell densities are observed (fig. 31).

For *the elastomeric ligatures GAC* similar morphological aspects are observed and also cells densities to those obtained for the elastomeric ligatures Orthoclassic with high elasticity, after 3 days. The cell density is higher close to the ligature than at distance from it (fig. 32). At 6 days, normal fibroblasts are observed only at distance from the ligature. Cells disappeared completely around the ligature (fig. 33).

Discussions

In this experiment we assessed the cellular responses of human fibroblast cultures to dental copper amalgam, the elastic chain and the elastomeric ligatures from the fixed orthodontic appliances components. The estimation of viable cells was obtained by comparing the maximum cell density from the first well (control sample) with other wells. The periods at which the readings were determined were compared with the duration of use in the oral cavity.

In this study, the fragments of dental copper amalgam had a strong cytotoxic effect due to the presence of copper in the composition, as previous studies have shown Kaga, *et al*., 1991). The high degree of cytotoxicity of the elastic chain determined morphologic cells changes, which may be due to higher latex content and greater contact with the cells' surface. These aspects are more severe compared to those presented in literature (Santos *et al*., 2010; Holmes *et al*., 1993; Pithon *et al*., 2010).

In this study, the elastomeric ligature with low elasticity had a relative biocompatibility, and the elastomeric ligature with high elasticity had good compatibility, and the toxicity seems to be absent. The GAC elastomeric ligation showed moderate compatibility after 3 days and the toxicity seems to be reduced, but after 6 days this material was toxic to the cells.

Previous studies indicated that the main drawback of these latex-based biomaterials is that the toxic products can be ingested and thus to determine various diseases by the accumulation of toxic substances (Schmalz, 1994). There are no studies showing the connections between the cytotoxic and the allergic potential of latex, although the substance is being widely regarded as an allergen. The studies of Santos *et al*. (2008-2010) demonstrated the cytotoxic potential of latex. The data corresponds to those of present study.

Further researches are needed to assess the influence of colours used in the development of cytotoxicity. An *in vivo* study would have a greater complexity and a number of additional variables, like the presence of saliva and of the bacterial plaque, the effect of the oral pH, temperature and blood flow changes and also patient specific immunological reactions.

Conclusions

In this experiment, all analyzed materials determined cellular changes, demonstrating the cytotoxic potential of the four types of biomaterials on the latex. The elastic chain showed the most pronounced cytotoxic character. The elastomeric ligatures had a cytotoxic character dependent of their chemical composition. The study has practical significance by reducing the possibility of adverse reactions due to knowledge of the cytotoxic nature of the used materials.

CHAPTER 6
Experimental researches to improve the quality of orthodontic biomaterials and antibiotics used in oral cavity infections

The properties of orthodontic biomaterials can stimulate or counteract protein interactions and also intercellular or cell-substrate interactions (Towler *et al*., 2004). Some biomaterials require complex interactions with cell systems for the best biointegration, while other biomaterials must have minimal interactions with cellular/tissue systems.

The oral cavity is usually colonized by viruses, fungi and over 700 bacterial species of which some may be pathogenic, others are commensal or symbiotic (Aas *et al*., 2005). When ordinary flora of the oral cavity is destroyed, indigenous bacteria can transform into pathogenic ones, leading to oral structures diseases. Also, the insertion of orthodontic appliances can change the microflora of the oral cavity (Zheng *et al*., 2016). Oral cavity infections can be odontogenic (dental caries and periodontitis) and non-odontogenic (extra-oral and systemic) (Dewhirst *et al*., 2010; Levi & Eusterman, 2011; Marsh & Devine, 2011).

6.1. Researches for the optimizing quality of the metallic orthodontic devices

The original purpose of placing micro-implants was to achieve maximum control of orthodontic anchorage, a decisive factor in successful treatment (Park *et al*, 2005; Lee *et al*., 2007; Candido *et al*., 2013). Over time, orthodontists have tried multiple types of orthodontic anchorages, involving either permanent molars or accessory devices which have been poorly tolerated by patients (Angle, 1900; Gainsforth & Higley, 1945; Brånemark *et al*., 1969; Linkow, 1969; Sherman, 1978). Because of this, researchers have tried to find the so-called ideal anchor. With the evolution of biomaterials, surgical techniques and anesthetics, it was concluded that this ideal anchorage should be independent of the teeth and that it should be located strictly on the bone. Starting from these considerations, plates and orthodontic implants were imagined as anchor means (Linkow, 1970; Turley *et al*., 1980; 1988; Creekmore & Eklund, 1983; Roberts *et al*., 1989; Wehrbein *et al*., 1996; Kanomi. 1997; Block, 2000; Park *et al*., 2001; Sethi *et al*., 2003; Melsen, 2005; Lee *et al*., 2007). The plates provide an excellent anchorage and have a low loss rate, but instead they have the disadvantage of a more laborious technique, with the need for a flap, insertion and a more difficult removal and possible complications. Mini/micro-implants provide a good anchor, they can be easily inserted and removed, but they have an increased loss index. From this point onwards, the attention of the researchers has been directed towards the obtaining of biocompatible and biointegrate implants with the most easy handling as possible, a healing time as low as possible and a maximum capacity to resist under the action of orthodontic forces (Albrektsson & Zarb, 1993; Pioletti *et al*., 2003).

Micro-implants have numerous advantages: continuous power, simple mechanism, good results, the decrease of the treatment by 40%, decrease of extractions number, and patient's minimal cooperation. Failures and problems that may occur may be due to the implant (wrong choice, fracture, gingival infection), to the operator (excessive application of force, pushing the total bone screw, wrong implant angle, wide tunnel milling, septic technique, tissue damage or nerve root), to the patient (low bone density, thin mucus, systemic diseases, smoking excessive, mouth breathing, poor hygiene, diabetes, osteoporosis).

Implant loss may be due to its shape, insertion technique, patient hygiene and the quality of the implant surface. Therefore, the qualities of the implantation surface have been extensively studied and improved. Researches turned their attention to obtaining implantation surfaces that would allow the attachment of complex molecular species with appropriate functionality. It is fundamental that attachment situs and interactions at the implant surface do not interfere with the functional structure or active situs of the attached biomolecules (Tanabe *et al*., 2008). Implant surface microtropography is important in cellular response by a phenomenon called contact guidance, involving changes in cellular form, orientation and polarities of cellular functional behavior (Linkow, 1969). However, cell attachment is a complex phenomenon, not only being the

result of the surface roughness of the implant. Thus, modulation of osteogenesis at the bone-implant interface has been demonstrated that it is done by cytokines, systemic hormones and pharmacological manipulation (Dubova *et al*., 2006).

Another issue was the contamination of the impurities at the implantation surface level, and thus extremely elaborated clinical protocols have been developed. Also, chemical composition and various methods of treating implant surfaces are important. Thus, the most frequent chemical elements that contaminate the surfaces are hydrocarbons, organic/ inorganic nitrogen, and sulphur components, along with other elements such as phosphorus, silicon, sodium and chlorine. The short-term effect of low levels of impurities is not well-defined (Haddad *et al*., 2007; Zorn *et al*., 2007).

These aspects have been studied in multidisciplinary teams using advanced methods and techniques (SEM-EDX, X-ray diffraction, Fourier-transform infrared spectroscopy, transmission electron microscopy, electrochemical impedance spectroscopy, linear and cyclic polarization. polarization and differential scanning calorimetry), and through experiments we tried to improve the biomaterials most used in orthodontics, by dealing with NiTi and Iron alloys.

6.1.1. The influence of hydroxyapatite layer of Titanium-based micro-implant on osseo-integration

The deposition of hydroxyapatite thin layers in order to improve the biocompatibility of implants titanium (Ti)-based alloy represents a natural and proper solution for osseo-integration process (Sanden *et al*., 2002; Huang *et al*., 2004; Luo *et al*., 2013). The mechanical stability of those hydroxyapatite deposits on the dental implants is a problem that can be solved by various technical methods of depositing of the ceramic material, after preparing the metallic substrate (Chua *et al*., 2008).

The first experiment aimed was to obtain a thin hydroxyapatite layer on the Ti6Al4V implant substrate through electrophoretic process and to assess the morphological aspects, thickness, and chemical characteristics of deposited layer.

The second experiment aimed was to increase the effective surface area of the Ti6Al4V implant through sandblasting operation, calculus of the new effective area, deposition a thin hydroxyapatite layer by electrophoresis method and to analyze of the structural, chemical and mechanical properties of the Ti6Al4V-HA new material.

Paraschiv C, Stirbu I, Cimpoesu R, Bernevig M, Nejneru C, Manole V, Cimpoesu N, **Zegan G**. Preliminary results on hydroxyapatite growth on advanced Ti-base alloy using electrophoretic deposition process. *Optoelectronics and Advanced Materials-Rapid Communications,* Jan-Feb 2016; 10(1-2): 87-90.

Zegan G, Cimpoesu R, Agop M, Stirbu I, Chicet DL, Istrate B, Alexandru A, Anton Prisacariu B. Improving the HA deposition process on Ti-based advanced alloy through sandblasting. *Optoelectronics and Advanced Materials-Rapid Communications,* Mar-Apr 2016; 10(3-4): 279-284.

Material and methods

Ti-based alloys (Ti6Al4V) purchased from Zirom Giurgiu brand under bars form with 10 mm diameter and 20 mm length were used.

In the first experiment, for the deposition of the hydroxyapatite layers an electrophoretic equipment with a Consort EV 261 power supply, and an own deposition cell were used. Superficial hydroxyapatite layers were deposited using a solution with hydroxyapatite powders in isopropyl alcohol stabilized with a superficial agent type Tween 80. The thin layers obtained were analyzed using SEM and EDX analyses.

In the second experiment, samples were sandblasted using a Shot Blasting Cabinet model SB974 equipment and classical foundry sand at 100 psi (7 bar) air pressure for distance between the equipment gun and sample of 100 mm with 5, 10 and 15 seconds interaction time. The chemical

composition of classical foundry sand is 95% CO_2 and 5% $Al_2O_3Fe_2O_3$. For the deposition of hydroxyapatite layers, the same equipment and method was used as in the first experiment. The structural properties of Ti6Al4V-HA material were analyzed using SEM, EDX and X-ray Diffraction (XRD), and the mechanical properties with the micro-indenter equipment type CETR-UMT, Bruker.

Results

In the first experiment, the SEM analysis of the hydroxyapatite thin layer morphology deposited on Ti6Al4V implant show good homogeneity (fig. 34a), with an average thickness of 28 μm (fig. 34b) and with macro-cracks at the edge of the layer with 810 μm depths (fig. 34c). EDX analysis of the chemical composition determination show a Ca:P ratio with a mean value of 1.76 (fig. 35).
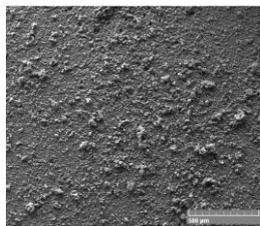
| a) general 2D image of the layer (100x) | b) 2D image on the edge of the sample (500x) | c) 3D image of deposited layer (500x) | **Fig. 35. EDX analysis of hydroxyapatite layer** |
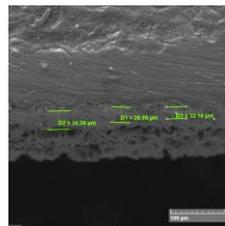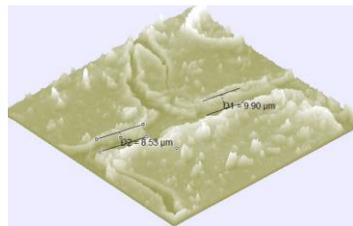
Fig. 34. SEM analyses of hydroxyapatite layer

In the second experiment, the SEM analysis of the Ti6-Al4-V alloy after sandblasting show numerously structural modifications of surface, with big and irregular depths (fig. 36) and chemical contaminants with sand particles, as the EDX analysis shows (fig. 37). Hydroxyapatite layers are homogeneous macro-structural, but with some micro-structural defects like pores (fig. 38). The XRD spectra identify the present of sand (SiO_2) and titanium oxide (TiO_2), and show the α and β phases of the characteristic peaks of the substrate (fig 29).
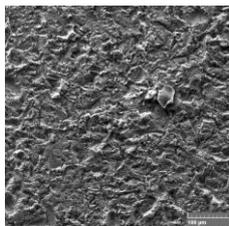
| a) 2D image of the surface | b) 3D image of the surface | **Fig. 37. Contaminated surface of Ti6Al4V with SiO_2 particles** | **Fig. 38. The hydroxyapatite layer deposited on sandblasted Ti6Al4V alloy for 15 seconds** |
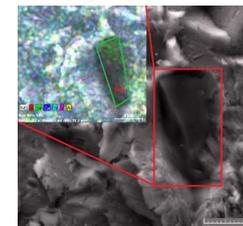
Fig. 36. SEM analyses of Ti6Al4V alloy after sandblasting for 15 seconds (500x)
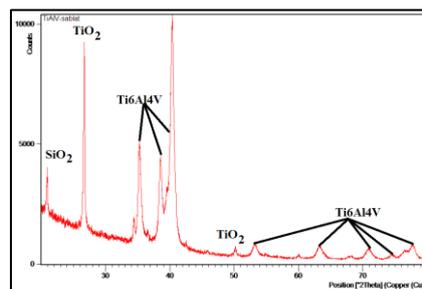
Fig. 39. XRD graph on sandblasted Ti6Al4V alloy

Discussions

The implant Ti6Al4V is common used in dental and orthopedic therapies, with a hydroxyapatite coating and represents a solution for good medical results. The microstructure of hydroxyapatite is one of the most frequently used bio-ceramics for bone and dental tissues reconstructions (Luo *et al.*, 2013).

In the first experiment, for the deposition of hydroxyapatite as thin layers on Ti6Al4V implant metallic substrate we used electrophoretic process techniques. The experimental results revealed that both compactness and porosity of the hydroxyapatite layer require further experimental investigations. In order to increase the osseo-integration process some implants need the porous state of the hydroxyapatite. The thin layer obtained through electrophoretic deposition process was chemically homogeneous and had mean value of Ca:P ratio appropriate of human hydroxyapatite.

Deposition through electrophoretic technique represents a method that uses the loaded particles movement in a suspension in the presence of an electric field, which allows the formation of well-established particles in thin layers (Van Tassel *et al.*, 2006). This deposition method evolves like an important instrument in nano-technology domain, like a technique that permits the industrial scale production of components and with nanometric scale dimensions, like particles, tubes or rods (Besra & Liu, 2007).

Nowadays, a Ti-based alloy used in dentistry as mini/micro-implant has a few months necessity to integrate at least partially with its adjacent bone. The retrieved Ti implants analysis of the patients showed that the contact ratio between bone and implant is not perfect, heaving a mean percentage of 60 and 80%, even for good successful implants that had used for up to 17 years (Miyakawa *et al.*, 1996), fact that shows the incomplete character of the osseo-integration process during a long time.

In the second experiment, the Ti-based alloys surface was prepared by sandblasting to deposit a thin layer of hydroxyapatite by electrophoresis. The experimental results showed irregularities of the Ti6Al4V surfaces, which allowed a better adhesion of the hydroxyapatite layer to the metallic substrate. Also, the indents created by sand have influenced the homogeneity of the thin layer of hydroxyapatite, which caused micro-cracks in the deposited layer, making it a more resistant material with a plasticity of the dislocation between the two layers, as evidenced from the mechanical tests. On the other hand, the chemical contamination of the metallic substrate had a negative influence toward titanium's resistance to corrosion and its biocompatibility. The β phase of the characteristic peaks of the substrate indicated line shift that suggested lattice distortion, especially after the sandblasting process, helpful for the mechanical stability of hydroxyapatite deposits.

The operation includes three specific purposes: the cleaning surface contaminants, the roughening surfaces to increase effective surface area, and the producing of beneficial surface compressive residual stress. As a result the modified surfaces exhibit bigger activation energy of the surface, conducting to higher surface chemical and physical activities and improving fatigue strength and life. For effective area estimation of a surface that increase by sandblasting with particle with 300 μm average than a tenth part (around 30 μm) will be indented in the surface (Ratoi *et al.*, 2015). Even if hydroxyapatite have a high resistance to dislocation at room temperatures, similar pop-ins in the force-depth curve has been observed in sapphire, aragonite, GaAs and ZnO (Kucheyev *et al.*, 2002; Kearney *et al.*, 2006; Wasmer *et al.*, 2007). The good bonding of couple Ti implant and the hydroxyapatite (HA/Ti bonding or bone/Ti implant) can be explained by a favorable result of blasting during sandblasting (a high number of small indentations on the metallic surface), which increase the effective surface area (Piattelli *et al.*, 1996).

These experiments are helping to improve the osseo-integration process with the adjacent tissues and the stability of the thin layer of hydroxyapatite deposited on the implant, which provides two technical solutions for obtaining some materials with new properties.

Conclusions

In the first experiment, the morphological and chemical homogeneity and the porosity of the thin layer of hydroxyapatite deposited on the substrate of the Ti6AI4V implant by electrophoretic technique indicate physicochemical properties that can improve the osseo-integration process of the implant with the adjacent tissues.

In the second experiment, creating irregularities on the substrate surface of the Ti6AI4V implant by sandblasting led to the formation of indentation in the hydroxyapatite layer, increasing its mechanical stability and dislocation resistance.

Future experimental and clinical tests would be useful for checking these qualities through other technologies to become industrial products marketed by dental materials firms.

6.1.2. The influence of titanium and zinc oxides and silver-doped hydroxyapatite of micro-implants on infections prevention

The implant longevity on dental arches depending on the structural and morphological surfaces characteristics, which will provided the osseo-integration in adjacent tissues, which will ensure osseo-integration into the adjacent tissues and will prevented the multiplication of bacteria (Mavrogenis *et al*., 2009; Migliorati *et al*., 2013; Oltramari-Navarro *et al*., 2013; Nienkemper *et al*., 2016). Nowadays, researchers are looking for solutions to improve specific properties of implants by coating their surfaces with particulate thin films (Moioli *et al*., 2007; Chua *et al*., 2008).

The first experiment aimed was to synthesize titanium oxide (TiO_2) and zinc oxide (ZnO) by the sol-gel method and to assess their structural, morphological and chemical characteristics.

The second experiment aimed was to obtain silver-doped hydroxyapatite thin films on a Ti dental micro-implant through ion beam sputter deposition and plasma based ion etching, and to assess of them physico-chemical properties.

Zegan G, Anistoroaei D, Golovcencu L, Cernei ER, Dascalu CG, Carausu EM. Physicochemical properties of advanced nanostructured materials for dental micro-implant coatings. *Revista de Chimie* (Bucharest)*,* Sep 2017; 68(9): 2052-2054.

Zegan G, Carausu EM, Golovcencu L, Botezatu-Sodor A, Cernei ER, Anistoroaei D. Nanoparticles deposition on mini-implants for osseo-integration and antibacterial properties improvement. *Revista de Chimie* (Bucharest)*,* Dec 2017; 68(12): 2929-2931.

Material and methods

In the first experiment, the ZnO and TiO_2 particles synthesis was carried out using sol-gel method (Ficai & Grumezescu, 2017).

For *ZnO synthesis* 4.3g $ZnSO_4.7H_2O$ and 1.2g diethylene glycol were used, mixed into a solution containing 10 ml pure ethanol and 300 ml double distilled water. The entire solution was vigorously stirred at 85°C for 2 hours, leading to gel formation. After this stage, the obtained gel was subjected to drying at 220°C for 2 hours. The obtained product was ground into fine particles and finally treated at 500°C for 5 hours.

For *TiO_2 synthesis* 20 ml $TiCl_4$ were used, added gradually in a 40 ml HCl solution of 37% concentration. The mixed solution was cooled in an ice bath at 5°C. The obtained solution was added to 600 mL double distilled water. To precipitate the amorphous TiO_2, the reaction mixture was treated with an aqueous solution of ammonia till pH value reached at 8. The suspension was subjected to ageing stage for 48 hours at room temperature then dried at 80°C for 24 hours. The final white powder was grounded for thermal treatment at 550°C.

The synthesized samples were analyzed about the structural aspects by XRD analysis and FTIR spectrometry, and the morphological aspects by SEM-EDX and Transmission Electron Microscopy (TEM).

In the second experiment, for the deposition of silver-doped hydroxyapatite thin films on Ti micro-implants a process was used representing a combination of ion beam sputter deposition and plasma based ion etching. The structural and morphological characteristics of silver-doped

hydroxyapatite thin films deposited on Ti substrate were assessed, using EDX, SEM and XRD analyses.

Results

In the first experiment, the XRD spectra of ZnO and TiO_2 shows the diffraction peaks at angles 2θ and their reflections, corresponding of the crystalline phase particles (fig. 40). The FTIR spectra shows a strong absorption peak at around 3500 cm^{-1} for stretching vibration belonging to OH group nonchemical bonded and at around 1600 cm^{-1} associated to HOH bending vibration (fig. 41). SEM-EDX analyses shows a high homogeneity, the absence of particle agglomeration and spherical shape of crystals, the size ranging from 5-20 nm to ZnO and 5-10 nm to TiO_2 (fig. 42). TEM analyses confirm the size and the uniformity of ZnO and TiO_2 particles (fig. 43).
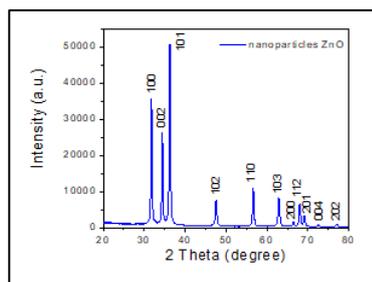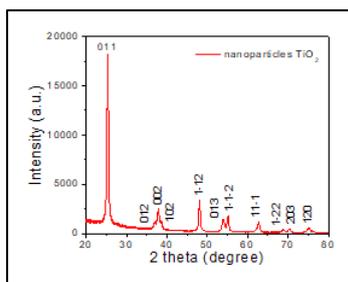
a) ZnO particles	b) TiO_2 particles

Fig. 40. XRD graphs of ZnO and TiO_2 particles
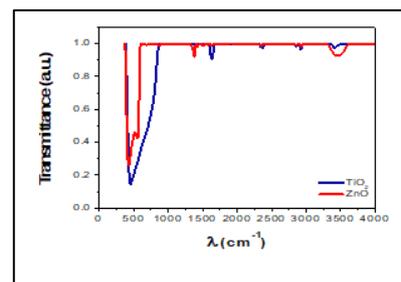
Fig. 41. FTIR spectra of ZnO and TiO_2 particles
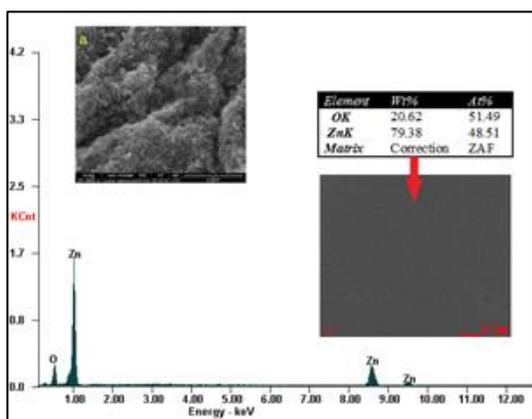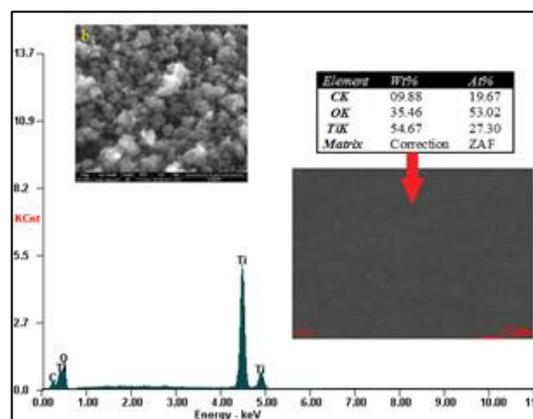
a) ZnO particles	b) TiO_2 particles

Fig. 42. SEM-EDX analyses of ZnO and TiO_2 particles
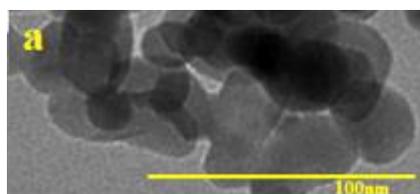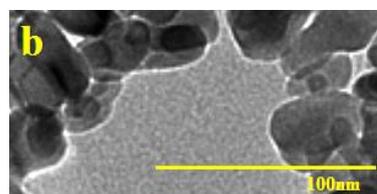
a) ZnO particles	b) TiO_2 particles

Fig. 43. TEM images of ZnO and TiO_2 particles

In the second experiment, EDX analysis determined the silver concentration of S1 and S2 samples from the silver-doped hydroxyapatite thin films on metallic substrate. The S1 sample shows a lower silver concentration of 0.64±0.28% wt and the S2 sample shows a higher silver concentration of 1.52±0.34% wt, remaining unchanged after thermal heating. SEM analysis of S1 and S2 samples shows small cracks formed in hydroxyapatite film after thermal treatment (fig. 44). The XRD analysis shows the peaks corresponding to hydroxyapatite and silver to the two samples (fig. 45).
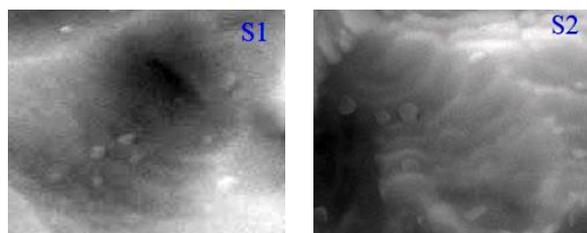
a) S1 sample   b) S2 sample
Fig. 44. SEM analysis of S1 and S2 samples (25000x)
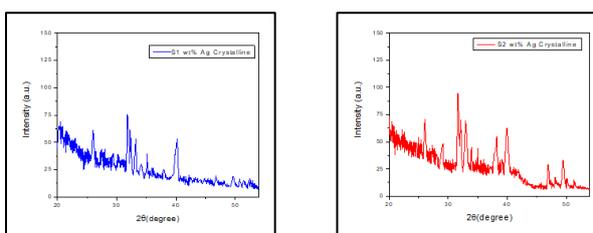
a) S1 sample   b) S2 sample
Fig. 45. XRD graphs of S1 and S2 samples

Discussions

Nano-technology can create antimicrobial particles for implant coatings, with controlled properties and biological interactions, such as composition, structure, dimension, morphology, porosity, pores size distribution, specific surface area, and uniformity and agglomeration degree (Zarb & Schmitt, 1990; Mendonça *et al*., 2008; Sharma *et al*., 2010).

Titanium and its alloys are often used in orthodontic devices due to their good biocompatibility with bone tissue and high resistance to corrosion. An essential condition of orthodontic micro-implants is biocompatibility toward adjacent tissues and preventing oral infection that will lead to implant rejection. Several researchers related that metal or metal oxides particles, like ZnO, TiO_2, gold, silver, silica (SiO_2) and copper/copper oxide (Cu/CuO) (Spencer *et al*., 2009; Sodagar *et al*., 2013; Ramazanzadeh *et al*., 2015), have the most effective antibacterial activity and can cover micro-implants or brackets in orthodontics as antimicrobial agents (Ahn *et al*., 2009; Allaker, 2010).

In the first experiment, ZnO and TiO2 particles were synthesized by the gel-soil method, and the morphological and chemical properties were analyzed to improve the biocompatibility of the implants. The sol-gel process is a wet chemical technique, also known as a chemical solution deposition, and it involves several steps, in the following chronological order: hydrolysis and polycondensation, gelation, aging, drying, densification, and crystallization (Sakka, 2013; Neacsu *et al*., 2016). In the present experiment, the synthesis and analysis of ZnO and TiO2 particles offered information about their specific structural and morphological properties, being an alternative for micro-implants coating, as a biocompatible and bactericidal variant by controlling their size.

Hydroxyapatite with chemical formula $Ca_5(PO_4)_3OH$ is a bioactive and biocompatible material representing nearly 70% of inorganic component of bone matrix, constituting the mechanical strength of the bone tissue (Huang *et al*., 2004; Ong *et al*., 2006; Rabiei *et al*., 2007). Yang *et al*. (2005) used for the first time hydroxyapatite in clinical trials and that revealed an osseo-integration increase after 10 days post insertion of an implant. Silver is used in medicine to serve as an antimicrobial agent. Due to this property, silver represents a solution for implant coating with antimicrobial activity, which includes binding to bacterial DNA, thus preventing bacteria replication (Silver, 2003; Chen *et al*., 2006; Lansdown, 2006). To date, the only commercially accepted method of applying hydroxyapatite coatings to metallic implants is plasma spraying (Geetha *et al*., 2009).

In the second experiment, the combination of ion beam sputter deposition and plasma based ion etching (Mayer & Barker, 1982; Plasma Process Group, 2017; Angstrom Engineering, 2017) produces a unique topography surface of silver-doped hydroxyapatite thin films on Ti micro-implant, with adequate properties to intensify the osseo-integration process and to inhibit bacteria multiplication. The thermal treatment by annealing in air increased thin films stability of layer in dry or aqueous environment. After the thermal treatment, the appearance of small cracks in hydroxyapatite film was due to residual stresses during annealing or to variations in thermal expansion between implant surface and hydroxyapatite thin film. The hydroxyapatite significant peaks appeared on XRD spectra was indicated that thin films have undergone a high amount of recrystallization.

These experiments are contributing to improve the process of osseo-integration and preventing infections around the implant, which offer current technical methods for obtaining materials with special properties.

Conclusions

In these experiments, the settlement of particles on the surface of Ti implants provided the possibility of designing new materials with advantageous structural, morphological and chemical characteristics to prevent infections caused by pathogenic microbial agents in the body. Future studies should refer to tests about the investigation of the behavior of microbial germs in the oral cavity on these types of implants, as well as their stability in cellular breeding environment.

6.1.3. Electrochemical corrosion rate of new alloy based on Iron

Nowadays, new metallic biomaterials for medical applications were reported. Iron (Fe)-based materials are a solution for orthodontic applications based on their mechanical and chemical properties, but it is necessary to control the corrosion rate in the salivary environment. In medical practice, magnesium (Mg)-based alloys are successfully used (e.g. MAGNEZIX approved in 2013 for human patients) (Im *et al.*, 2017), but Fe-based alloys are an alternative to Mg-based alloys due to their qualities for a longer period of use.

In this experiment, a new Fe-based alloy Fe10Mn6Si with two additional elements Ca and Mg was obtained and analyzed in biological environment by immersion with simulated body fluid solution (SBF) and the electrochemical corrosion rate was evaluated.

Cimpoesu N˛ Sandulache F, Istrate B, Cimpoesu R, **Zegan G.** Electrochemical behavior of biodegradable FeMnSi–MgCa alloy. *Metals,* Jul 2018; 8(7): 541.
Work awarded by UEFISCDI in December 2018

Material and methods

For obtaining the new metallic material FeMnSi-MgCa, high purity metallic elements were used like Fe (Armco 99.99 %), Mn (99.98%), Si (99.9%), Mg (99%) and master alloys like SiCa (60-40% wt) and MgCa (85-15% wt). The obtaining process was done in two stage: the melting of Fe, Mn and Si elements in an electric arc furnace (RAV MRF ABJ_900); and the addition of Mg and Ca elements in a vacuum induction furnace (UltraCast) in ceramic crucible (Windhagen *et al.*, 2013).

The experiments were realized by immersion (7, 14, and 30 days) in SBF solution (artificial blood plasma) (Hashmi & Saqlain, 2014), at 37°C and a constant pH (around 7). Material surfaces before and after corrosion tests were analyzed through SEM, EDX, and XRD techniques.

The immersed samples were abraded with abrasion papers of SiC up to 2000 grit and the surface was cleaned in an ultrasound bath (30 min.) in industrial alcohol. The products of corrosion were removed through the immersion in a solution (20g of Sb_2O_3 and 50g of tin chloride were dissolved in 1000 ml of hydrochloric acid and sonication for 30 min.) (Zheng *et al.*, 2014; Armarego, 2017). Corrosion rates were calculated (Zheng *et al.*, 2014) after immersion and after the cleaning procedure of the immersed FeMnSi-MgCa alloy.

The electrochemical tests were done: electrochemical impedance spectroscopy (EIS), linear polarization (LP) and cyclic polarization (CP). The corrosion's current density (i_{corr}) is represented by the degradation degree of the alloy. Electrochemical measurements were performed using a Dynamic Electrochemical System VoltaLab 40 (PGZ301), VoltaMaster 4 software (Radiometer Analytical SAS, Lyon, France) and ZSimpWin software (Princenton Applied Research, Farnborough, Hampshire, UK.).

Results

SEM analysis of the new alloy FeMnSi-MgCa after the immersion in SBF solution show a deep corrosion of the surface (microns order), to type quite agglomerated, with the unification of

the holes. After 7 days of immersion, 50% of the area is affected by corrosion and the reaction compounds increase with the immersion period (fig. 46).
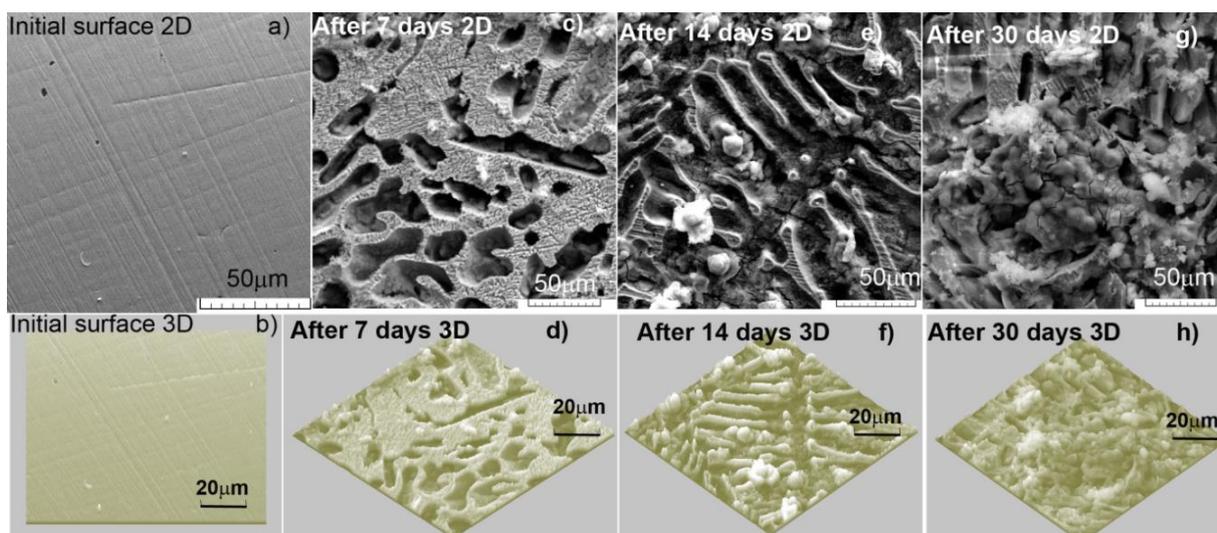

Fig. 46. SEM analyses of the surface of FeMnSi-MgCa alloy (1000x)
a-b) initial surface; c-d) the surface after 7 days; e-f) the surface after 14 days; and g-h) the surface after 30 days

*EDX analy*sis shows the distributions of the elements Fe, Mn, Si, Mg and Ca characteristic of the immersed alloy and the distributions of the elements Na, Cl, O, and C that were identified to qualitative analysis of the surface. Oxides ($FeO.nH_2O$, $Fe_3O_4.nH_2O$ and $Fe_2O_3.nH_2O$) (higher quantities for the 7 days immersion period) and Na salts and carbonates ($MgCO_3$ or $CaCO_3$) (higher quantities for the 30 days immersion period) were present, resulting from interaction with the immersion solution (fig. 47).
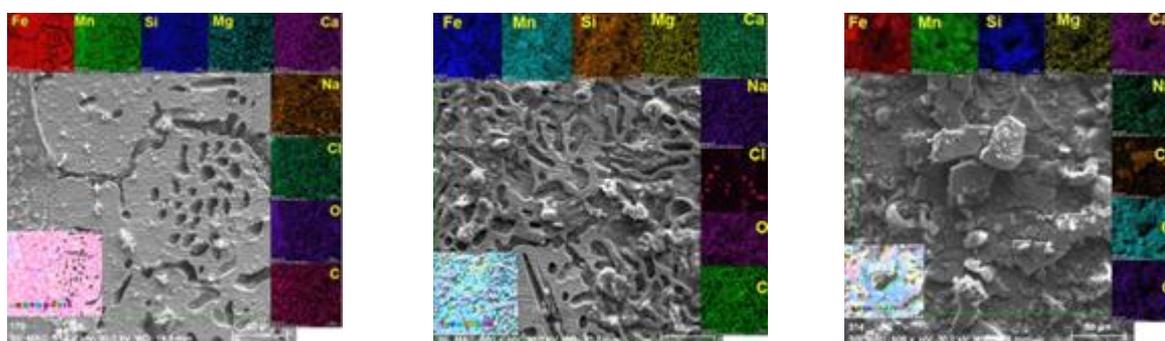
Fig. 47. EDX analyses of the metallic surface after 7, 14 and 30 day immersion period

After the cleaning procedure, EDX analysis shows: higher quantities of Si, Fe and Mn oxides, and the absence of salts, Mg, Ca and compounds based on carbon at the immersed sample for 7 days; lower quantities of Si, Fe and Mn oxides and the appearance of Na salts and Ca carbonates at the immersed sample for 14 days; higher quantities of Si oxides, salts and compounds based on carbon and Mg, Ca elements appear at the immersed sample for 30 days (fig. 48). XRD analysis identifies characteristic peaks for the oxides Fe_3O_4 and SiO_2, beside the main elements peaks (fig. 49).

The values of the corrosion rate after immersion are: 491.015 µm/year after 7 days; 355.463 µm/year after 14 days; respectively 211.018 µm/year after 30 days; the average rate of degradation is 352.50 µm/year. The corrosion rate values after the removal of the non-adherent compounds on the material surface are: 768.294 µm/year after 7 days; 767.800 µm/year after 14 days; respectively 308.99 µm/year after 30 days; the average rate of degradation is 615.028 µm/year.

Quantitative, electrochemical tests indicate the negative corrosion potentials for the four samples, placing this alloy in the category of being easily corrodible in the immersion solution. Qualitatively, the thermodynamic tendency of corrosion of the alloy is greater when the surface of the alloy is freshly polished and decreases when the alloy is longer in contact with the corrosion medium, the corrosion rate decreasing approximately 12 times.
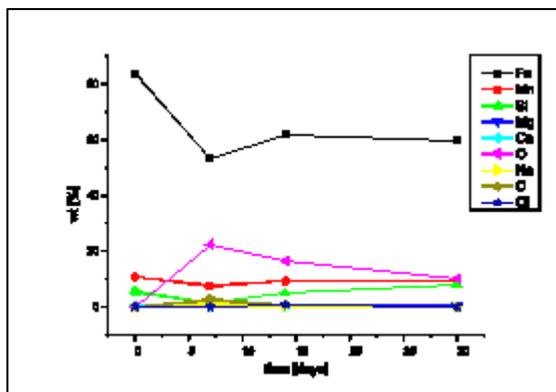

Fig. 48. EDX analysis of chemical elements after immersion and sonication cleaning
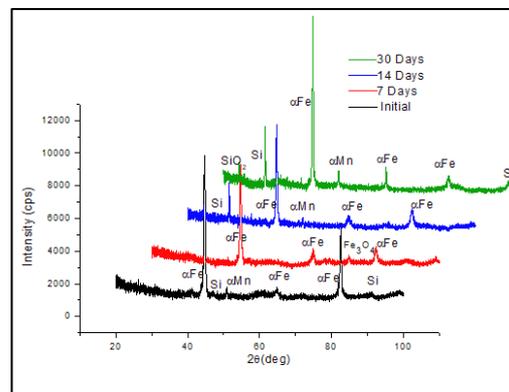
Fig. 49. XRD graphs of the FeMnSi-MgCa alloy's initial state and after different immersion periods in SBF solution

Discussions

In order to control the corrosion rate of the Fe10Mn6Si alloy, the use of two additional elements were proposed, such as Ca and Mg, as corrosion promoters. The material was analyzed *in vitro* from a corrosion resistance point of view.

The experimental outcomes present preliminary information about the degradation rate of the new alloy. The state of the surface was highlighted through microscopy before and after the tests of resistance at corrosion through immersion and linear and cyclic potentiometry. Using the chemical analysis the chemical compounds formed on the surface were determined and the chemical evolution of the alloy was followed at the interface with the biological/electrolyte solution. The potentiodynamic polarization and the impedance measurements are the methods used to determine the corrosion rate of the biodegradable metallic materials.

The new Fe-base alloy surface was exposed to SBF solution, and a complex oxide layer (FeO/MnO/SiO/CaO/MgO) immediately grew on the surface. The stability of the new layer decreased in time in the presence of the SBF solution and after a time period passed to the solution. Fe_3O_4 partially changed to $Fe(OH)_2$, through precipitation and dissolution mechanisms. The presence of chloride on the interface metal/solution (for all samples), maintained a near equilibrium state (homeostasis) between the dissolution of some compounds and the precipitation process and passing to solution. In one case, $Fe(OH)_2$ reacted with chloride and formed soluble $FeCl_3$.

After the cleaning stage, no further chloride was identified on the surface. CaO and MgO products were extremely unstable in SBF solution. Both oxides became $Ca(OH)_2$ and $Mg(OH)_2$ and interacted with CO_3^{2-} and precipitated on the surface. It appeared that a major part of these compounds was not stable on the surface and passed to solution after the sonication cleaning stage (Stanciu *et al.*, 2017). Each time the oxides layer is formed on the top of the alloy surface and meets the simulated body fluid solution, it cannot prevent the diffusion of the solution ions and the degradation will continue till the end of the metallic material. In conclusion, the passivation layer protects the metallic surface only for a short time period and the galvanic corrosion will take place until the total dissolution of the material (Liu *et al.*, 2015).

In this experiment, the complex oxide layer formed at the beginning protected the metallic material, as reported by previous studies (Hermawan & Mantovani, 2013; Zhen *et al.*, 2013; Chelariu *et al.*, 2015; Asgari *et al.*, 2016). Then, the oxides interacted with the solution and formed hydroxides and grew a new layer with a lower stability that continuously interacted with chloride ions and soluble compounds that passed to the solution were formed. At the same time, the

carbonate compounds attached to the alloy surface and decreased the corrosion rate because of their non-conductive nature. In the last stage, the metallic surface had metallic material parts close to spalling, metallic material holes after the alloy passes to solution, with oxides, hydroxides, carbonates and salts on the surface. After sonication, an important part of these compounds was removed from the surface.

Conclusions
In this experiment, a new alloy was obtained, based on Fe with a corrosion rate (around 700 µm/year) smaller than the Mg-based alloys, but higher than the usual Fe-based alloys, suitable for medical applications with elements with thicknesses between 500 µm and 1000 µm. Further work *in vivo* tests of this new material is proposed to evaluate the behavior of the material in real conditions in short, medium, or long periods.

6.1.4. Use of the effect of NiTi alloy shape memory in medical equipment
Snoring and sleep apnea are frequent problems not only in adults, but also in children and adolescents, these being symptoms of the sleep-disorder breathing (SDB) and obstructive sleep apnea (OSA) (Erler & Paditz, 2004; Katyal *et al*., 2013). In paediatric patients, the incidence of SDB is 2% and for OSA is 7-8% (Filiaci *et al*., 2014). In adults, SDB prevalence is 20% in Western countries and 32.8% in South America (Young *et al*., 2002; Tufik *et al*., 2010). The clinical symptoms are shown on the polysomnographic test (Weathely *et al*., 2004; Shayer *et al*., 2014; Muzet *et al*., 2016; Blackwell *et al*., 2017). Depending of the doctor's diagnosis different intervention measures can be taken. One of these, in the urgent cases of intervention, implies using equipment of backing up the respiration during sleep.

In this experiment, a medical equipment type belt is proposed, to assure the respiratory rhythm, which uses the effect of shape memory of the NiTi alloys under the form of wire, through optoelectronic command.

Enache A, Mihaescu T, Cimpoesu N, Manole V, Prisecariu BA, **Zegan G***, Stratulat SI. Programmable belt type device with optoelectronic command to improve the artificial ventilation during sleep. *Optoelectronics and Advanced Materials – Rapid Communications,* Nov-Dec 2018; 12(11-12): 700-704.

Material and methods
Wires of 1 mm diameter were purchased from Saes Getters, having the chemical composition 54.5% wt Ni-45.5% wt Ti (% mass) equivalent to Ni49.35Ti50.65. The wires were treated by means of a thermo-mechanical treatment, comprising heating cycles and turned to an arch wire shape on a mechanical device. The fragments of the wire were cut and analysed through differential scanning calorimetry performed on a Netzsch DSC 200 F3 Maya.

Results
The proposed equipment has a double command: the medical prescription from polysomnography apparatus is taken by software or is introduced by the physician. Thus, the parameters are transmitted to the acquisition board (Arduino type) that controls the frequency parameters of the opto-coupler, led-photodiode, and the photo resistance will modify the value (Cimpoesu *et al*., 2010). These variations lead to the changing the alternative voltage/ pulse rate continuously from the power supply. This tension with well-established parameters of frequency will be used to command the ventilation belt (fig. 50). The power supply for the NiTi springs done in a dimmer, coupled serially on the secondary of the transformer for a current of maximum 2A and an alternative tension of 24V (fig. 51). The ventilation belt is formed from the fixing element (harnesses) and the active part (plastic carcass) from the NiTi springs, which at heating make the machine work, equipped with a ventilator necessary to cool these elements. In order to obtain the mechanical tension in harnesses of approximately 10 kgF (100 N), six NiTi springs are necessary.

During the expiration, the NiTi springs powered with voltage are in tight position, and the mechanical tension work on the abdomen and harnesses. During the inspiration, the NiTi springs are in relaxed, elongated position (fig. 51). The main physical characteristic of the NiTi alloy used is the temperature of phase transformation martensitic-austenitic, at which the springs modify their shape form elongated in tight and vice versa (fig. 52).
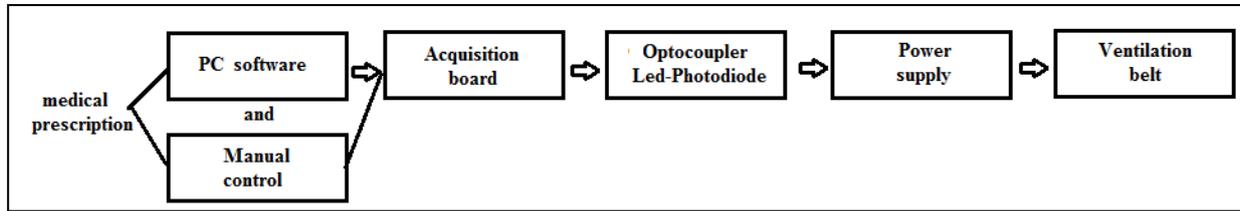

Fig. 50. The block diagram of the device
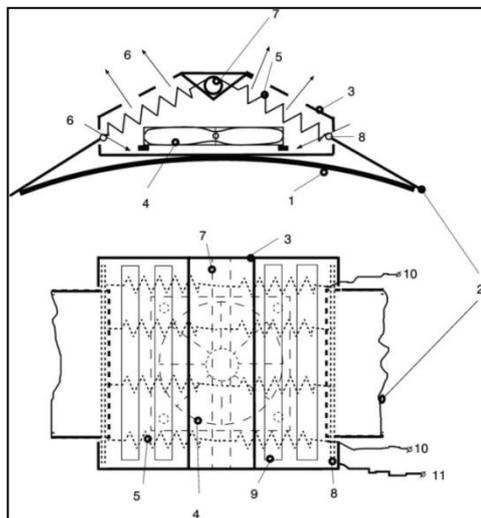
a) The compounds: 1-abdomen, 2-harnesses, 3-carcass, 4-ventilator, 5-NiTi spring, 6-flow of air to cool the springs, 7-insulated support to maintain the NiTi springs, 8-rod for connecting spring and harness, 9-slit for the cooling air, 10-power for ventilator
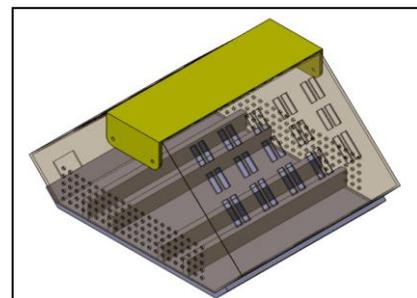
b) the 3D design of the plastic carcass
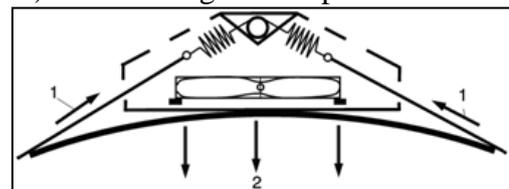
c) the distribution of the mechanical tensions on the abdomen: 1- harnesses forces and 2 - on the chest

Fig. 51. The ventilation belt
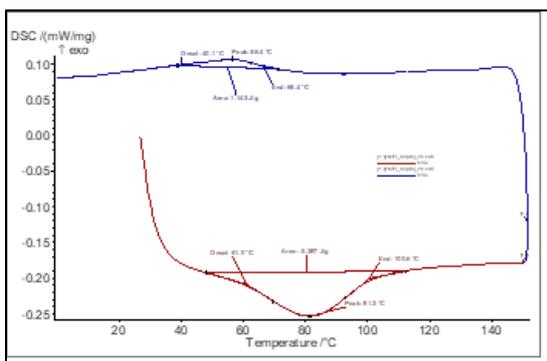
a) initial state (during the inspiration)
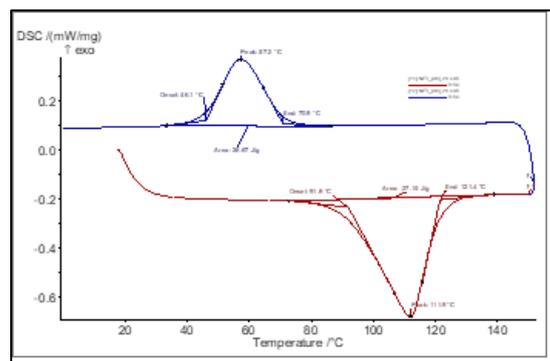
b) after thermal treatment evaluation at 20°C/min heating rate (during the expiration)

Fig. 52. Calorimetric DSC charts of NiTi wire (d=1 mm)

Discussions

Clinically, OSA is defined by the occurrence of daytime sleepiness, loud snoring, witnessed breathing interruptions, or awakening due to gasping or choking. The apnea-hypopnea index (AHI)

measures the OSA severity: normal (AHI <5 apnea-hypopnea per hour of sleep), mild sleep apnea (AHI 5-15 apnea-hypopnea per hour of sleep), moderate sleep apnea (AHI 15-30 apnea-hypopnea per hour of sleep); severe sleep apnea (AHI >30 apnea-hypopnea per hour of sleep) (Park *et al.*, 2011; Banabilh, 2017).

OSA is a multifactorial disease and the etiology of OSA is diverse and complex. There are certain behavioral and systemic factors that can contribute to sleep apnea (obesity, kyphoscoliosis, respiratory and neuro-motor disorders). Also, there are anatomic factors that can contribute to OSA, such as maxillary or mandibular retrognathism, increase of lower face height, large tongue, elongated soft palate, an inferiorly positioned of hyoid bone, narrowed nasal cavities, and tonsil hypertrophy. Angle Class II of malocclusion was associated with OSA of patients (Young & McDonald, 2004; Lam *et al.*, 2005; Khassawneh *et al.*, 2009; Banabilh *et al.*, 2007; 2010).

Today, the diagnosis and treatment of OSA depend on a multidisciplinary team of professionals, in which orthodontists have a fundamental role in the OSA diagnostic, through examination of lateral cephalometry (assessing the pharyngeal airways, the skeletal and soft tissue structures) (Ceylan *et al.*, 1995; Battagel *et al.*, 2000; Capistrano *et al.*, 2015). Some authors suggest an index for the orthodontic treatment useful for paediatric patients with OSA (Altalibi M, *et al.*, 2014).

The shape memory NiTi alloy with near equiatomic concentrations is known for their high physical, mechanical and functional properties, as well as to their super-elasticity and excellent corrosion resistance in different environments (Khol, 2014; Sreekumar *et al.*, 2007; Tadayyon *et al.*, 2017; Laplanche *et al.*, 2017).

In this experiment, the concept of an equipment of regulating the respiration during the sleep apnee was presented, equipment made of plastic with light elements designed to 3D imprint and intelligent active elements of NiTi (shape memory alloy). The proposed equipment can work separately or in synchronization with the ventilation apparatus only for the expiration to improve some physical parameters: the respiratory effort decreases, the snore is eliminated; the arterial saturation with oxygen increases, the pulse rate and the blood pressure oscillations are stabilized.

Applying a treatment of educating the warm form of the alloy wires with shape memory leads to the modification of the domains of transformation temperatures, especially on the reversion of the martensite (M→A), with a difference of approximately 20°C between the values A_{50} in austenite although the direct transformation (A→M) takes place in approximately the same domain (differences lower than 1°C between the values of temperature M_{50}).

After the stage of inspiration of the air, the helical arcs from shape memory alloy NiTi are contracted, creating a pressure under the thorax of the patient, necessary to eliminate the air with CO_2 out of the body. Depending of the number and sizes of springs, its can obtain the whole range of pressures till the highest level. Establishing the parameters in the program will be made according to the data registered in the polysomnography apparatus (Shayer *et al.*, 2014; Blackwell *et al.*, 2017).

This equipment with intelligent elements can act only upon the expiration, the pressure being exerted from the outside to the inside, through the compression of the thorax under the action of NiTi springs. These elements are electronically activated using a computer, an acquisition plate and a variable power source. Using the computer program, the time parameters can be optimized (2 times- inspiration and expiration), and the intensity of the abdominal pressure will be indicated by the physician.

Conclusions

In this experiment, a device with medical applications that uses active elements with shape memory under the form of wire is proposed, electrically activated by an optoelectronic system, and the heating behavior of the elements by differential calorimetry was tested and their thermo-mechanical response.

6.2. Antibiotic controlled delivery systems for the treatment of oral infections

The benefits of orthodontic treatment are numerous and in most cases, the benefits outweigh the possible disadvantages. The orthodontic treatment can play an important role in enhancing esthetics, function, and self-esteem in patients. Apart from its aesthetic effect, this treatment has the potential to cause unwanted side-effects. The side-effects can be intra-oral, extra-oral, and systemic. The intra-oral side-effects can be on the teeth (white spot lesions, tooth caries, attrition, root resorption); on the gum tissues (gingivitis, gun recession, gingival hyperplasia, resorption of alveolar crestal bone); and on the soft tissues (trauma, allergy). The extra-oral side-effects can be allergies to orthodontic appliance components, headgear and temporomandibular dysfunction. The systemic side-effects can be crossed infections (Gorelick *et al.*, 1982; Malmgren, 1988; Boyd & Baurmind, 1992; Levander & McGuinness, 1992; Sadowsky, 1992). These potential complications are easily avoidable by undertaking certain precautions and timely interventions by both the orthodontist and the patient. The decision whether to proceed with the orthodontic treatment is essentially a risk-benefit analysis, where the perceived benefits of commencing treatment outweigh the potential risks (Talic, 2011; Meeran, 2013).

For the protection of both doctor and patient, infection control is very important, because more microorganisms are found in the oral cavity than in any other part of the body (Kalra *et al.*, 2015). Local therapy of most common oral affections has several advantages compared to systemic drug administration being targeted directly to the injured area while reducing adverse effects (Holpuch *et al.*, 2011; Sankar *et al.*, 2011). Antimicrobial resistance represents a big worldwide concern. The practices regarding the antibiotic prescription for therapeutic or prophylactic purposes, including those recommended by dentists, contribute to the rational use of antibiotics (Palmer, 2016).

The polymicrobial nature of odontogenic infections needs the therapy with antibiotic against aerobic and anaerobic bacteria respectively. The most commonly prescribed antibiotics by dentists (Al-Mubarak *et al.*, 2004; Ogunbodede *et al.*, 2005; Al-Haroni *et al.*, 2006) for infections of oral cavity are amoxicillin (Palmer *et al.*, 2000; 2001), penicillin (Yingling *et al.*, 2002; Demirbas *et al.*, 2006; Al-Haroni *et al.*, 2007), metronidazole (Palmer *et al.*, 2000; 2001), amoxicillin and clavulanic acid (Poveda Roda *et al.*, 2007), as well as erythromycin with clindamycin as a choice in individuals which are allergic to β-lactam antibiotics.

Nowadays, worldwide research is being done to develop new drug delivery systems. In conventional therapy, the drug is released immediately after administration, resulting in rapid increase in blood plasma concentration, sometimes at levels that exceed the toxic level. Controlled-release systems are designed to enhance drug therapy, through superior control of drugs exposure over time, to protect drug from physiological degradation or elimination, (Siegel & Rathbone, 2012).

The controlled release of drugs aims at: maintaining a constant blood concentration with minimal fluctuations of the therapeutically active compounds; predictable and reproducible delivery rates over a long period of time; the protection of bioactive compounds with a very short half-life; eliminating side effects of drug residues and frequent dosing; optimised therapy and patient tolerance and solving the problem of drug stability. Depending on the purpose, there are several types of controlled release drug profiles, the most important being: type I- release rate decreases exponentially with time; type II- kinetics of the order 0 with the constant release rate; type III- Release 0 with significant delay; type IV- delayed pulsatile release; type V- multiple release with constant delay between releases (Langer & Peppas, 2004).

The controlled drug delivery technology has progressed over the last six decades. It began in 1952 with the introduction of the first sustained release formulation. The first generation (1950-1980) was focused on developing oral and transdermal sustained release systems and establishing the controlled drug release mechanisms (such as different drug release mechanisms including dissolution-, diffusion-, osmosis-, and ion exchange-based mechanisms). Attention of the second generation (1980-2010) was dedicated to development of zero-order release systems (does not result in maintenance of the constant drug concentration in the blood), self-regulated drug delivery

systems, long-term depot formulations, and nanotechnology-based delivery systems. So, in the 1980s "smart" polymers and hydrogels were developed so that the drug kinetic release is triggered by the changes in environmental factors (such as pH, temperature, or glucose). Then, in the 1990s, a new trend emerged in dealing with nanoparticles made of biodegradable polymers, polymeric micelles, lipids, chitosan, and dendrimers. From the beginning of the new century, the research topics have focused on nanotechnology and have advanced until they manipulated nano-sized delivery vehicles for targeted drug delivery (Park, 2015; Zhong *et al.*, 2018).

Particles antibiotic delivery carriers were designed for drug targeting to the affected site for better efficacy. Encapsulation of drugs in particles as delivery systems can improve their efficiency by enhancing their bioavailability, minimizing side effects and by facilitating the prolonged release of encapsulated drug (Tilakaratne & Soory, 2014). Hybridization of drugs with hydrotalcite type nanomaterials results in remarkable efficiency and stability. Anionic clays may be considered carriers that enhance antibiotic bioavailability and allows the controlled release of the drug with fewer side effects.

The burst effect is a major consideration in designing controlled release systems. In most pharmaceutical applications, the burst effect is regarded as an event that should be avoided, even at the cost of using an overcoat to reduce the initial burst drug. At minimum, burst release leads to a loss in treatment efficacy, as the drug is lost in an uncontrolled and unpredictable pattern (Brazel & Huang, 2004). Although the significance of burst release in controlled delivery systems has not been entirely ignored, no successful theories have been put forth to fully describe this phenomenon (Huang & Brazel, 2001). By means of polymeric devices, drugs with a variety of physicochemical properties can be predictably released in therapeutic doses (Laurencin & Langer, 1987). An interdisciplinary approach to the drug delivery systems will provide safer and more effective therapies in the future.

For this context, a part of my researches focused on experiments that investigated several preparations with prolonged release antibiotics effects at the local level of oral cavity, in case of the occurrence of some odontogenic and non-odontogenic infections during orthodontic treatment, as unwanted effects or experiments which we have done with other specialists in related fields.

6.2.1. Prescription of antibiotics in dental practice

Most of the studies have investigated the knowledge, attitude and/or patterns of the prescription of the antibiotics among dental practitioners that provide data regarding the practices in special situations (Falkenstein *et al.*, 2016; Mansour *et al.*, 2018; Kaul *et al.*, 2018). There are also studies evaluating the current general practice (Halling *et al.*, 2017). Only a few studies provided data prescribing trends in dentistry for long periods of time (Marra *et al.*, 2016; Ford *et al.*, 2017), the longest period being of 17 years (Marra *et al.*, 2016).

The aim of this literature review was to analyse the modalities of the evaluation of the prescription patterns of antibiotics among dentists.

Hunea IM, Cretu A, Ghiciuc CM, Anistoroaiei A, **Zegan G**. Methods of evaluation of antibiotic prescription in dentistry. *Romanian Journal of Medical and Dental Education,* Mar 2019; 8(3): 30-35.

Material and methods

141 relevant studies from MEDLINE/PubMed database, published in English were selected, in the interval 1980-2019, including 28 studies which evaluated the prescription patterns of antibiotics in dentistry. We excluded the studies presenting only data about the knowledge regarding antibiotics in dental practice. We recorded the type of study, the modality of collection of data, the response rate, and the characteristics of the prescription.

Results

There are various modalities to evaluate the prescribed antibiotic in dental practice: questionnaires-based studies, analysis of prescriptions issued by dentists or the analysis of the prescriptions received by the patients for a particular situation (prophylaxis for infective endocarditis, dental treatment) (Konde *et al*., 2016; Peric *et al*., 2015; Khalil *et al*., 2015).

Most of the studies reported broad spectrum penicillin's as first-line drugs. Amoxicillin is the first broad spectrum penicillin's used, followed by amoxicillin with clavulanic acid. Macrolides are first-line drugs for patients allergic to penicillin's. Clindamycin is the preferred macrolide. Metronidazole was used alone or in association with other antibiotics (Amoxicillin or Spiramycin). Other option of antibiotic treatment, less preferred by dentists, is represented by tetracycline's (doxycycline). Some studies evaluated the use of antibiotics for particular therapeutic or prophylactic indications in dental practice (Lee *et al*., 2009; Kaptan *et al*., 2013; Arteagoitia *et al*., 2016; El-Kholey *et al*., 2018).

Discussions

This review analyzed the modalities to evaluate antibiotic prescription patterns in dentistry. Guidelines and advice for prophylaxis before undergoing treatment are needed. Studies evaluating the self-medication with antibiotics were excluded from this analysis. Also, the studies evaluating only the knowledge or perception regarding the use of antibiotics or antibiotic resistance were excluded. There are many ethical dilemmas in medicine and prescribing antibiotics to patients in dentistry (Lacatusu, 2014). Using modern techniques in hospitals and dental offices, it is important to choose the right antibiotic, in order to contribute to the reduction of antimicrobial resistance (Jelihovschi, 2017).

Conclusions

Routine prescription of antibiotics should be discouraged also in dental practice. There is a need to for more research studies and for educational initiatives to prevent inappropriate recommendation of antibiotics in dentistry.

6.2.2. The obtaining of antibiotic-loaded chitosan particles by ionotropic gelation

Chitosan appeared as a potential biomaterial for dental uses due to its special properties such as bioactivity, biocompatibility and antimicrobial (Chen *et al*., 2011; De Carvalho *et al*., 2011; Konovalova *et al*., 2017). The antibiotic encapsulated chitosan particles have higher surface area and reactivity to facilitate drug release, due to their bacteriostatic and bactericidal action for a wide variety of oral microorganisms (Li *et al*., 2005; Samprasit *et al*., 2015).

These experiments aimed to investigate the possibilities to improve the antibiotic release from the antimicrobial common treatment of oral infections, through obtaining antibiotic-loaded chitosan particles and liposome-loaded chitosan hydrogels.

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Paun VA, Popa M, Desbrieres J, Peptu CA, Dragan SV, **Zegan G***, Cioca G. Liposome loaded chitosan hydrogels, a promising way to reduce the burst effect in drug release. A comparative analysis. *Materiale Plastice*, Dec 2016; 53(4): 590-593.

Material and methods

The obtaining of antibiotic-loaded chitosan particles

Synthesis of the chitosan-tripolyphosphate complex by ionotropic gelation. The 0.2% (w/v) chitosan powder was dissolved in 0.25M acetic acid and magnetically stirred at 400 rpm at room temperature. Thus, the amine group of chitosan molecule was protonated by the acetic acid for a stronger interaction with the crosslinking agent and the drugs. Sodium tripolyphosphate (TPP) was prepared in distilled water by dissolving the powder in 0.25M acetic acid, then added to chitosan solution under stirring followed by 20 minutes of sonication, so the resulting suspension being centrifuged at 15000 rpm for 10 minutes. The obtained pallets were subsequently re-suspended in deionised water by sonication, then centrifuged and dried at 25°C.

Antibiotic-loaded particles were spontaneously formed by dropwise addition of 12 ml TPP 0.4% to 20 ml chitosan solution 0.35% (w/v) containing antibiotic (5 mg/ml in a mass ratio of 7:1) under magnetic stirring conditions followed by sonication. The resulting particles suspension was centrifuged at 15000 rpm for 4 times (15 minutes each), washed with distilled water and then dried.

The first preparation with amoxicillin and clavulanic acid marked as Amox/Clav.ac.-CNP6 (with 0.60% w/v TPP) was compared with drugs encapsulated denoted as Amox/Clav.ac.-CNP4 (with 0.40% w/v TPP), using Dynamic Light Scattering (DLS), the FTIR spectrometry and adsorption-desorption isotherms. The second preparation with doxycycline marked as Doxy-CNPs6 (with 0.60% w/v TPP) was compared with drugs encapsulated denoted as Doxy-CNPs4 (with 0.42% w/v TPP), and antibacterial activity was evaluated by minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC).

The obtaining of liposome-loaded chitosan hydrogels

Preparation of liposomes. For calcein-encapsulating phosphatidylcholine (PC) were used Multi-Lamellar Vesicles (MLV) were used, prepared by the thin film hydration method (Peptu *et al*., 2010) and Small Unilamellar Vesicles (SUV) prepared by sonication method (Popescu *et al*., 2011). In each hydrogel a certain volume of liposomes suspension was added before the glutaraldehyde addition and the total amount of included calcein was calculated by breaking the liposomes with the surfactant Triton X-100. For the liposomes distribution studies throughout the polymeric matrix, Rhodamine B labelled liposomes were prepared.

Preparation of polymer-liposomes-calcein systems. Calcein was encapsulated in liposomes. The suspension of calcein loaded liposomes was added to the polymer solution prior to the introduction of glutaraldehyde as covalent crosslinking agent (Peptu *et al*., 2008). Subsequently, the aqueous solutions of specific amounts of glutaraldehyde were added under vigorous stirring and the crosslinking process continued with the introduction of ionic crosslinker (sodium sulphate or TTP) (Ciobanu *et al*., 2014). The release of calcein was studied comparative, according to the ionic crosslinking agent, sodium sulphate (CG-S) or TTP (CG-T).

Results

The analysis of antibiotic-loaded chitosan particles

Amox/Clav.ac.-CNP6. The DLS analysis shows Amox/Clav.ac.-CNP4 particles with an average particle diameter of 45 nm and a wide particle size distribution, with spherical in shape and smooth edges. Amox/Clav.ac.-CNP6 particles are an average diameter of 270 nm and a narrow particle size distribution, with spherical in shape and not so smooth edges (fig. 53a). FTIR analysis shows typical absorption peaks for drugs (amoxicillin and clavulanic acid, and Amox/Clav.ac.-CNP6), and different spectra for chitosan particles (fig. 53b). Drug release profile: for Amox/Clav.ac.-CNP4, the release occurred within the first 5 hour and for Amox/Clav.ac.-CNP6 within the first 4 hour (fig. 53c).

Doxy-CNPs6. The DLS analysis shows Doxy-CNPs4 particles with an average particle diameter of 45 nm and a wide particle size distribution, with spherical in shape and smooth edges. Doxy-CNPs6 particles are an average diameter of 280 nm and a narrow particle size distribution, with spherical in shape and not so smooth edges (fig. 54a). FTIR analysis shown typical absorption peaks for drugs (doxycycline and Doxy-CNPs), and different spectra for chitosan particles (fig.

54b). Doxy-CNPs6 released more antibiotic amount than Doxy-CNPs4 by the end of the 24 hour monitoring period (fig. 54c). Antibacterial activity after four hours incubation at 37°C was 16µg/ml for Doxy-CNPs4 and 13µg/ml for Doxy-CNPs6. For both samples more than 90% bacteria growth inhibition was observed. Bactericidal activity was 48µg/ml for Doxy-CNPs4 and 40µg/ml Doxy-CNPs6.


| a) DLS analysis of particles size distributions | b) FTIR spectra of amoxicillin and clavulanic acid (A), Amox/Clav.ac.-CNP4 (B), Amox/Clav.ac.-CNP6 (C) and chitosan (D) | c) the amount of drugs release over a 24-hour period |
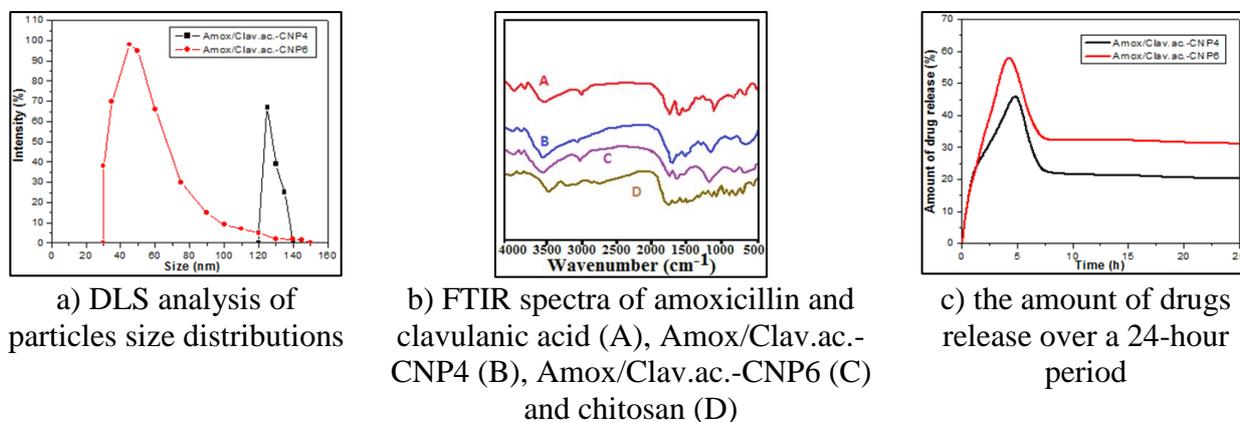
Fig. 53. The analyses of Amox/Clav.ac.-CNP4 and Amox/Clav.ac.-CNP6

| a) DLS analysis of particles size distributions | b) FTIR spectra of chitosan (A) doxycycline (B) and Doxy-CNPs (C) | c) the amount of antibiotic release over a 24-hour period |
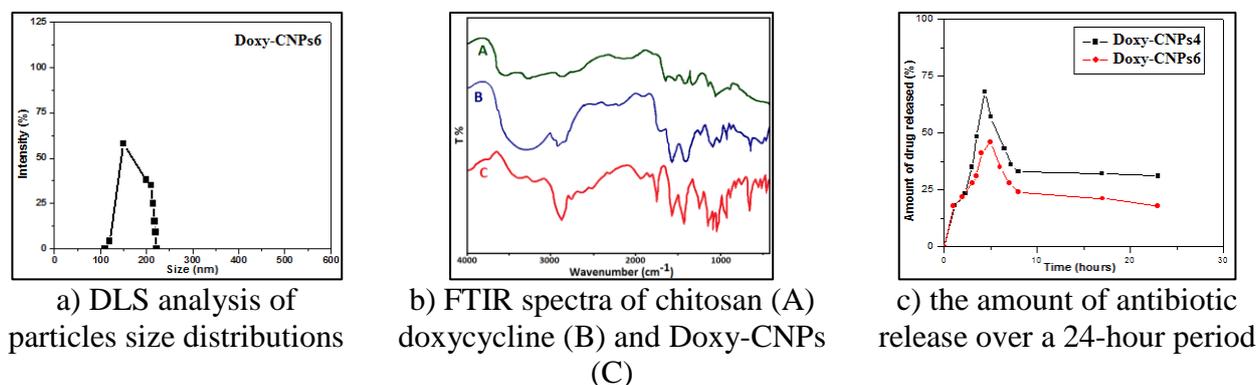
Fig. 54. The analyses of Doxy-CNPs4 and Doxy-CNPs6

| a) with sodium sulphate | b) with sodium TTP |
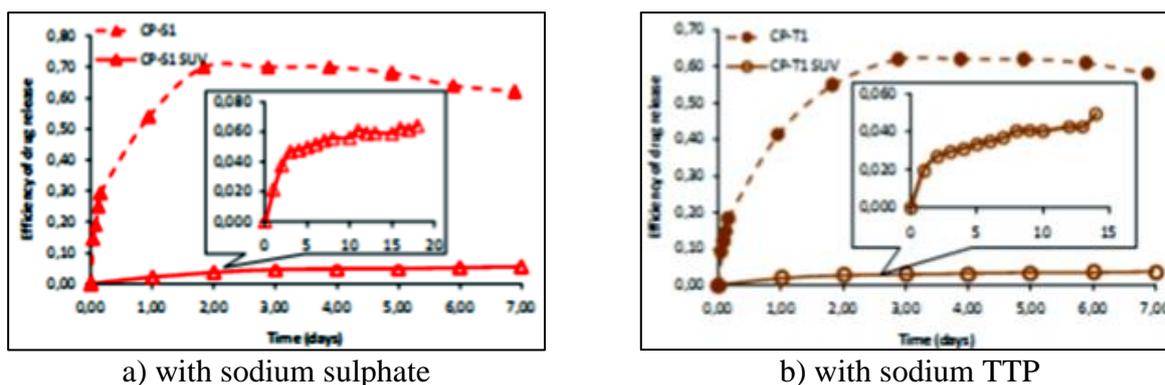
Fig. 55. Release kinetics of calcein from chitosan based hydrogels

The analysis of liposome-loaded chitosan hydrogels
The DLS analysis shows mean diameters of 1.266±0.237 mm for MLVs and 1.266±0.237 mm for SUVs and a wide vesicles size distribution. The liposomes are well distributed throughout the whole surface area of the hydrogels. The FTIR analysis shows for the CG-S hydrogels that about 80% of the loaded drug was gradually released into the supernatant over 7 days (fig. 3a). A similar behavior was observed for CG-T hydrogels, but there was a lower efficiency of calcein release, about 65% (fig. 3b).

Discussions

The oral cavity is a complex environment for drug delivery systems. Therapeutic efficacy at the targeted site of action can be improved by delivering drugs directly to the oral cavity. Therefore, systemic dose of the drug can be reduced thus decreasing side effects.

Particles used in dentistry have a wide range of pharmaceutical uses since their physico-chemical properties can be controlled accordingly to their target. Novel hybrid particulate formulations based on drug incorporated particles for local application in dentistry can be provided as an aqueous suspension or incorporated into a paste or gel creating products with easy administration (Hannig, 2010).

Ionic gelation, known as ion-induced gelation, results in particles with defects, such as improper surface morphology, fragile particulate system, high dispersibility index, and lack of proper surface modification sites to attach functional moieties (Patel *et al*., 2007; Badawi *et al*., 2008; Wu *et al*., 2009; Kunjachan *et al*., 2010).

Amoxicillin and clavulanic acid are two of the most commonly prescribed antibacterial worldwide for treating oral infectious diseases (Brook *et al*., 1991; Martínez *et al*., 2004; López-Píriz *et al*., 2007). Doxycycline is an antimicrobial drug used to treat extracellular and intracellular infection caused by Gram(-) and Gram(+) bacteria as well as spirochetes, Chlamydia, mycobacteria and mycoplasma (Joshi & Miller, 1997; Riond & Riviere, 1998). This antibiotic is one of the most prescribed medicine worldwide due to its antibacterial effect on a various pathogens (Cunha *et al*., 2000; Angelakis *et al*., 2015). Systemic administration of doxycycline may cause side effects resulting in tissues altering and blood vessels.

Chitosan is a multipurpose natural biomaterial investigated as a pharmaceutical excipient in drug formulations. Chitosan is a natural aminopolysaccharide comprising glucosamine and N-acetyl glucosamine units. This biopolymer is widely used for the development of biomedical applications and it shows diverse biological activities towards mammalian cells, including muco-adhesion, the ability to condense and transport oligonucleotides, and adjuvant activity. Antibiotics or other drugs used in oral diseases treatment can be loaded on chitosan particles having possible applications in orthodontics as materials deposited on mini-implants for a better osseo-integration and prevention on microbial infections (Deng *et al*., 2013; Norowski *et al*., 2015; Huang *et al*., 2016; Qasim *et al*., 2017).

In this experiment, the DLS analysis of the antibiotic-loaded chitosan particles revealed that the sodium TPP concentration determined the particle size, and narrow particle size distribution produced an equally distributed among the particles, thus normalizing rate of antibiotic release. FTIR different spectra of chitosan particles demonstrated that the antibiotic was successfully encapsulated in chitosan particles. Antibiotic release profile revealed a burst effect, followed by a slow controlled release until 24 hours, perfect for microbial infections treatment.

Hydrogel is a colloidal gel in which the dispersion medium is water. Liposomes are spherical self-closed structures, composed of curved lipid bilayers, which enclose part of the surrounding solvent into their interior. The simplicity of production, their biocompatibility, absence of toxicity (Vlahovska *et al*., 2009; Mufamadi *et al*., 2011), the size and similar composition to cell make them a revolutionary tool in medicine and biomedical domains.

In this experiment, the role of the type of liposomes was studied. The systems MLVs released higher amounts of calcein compared to the systems SUVs, although these liposomes were more stable in the matrix and diffused with difficulty. Although MLVs are more stable than SUVs, they transported a much higher quantity of calcein (in relation with their volume). Moreover, they presented a better efficiency in controlling the burst effect and the release kinetics. From kinetics studies and modelling of kinetics curves we have shown that interpenetrated networks based on double-crosslinked chitosan are capable of releasing hydrophilic drugs through a multi-scale mechanism, characterized by four distinct phases, each characterized by a different kinetics model (Bacaita *et al*., 2014). The use of liposomes entrapped within chitosan hydrogels allowed a strong decrease of the burst effect as well as a better control of release kinetics. Sodium TPP was a

stronger ionic crosslinking agent than sodium sulphate, leading to a denser hydrogel matrix and, furthermore, to longer stability and lower efficiency of CGT hydrogels compared to CG-S ones.

Chitosan hydrogels are able to provide local delivery of drugs, but their delivery can be rapid and uncontrollable in time, due to the burst effect. This leads to a loss in drug efficiency and lifetime. The results showed that the use of liposomes entrapped within chitosan hydrogels allowed a strong decrease of burst effect, consisting of drug diffusion through the swollen hydrogel and/or pores with water filled, being continuously fed by calcein loaded in liposomes.

Conclusions

In these experiments, several few novel drug delivery systems were designed for oral treatment and prophylaxis of different infections in the oral cavity. Particulate of antibiotic-encapsulated chitosan for oral infections treatment indicated a slow, sustained and controlled release, effective for the treatment of oral microbial infections. The complex hydrogels formations were made in order to check the effect of the liposomes as extra barrier for the calcein controlled release. To overcome the consequences of burst effect, systems involving liposomes incorporated into chitosan hydrogels may appear as a promising material in tissue engineering, regenerative medicine and drug loading systems.

6.2.3. The obtaining of hydrotalcite particles intercalated with antibiotics/ ascorbic acid by co-precipitation

In the last few years, inorganic type's hydrotalcites particles have been introduced into the pharmaceutical industry as controlled release drug delivery systems due to their biocompatibility, stability and anion exchange properties (Wang & Zhang, 2012; Rives *et al*., 2013). These layered materials control the release pharmacokinetic profile by replacing metal ions in the host hydrotalcite layers (Khan & O'Hare, 2002; Chakraborti *et al*., 2012; Tugui *et al*., 2015). Choosing metal layers, pH stability and antacid performance are controllable by restricting molecular interactions and depends on their diffusion through particle in controlling drug release rapidity (Choy *et al*., 2001; Ambrogi *et al*., 2003; Wei *et al*., 2004). A lot of drugs have been successfully incorporated into hydrotalcites, including the antibiotics (Yang *et al*., 2016).

The aim of these experiments was to synthesize and characterize the structure of inorganic type's hydrotalcites particles intercalated with antibiotics/ ascorbic acid, using in the treatment of oral diseases.

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Zegan G, Golovcencu L, Cernei ER, Carausu EM, Anistoroaei D. Structural and morphological characteristics of hybrid nanomaterials type ascorbic acid-hydrotalcite used for saliva stimulation. *Revista de Chimie,* May 2018; 69(5): 1244-1246.

Material and methods

Hydrotalcite synthesis was obtained by the co-precipitation method, using metal salts with a different molar ratio as precursors and $NaOH/Na_2CO_3$ as precipitates. These were added dropwise until complete precipitation at pH=9. The resulting precipitate was aged at 60°C for 24 hours under

stirring to achieve the aging step. The obtained precipitates were separated by filtration, washed extensively with deionized water until sodium free and dried under vacuum at 60°C. The hydrotalcite was calcined at 500°C for 5 hours, in order to be used for drug intercalation by rehydration. Thus, Fe-hydrotalcite, MgAl-hydrotalcite and ZnAl-hydrotalcite were synthetized.

Drug intercalation into anionic clay gallery was carried out by reconstruction method using calcined samples Fe-hydrotalcite and by anion exchange method (co-precipitation) using MgAl-hydrotalcite and ZnAl-hydrotalcite parent samples. The amount of drug (amoxicillin, metronidazole, ampicillin or vitamin C) was optimized to overcome 2 times the anionic exchange capacity of the clay. The hydrotalcite was added to a 100 ml solution of drug dissolved in deionized water. The final suspension was vigorously stirred at 60°C for 24 hours, filtered, washed with distilled water and dried at 40°C. Thus, amoxicillin-Fe-hydrotalcite, metronidazole-MgAl-hydrotalcite, ampicillin-MgAl-hydrotalcite, ampicillin-ZnAl-hydrotalcite and vitamin C-ZnAl-hydrotalcite were synthetized.

Structure of preparations was analyzed using SEM, FTIR spectroscopy XRD and adsorption-desorption isotherms.

Results

SEM analyses of Fe/ MgAl/ ZnAl-hydrocalcites shows a typical morphology of particles, with approximately hexagonal shape and wide cavities between particles. Mean value of particles size was found to be 100 nm for Fe-hydrocalcite, 130 nm for MgAl-hydrocalcite and 90 nm for ZnAl-hydrocalcite. After drug intercalation in hydrotalcite, SEM micrographs indicate a compact, nonporous solid structure, a stronger cohesion between the particles, being the result of changing the initial hydrotalcite type structure (fig. 56).
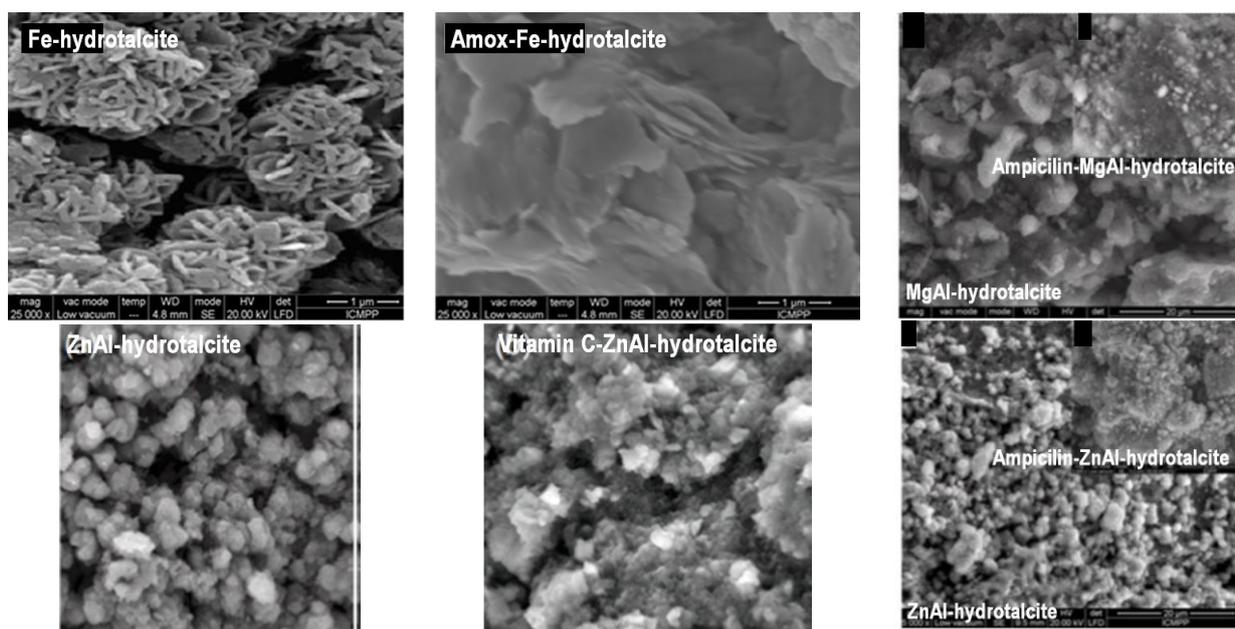

**Fig. 56. SEM micrographs of obtained preparations**

FTIR analyses shows the typical peaks of hydrotalcites attributed to the stretching vibrations to the -OH groups, nitrate anions and M-O and MO-H groups layers in the hydrotalcite layer. After the drug intercalation in layered structure, several peaks were modified; others disappeared, being attributed to the stretching vibrations of the C-C, C-O and amide NH groups, confirming the binding of the antibiotic molecules to the hydrotalcite layers (fig. 57).

XRD analyses of hydrotalcites shows sharp and symmetrical peaks and few high-angle asymmetrical peaks, in agreement with the hydrotalcite type materials. After drug is released in an aqueous solution containing 0.2 M NaCl (fig. 58b) or 0.01 M and 0.1 M Na_2CO_3 (fig. 59b), the diffraction peaks have typical hydrotalcite feature.

Adsorption-desorption isotherms shown ununiformed small pores, mesoporous features being well defined by the particle shape and size and by the particle interconnect ways. After drug intercalated, pore size distribution curve is larger and modified, belonging to area of mesoporous characteristics. A reflection peak reveals the initial hydrotalcite phase specific to hydrotalcite structure. For hybrid structure, it is observed that the peaks remained unchanged after drug intercalation, but angle was stronger, implying a decrease of hydrotalcite interlayer distance (fig. 58c).

a) by anion exchange route   b) by reconstruction method
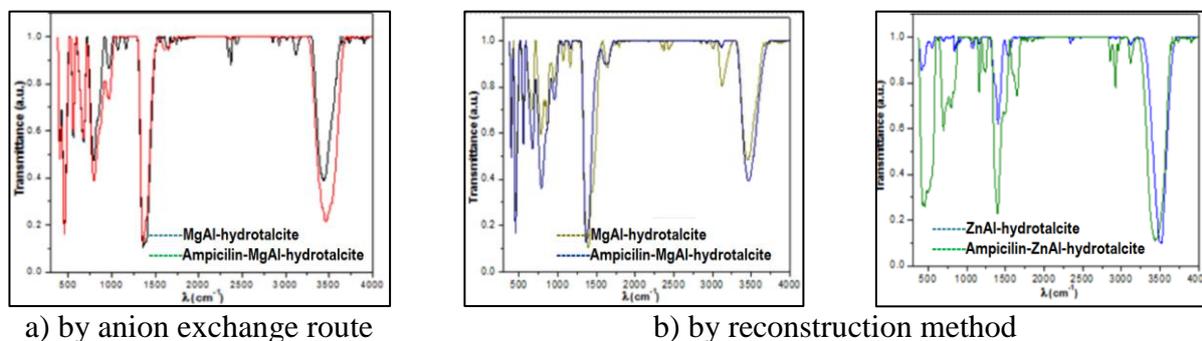
Fig. 57. FTIR spectra of hydrotalcite particles intercalated with ampicillin

a) FTIR spectra   b) XRD pattern   c) release profile of antibiotic
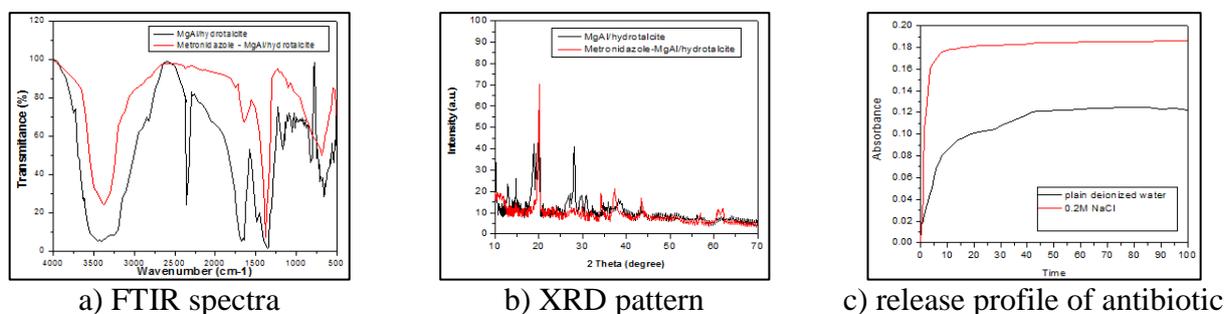
Fig. 58. Analyses of MgAl-hydrotalcite and metronidazole-MgAl-hydrotalcite

a) FTIR spectra   b) XRD pattern
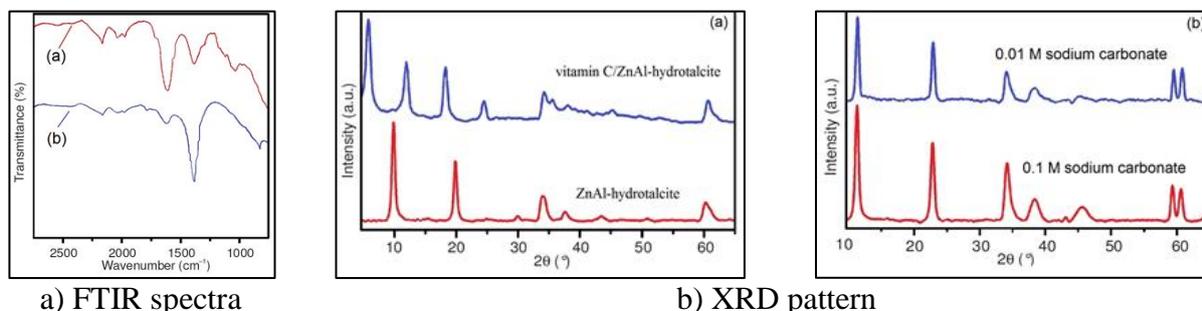
Fig. 59. Analyses of ZnAl-hydrotalcite and vitamin C-ZnAl-hydrotalcite

Discussions

Hydrotalcite was first described in 1842, and is a Layered Double Hydroxide (LDH) whose name is derived from its resemblance with talc and its high water content, being a class of anionic clays. The hydrotalcite has a porous structure, 3-layer rhombohedral (3R polytype) or 2-layer hexagonal (2H polytype). The carbonate anions located in the interlayer region are weakly bound, so hydrotalcite has anion exchange capabilities (Meyn *et al.*, 1990; Calabrese *et al.*, 2013). Hydrotalcites can act as inorganic vectors for different organic molecules negatively charged and intercalated into those.

Many negatively charged biomolecules can be incorporated between layers as charge compensating anions by ion exchange route or co-precipitation (Cavani *et al.*, 1991; Hwang *et al.*, 2001; Khan *et al.*, 2001). Co-precipitation is a very easy and convenient way to synthesize metal

oxides particles from aqueous metal salts solutions by the addition of a base under inert atmosphere at room/ elevated temperature (He *et al*., 2005; Gautam & Chattopadhyaya, 2016).

Amoxicillin and clavulanic acid are the only orally administered antimicrobials with adequate pharmacokinetic properties to be effective against the most commonly oral pathogens for the treatment of oro-facial infections (Dar-Odeh *et al*., 2010; Garea *et al*., 2010 *et al*., 2014). Ampicillin is one of the largest used spectrum antimicrobial agents used due to its irreversible bactericidal effect (Suárez *et al*., 2009; Parveen *et al*., 2012; Ball *et al*., 2013). Doxycycline has a wide use for the treatment of periodontal diseases, but because of bacterial resistance it is preferably to use metronidazole, which is a selective agent against anaerobic bacteria.

Saliva has many functions in oral cavity such as protective, digestive and trophic ones. There are individuals with impaired salivary secretion (or affected by xerostomia), which produces dry mouth, difficult speaking and masticating, diminishing the sense of taste and receptivity of dental caries, gingivitis mucosal lesions and infections vulnerabilities (Sahoo *et al*., 2003; Shafik *et al*., 2005; Mese *et al*., 2007). L-Ascorbic acid (vitamin C) is an important compound for human body, water soluble, having a wide variety functions, such as collagen biosynthesis, photo-protection, melanin reduction, antiviral effect and stimulation of salivary secretion, immunity increased, cancers and cardiovascular diseases risk decreased and prevents anaemia (Sahoo *et al*., 2003; Eder *et al*., 2004; Hutchinson *et al*., 2011).

In these experiments, drug active molecules were intercalated in the inner-spaces of layered double hydroxides, by co-precipitation method. The new synthesized hybrid materials were analyzed through different advanced techniques. The hybrid structure remained unchanged after drug intercalation. The drug intercalation into the hydrotalcite layers is reversible, the drug having a controlled released. This property is based on the phenomenon called "memory effect" which involves the regeneration of the hydrotalcite initial structure from their calcined form, when is dispersed in an aqueous solutions containing suitable anion (Miyata, 1980; Stanimirova *et al*., 2001; Arco *et al*., 2004). These results provide the implementation of antibiotic therapeutic concentration in the action site and reductions of drug side effects while ensuring the patient's oral health. Also, vitamin C has been successfully embedded in the hydrotalcite interlayer gallery due to strong hydrogen bond and electrostatic attractions. The study revealed that the ascorbic acid is stabilized in the hydrotalcite interlayer space, without any changes in its structure and can be controlled released. Therefore, ZnAl-hydrotalcite was an excellent host for vitamin C storage and an efficient delivery vehicle for this active molecule that is unstable under normal environmental conditions. Interlayer region of drug-hydrotalcite matrix can be regarded as a micro-vessel in which active molecules may be stored and then released by an intercalation action in a medium containing other anions.

Conclusions

In these experiments, new hybrid materials were synthetized with the controlled release of drugs with anti-microbial act for infections of oral cavity; and for stimulating salivary secretion. The stability of drug was significantly increased after they incorporation into the interlayer space. Structural and morphological analyses showed that anions of active substances were intercalated in the interlayer of hydrotalcite and had no effect on the hydroxide lattice of hydrotalcite, so this new material has a good drug local sustained release in the oral cavity and can be used with success for those indications.

SECTION II
DIRECTIONS FOR FUTURE RESEARCH

The development of my professional, research and academic career will contribute to the constant improvement of the field of orthodontics. These three directions are closely linked due to the fact that medical education requires direct contact with the patient, and scientific research without clinical trials has no purpose.

Directions for future research in this area will be sketched in the context of the first century of the 3rd millennium, marked by economic, social, cultural, political, educational and climate transitions and changes, as well as major technological, IT and medical advances.

The objectives of the future research are to continue the themes presented in this thesis and to develop new ones as I get original results in working teams with my colleagues, students, residents and professionals from other related fields.

1. The profile of the future orthodontic patient and the performance of the medical team

The place of the orthodontic practice in the context of the practice of dental medicine depends on: the incidence of dento-maxillary anomalies in the future population; the prevalence of Angle Class of malocclusions; the theoretical and practical, universitary and postgraduate training of students in dental medicine; practitioners performances on invisible techniques agreed by patients; the risks of therapeutic procedures that produce different types of anxiety in patients; and the equipment of the dental offices with advanced 3D technologies. All these aspects of the profile of the future orthodontic patient and the performance of the medical team may be future research themes, correlated with the future socio-economic and demographic factors of the population.

2. Future health education and its impact on children's quality of life

Health and the education of the population are important parameters of a society, reflected on the standard of living and life expectancy of individuals. In current medical view, dento- maxillary anomaly is not considered a disease but a deviation from normal and is more a part of aesthetic medicine. The impact of dento-facial aesthetics in human relationships can positively/ negatively affect the socio-economic evolution of the individual, providing benefits/ prejudice to personal and psychological well-being. The most common reasons of orthodontic patients for therapy are aesthetic, social or economic (acceptance condition in some special high schools, marriage, and job). Therefore, adolescent/ younger patients are motivated for orthodontic therapy, but most children are influenced by the authority of their parents. These aspects of child health education and its impact on quality of life can be future research topics, correlated with information and knowledge gained through social media networks and user-generated content.

3. Etiopathogenic and therapeutic implications of dental and eruption anomalies

Until today, studies have shown that the etiopathogenesis of number anomalies, especially hypodontia, is not clear and new genes involved are discovered. Thus, the balance leans on the part of the anatomical theory of the mutations genes responsible for the development of the teeth, rather than on the part of the evolutionary theory of the numerical reduction of teeth. At the same time, researchers are synthesizing teeth in laboratory from stem cells, and the question arises whether there will be dental anomalies or will there be many more? Practitioners give the best therapy solutions for the arches to have a normal aspect, but they are not ideal solutions for all the tissues involved in this imbalance created by hypodontia. On the other hand, the mechanism of the dental eruption is not elucidated and various factors are criticized for the occurrence of eruption anomalies. In this case, dento-alveolar surgery is the best solution for eruption anomalies, especially the discovery of the impacted teeth, and the orthodontic traction helps the tooth eruption. In both cases (hypodontia and impaction) there are risks caused by these treatments. All these themes can be researched in the future by multidisciplinary teams to solve these problems.

4. Diagnostic and therapeutic management of the patient with dento-maxillary anomalies

The United Nations Organization estimates that the world population will reach 9.8 billion by 2050. Most of this increase will take place in poorer countries in the world, and combined with the effects of global warming can lead to large migrations. We also witness a new biological age for the human species since the first genetically modified children appeared, which raises great controversy among scholars. The advances in technology, such as ultrasound, prenatal genetic testing, and genetic engineering, change the demographics and genetic potential of the human population. Thus, through selective abortion, fewer girls are born and the number of men is rising. In this context, we can hope that: patients with dento-maxillary anomalies will increase proportionally with the population; the number of dental crowding by dento-maxillary discrepancies will increase due to the crossing between human races; male patients will be more than women. Or we may be sceptical because there will be no patients with dento-maxillary anomalies due to controlled genetic changes or other types of anomalies will appear. In both cases investigation possibilities should be improved according with advanced imagery and digital technology as well as therapeutic possibilities to establish a complete early diagnosis with minimal time and cost. All these aspects of the diagnostic and therapeutic management of the patient with dento-maxillary anomalies are interesting long-term research topics that can be correlated with socio-demographic and environmental factors.

5. The allergic potential of new orthodontic biomaterials

Every day, dental materials companies send us the latest and most performing components of orthodontic appliances for the orthodontic therapies and the aesthetics of the orthodontic patient. The dental industry also markets products for every wallet, but not always of the wanted quality. On the other hand, new orthodontic techniques with new alloy arches, customized accessory elements and 3D insertion methods appear. At the same time, patients allergic to different foods, medicines, etc. are becoming more common in dental offices. In this context, new research directions are emerging on the allergic potential of new orthodontic biomaterials, if we apply the principle that each organism is unique and reacts individually. It is also possible to perform mathematical and computer modelling of the body's reactions to the orthodontic material allergens using fractal geometry applications to elucidate certain dynamic phenomena in this field.

6. Use of nano-particles in dental medicine

Biomaterials used during orthodontic treatment must have certain qualities to ensure therapeutic effect, to be resistant to salivary environment, to have only the desired interactions with adjacent tissues, and not to cause side effects. The effect of corrosion of nickel or iron alloys in salivary media is already proven. Manufacturing companies have found solutions for the biodegradation and biocompatibility of metals (arches, bands, brackets), by using nano-particles of different materials deposited on the metal substrate and have also given a physiognomic effect. On the other hand, the new surface coatings of materials used in orthodontics (mini-implants) allowed deposition of nano-particles layers of bioactive and biocompatible materials to optimize bio-integrative properties in tissues or anti-infectives. However, the ideal materials of these particles have not been found. Experimental research can continue on these topics to find optimal materials with these properties and to test the properties on biological tissues by working together in multidisciplinary mixed teams.

SECTION III
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