ORIGINAL ARTICLE

Different Profile of Peripheral Antioxidant Enzymes and Lipid Peroxidation in Active and Non-active Inflammatory Bowel Disease Patients

D. Achitei · A. Ciobica · G. Balan · E. Gologan ·
C. Stanciu · G. Stefanescu

Abstract

Background   The role of oxidative stress in inflammatory bowel diseases (IBD) has been extended lately from a simple consequence of inflammation to a potential etiological factor, but the data are still controversial. Active disease has been characterized before by an enhanced production of reactive oxygen species and the increased peroxidation of lipids, but patients in remission were generally not considered different from healthy people in terms of oxidative stress.

Aims   We evaluated the antioxidant defense capacity and lipid peroxidation status in the serum of patients with active and non-active disease compared with healthy matched control subjects.

Methods   The study included 20 patients with confirmed IBD in clinical and biological remission, 21 patients with active disease, and 18 controls. We determined the serum levels of two antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX), and a lipid peroxidation marker, malondialdehyde (MDA).

Results   Active disease patients had an increased activity of both SOD and GPX, as well as significant high values of MDA versus controls. Furthermore, patients being in remission had significantly lower values of antioxidant enzymes (SOD and GPX) and increased lipid peroxidation measured by MDA serum levels, as compared with healthy control subjects.

Conclusions   Our study confirmed the presence of high oxidative stress in active IBD. More importantly, we have demonstrated a lower antioxidant capacity of patients in remission versus control group. This may represent a risk factor for the disease and can be an additional argument for the direct implication of oxidative stress in the pathogenesis of IBD.

D. Achitei · G. Balan · E. Gologan · G. Stefanescu
"Gr. T. Popa" University of Medicine and Pharmacy,
16 Universitatii Street, 700115 Iasi, Romania
e-mail: dorin.achitei@gmail.com

G. Balan
e-mail: drbalanumfiasi@yahoo.com

E. Gologan
e-mail: elenagologan2007@yahoo.com

G. Stefanescu
e-mail: gabriela.stefanescu@gmail.com

A. Ciobica (✉)
Department of Biology, "Alexandru Ioan Cuza" University,
Bd. Carol I, nr. 11, 700506 Iasi, Romania
e-mail: alin.ciobica@uaic.ro

A. Ciobica · C. Stanciu
Center of Biomedical Research of the Romanian Academy,
Iasi Branch, Bd. Carol I, nr. 8, 700505 Iasi, Romania
e-mail: stanciucarol@yahoo.com

Introduction

It has been demonstrated that oxidative stress is involved in the pathogenesis of various metabolic, inflammatory, or tumoral diseases [1]. Inflammatory bowel diseases (IBD) are chronic conditions characterized by the inflammation of the colon mucosa (ulcerative colitis) or of any part of the digestive tract (Crohn's disease). Their evolution is usually marked by active phases and periods of remission. A lot of progress has been made in understanding the etiopathogenic pathways of IBD, but the exact causes remain unestablished [2, 3]. With the genetic, environmental, and immunological theories being the most consistent,

oxidative stress began to be considered a potential etiologic or triggering factor [4, 5].

The activated immune cells of the intestinal mucosa release numerous reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals [6]. These are highly reactive molecules that can produce tissue injury, especially if the antioxidant defense system is lowered or inefficient [7]. It has recently been mentioned that ROS themselves can lead to the accumulation of inflammatory cells, being not only a consequence but a trigger factor for inflammation [8]. However, there is not yet sufficient evidence for this theory.

Increased markers of oxidative stress (oxygen free radicals and lipid peroxides), as well as decreased antioxidant defense, had been demonstrated in many studies involving IBD patients and in a variety of biological systems (colon mucosa, plasma, serum, fecals, saliva) [9–11], but there are also studies in which no difference was observed [12, 13]. These modifications of oxidative stress parameters were generally established for patients with active disease compared with control groups, while patients in clinical and biological remission were not proved to be different from normal subjects in terms of antioxidant defense or lipid peroxidation [14, 15]. Still, the number of studies examining the oxidative stress in different stages of IBD remains small.

The aim of our work was to evaluate the specific activity of two peripheral antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX), and a lipid peroxidation marker, malondialdehyde (MDA), in the serum of patients with active and non-active IBD compared with matched control subjects. Secondly, we compared the oxidative stress markers between Crohn's disease and ulcerative colitis patients within the 2 main groups (active and inactive disease). Finally, we correlated the three oxidative stress parameters with C reactive protein (CRP) as a systemic inflammation marker.

Methods

We performed a prospective study which included 41 consecutive patients with a confirmed diagnosis of IBD recruited from the gastroenterology unit of St. Spiridon University Hospital, Iasi, Romania. Twenty-seven of them were suffering from ulcerative colitis (UC) and 14 had Crohn's disease (CD). They were divided into the active disease group (21 subjects) and the remission group (20 subjects). The remission was defined as a Crohn's Disease Activity Index less than 150 (for CD) or a Mayo total score less than 3 (for UC) [16, 17]. The control group consisted in 18 healthy subjects (students, hospital staff) matched to the patients by age, sex, and body mass index. Because of the known influence of smoking habits in oxidative stress

status, the percentage of smokers was also considered in the selection of control subjects [18].

The ongoing medication of the patients was not suspended before the tests. Those with other chronic decompensated diseases were excluded. All subjects taking antioxidant supplements were also excluded.

The analysis of covariance showed that the patients in remission and the active disease group did not differ significantly from the healthy comparison subjects with respect to age, gender, smoking habits, and BMI. We also observed no significant differences between the remission and active groups in terms of timespan from the initial diagnosis, the percentage of Crohn's disease or ulcerative colitis. Additionally, no significant differences were seen in the case of medication type within groups (Table 1).

The study was conducted according to the provisions of the Helsinki Declaration and was approved by the local ethics committee. All subjects gave their written informed consent.

Blood samples were collected in the morning, before breakfast, allowed to clot and centrifuged immediately. Serum was aliquoted into Eppendorf tubes and stored at −40 °C prior to measurement. All samples were measured in duplicate and averaged.

Determination of SOD

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water-soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide anions) after 20 min of reaction time at 37 °C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Determination of GPX

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

Determination of MDA

Malondialdehyde levels were determined by thiobarbituric acid reactive substances (TBARS) assay. 200 μL of serum

Table 1 Demographic and clinical data in the control, remission and active disease groups

	Control (n = 18)	Remission (n = 20)	Active disease (n = 21)	F^b	P value[b]
Age[a]	36.39 ± 13.7	35.5 ± 14.6	34.71 ± 11	0.08	0.924
Gender (male/female)	8/10	11/9	13/8	1.1	0.263
Active smokers	33.3 %	30 %	33.3 %	0.55	0.577
BMI[a]	24.2 ± 3.9	23.4 ± 3.7	21.7 ± 4.8	2.11	0.069
Time from initial					
Diagnosis (years)[a]	–	4.75 ± 5.53	3.47 ± 4.33	0.68	0.415
Crohn's disease (%)	–	30 %	38.1 %	1.3	0.077
Ulcerative colitis (%)	–	70 %	61.9 %	1.3	0.08
Medication					
5-ASA	–	75 %	61.9 %	2.3	0.057
Corticosteroids	–	20 %	33.3 %	2.19	0.06
Azathioprine	–	15 %	4.7 %	2.03	0.095
Biological agents	–	15 %	9.5 %	1.7	0.112

[a] Each value represents mean ± standard deviation

[b] Analysis of covariance

was added and briefly mixed with 1 mL of trichloroacetic acid at 50 %, 0.9 mL of Tris–HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73 %. After vortex mixing, samples were maintained at 100 °C for 20 min. Afterwards, samples were centrifuged at 3,000 rpm for 10 min and the supernatant read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed as nmol/ml [19, 20].

Determination of CRP

Serum levels of C-reactive protein (CRP) were measured by immuno-turbidimetric automated assay using the Hitachi 912 ISE system.

Data Analysis

The results for antioxidant enzymes activity and MDA level were analyzed using one-way ANOVA. All results are expressed as mean ± SEM. Post hoc analyses were performed using Tukey's honestly significant difference test in order to compare active and non-active IBD groups. Crohn's disease and ulcerative colitis patients were analyzed separately within the active and inactive groups also using one-way ANOVA. $P < 0.05$ was regarded as statistically significant. Pearson's correlation coefficient was used to evaluate the connection between the oxidative stress parameters and CRP. As above, only values for which $P < 0.05$ were considered statistically significant.

Results

Regarding the specific activity of SOD, which is the first line of defense against oxidative stress development, we observed a significant decrease in the remission group
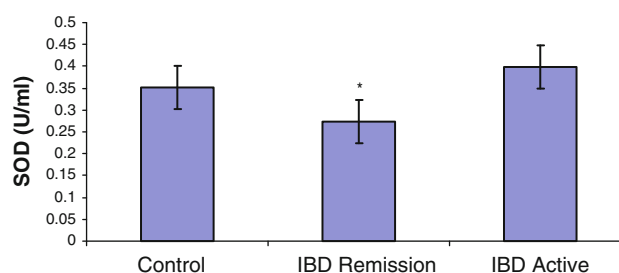

Fig. 1 Superoxide dismutase specific activity in the serum of control, IBD remission, and IBD active subjects. The values are mean ± SEM ($n = 18$ in control, 20 in IBD remission and 21 IBD active group). *$P = 0.03$

($F_{1,36} = 5$, $P = 0.03$), as compared to the control group (Fig. 1). Active disease patients had higher levels of SOD then control subjects, but this difference was not statistically significant ($F_{1,37} = 1$, $P = 0.27$). Additionally, post hoc analysis showed significant differences between the remission IBD group and the active group ($P = 0.004$).

When analyzing the subgroups of Crohn's Disease and ulcerative colitis, we noticed that general findings were more pronounced in CD patients, with significant decrease in SOD activity in remission patients versus the control group ($F_{1,22} = 8.03$, $P = 0.009$) and higher values of SOD in active disease compared with UC patients ($P = 0.36$). However, statistical analysis revealed no significant difference between the subgroups of CD and UC in remission ($P = 0.07$) or in active phase ($P = 0.35$).

Detailed results of the examined enzymes in study groups expressed as means ± SEM and P values are featured in Table 2.

Similarly, in the case of the other antioxidant enzyme (GPX), we noticed a significant decrease in its specific activity in the remission group by comparison with the control group ($F_{1,36} = 7$, $P = 0.01$). As in the case of

Table 2 Specific activity of SOD, GPX and MDA in active and non-active disease compared with control subjects

	SOD (U/ml)	P	GPX (U/ml)	P	MDA (nM/ml)	P
Control ($n = 18$)	0.351 ± 0.028		0.0167 ± 0. 0024		14,183 ± 0.712	
Non-active disease ($n = 20$)	0.273 ± 0.026	0.036*	0.0101 ± 0.0010	0.013*	15.473 ± 0.992	0.302
CD ($n = 6$)	0.202 ± 0.025	0.009*	0.0099 ± 0.002	0.134	14.517 ± 1.896	0.839
UC ($n = 14$)	0.304 ± 0.032	0.287	0.0101 ± 0.0013	0.035*	15.883 ± 1.165	0.202
Active disease ($n = 21$)	0.399 ± 0.030	0.274	0.0194 ± 0.0025	0.232	16.720 ± 1.148	0.047*
CD ($n = 8$)	0.436 ± 0.048	0.132	0.0183 ± 0.0026	0.682	16.760 ± 1.791	0.117
UC ($n = 13$)	0.376 ± 0.039	0.610	0.0200 ± 0.0038	0.444	16.695 ± 1.491	0.108

Separate values are given for CD and UC subgroups

Each value represents mean ± SEM

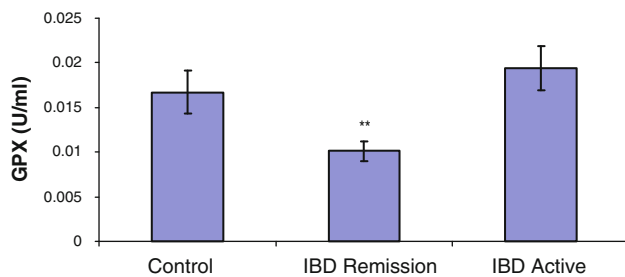*P value considered statistically significant if < 0.05

Fig. 2 Glutathione peroxidase specific activity in the serum of control, IBD remission, and IBD active subjects. The values are mean ± SEM ($n = 18$ in control, 20 in IBD remission and 21 in IBD active group). **$P = 0.01$
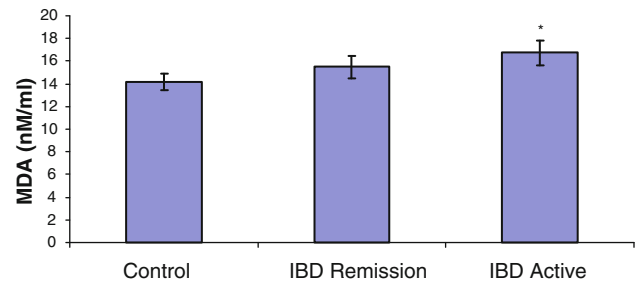

Fig. 3 Malondialdehyde concentration in the serum of control, IBD remission, and IBD active subjects. The values are mean ± SEM, ($n = 18$ in control, 20 in IBD remission and 21 in IBD active group). *$P = 0.04$

SOD, the active IBD group showed higher levels of GPX specific activity, but this difference did not reached the threshold of statistical significance ($F_{1,37} = 2$, $P = 0.23$) (Fig. 2). Post hoc analysis also revealed significant differences between the remission group versus the active group ($P = 0.002$).

The subgroups of CD and UC subjects showed very similar behavior of GPX-specific activity, with significant decrease in remission and consistent but still insignificant increase in active phase. In post hoc analysis, the differences between active and non-active patients were maintained for each subgroup ($P = 0.03$ for CD and 0.01 for UC).

Concerning the lipid peroxidation, a significant increase of the MDA concentration was observed in the active IBD group, when compared to control subjects ($F_{1,37} = 5$, $P = 0.04$). Still, no significant changes were noticed in the case of the remission group, when compared to healthy control subjects ($F_{1,36} = 1$, $P = 0.3$) (Fig. 3). Also, unlike the other two measurements, post hoc analysis revealed no significant differences between the remission IBD group and the active group ($P = 0.408$). In the subgroups of Crohn's disease and UC patients, no statistical difference was noticed between the two diseases in remission ($P = 0.53$) or in active phase ($P = 0.93$).

Analyzing the correlation between the three measured enzymes with the value of serum CRP, we observed in the case of GPX a small and non-significant positive correlation ($n = 41$, $r = 0.185$, $P = 0.245$). In contrast, the SOD levels had a moderate positive correlation with CRP which proved to be significant ($n = 41$, $r = 0.358$, $P = 0.021$). In the same way, MDA levels were positively correlated with systemic inflammation as measured by CRP values ($n = 41$, $r = 0.362$, $P = 0.020$).

Discussion

In this study, we demonstrate a significant modification of oxidative stress markers in the serum of patients with active IBD compared to matched healthy people. We also found that patients with non-active disease have a low antioxidant profile and increase lipid peroxidation.

SOD is an essential antioxidant enzyme which detoxifies the superoxide anion (O_2^-), generated by activated neutrophils and macrophages, by converting it to hydrogen peroxide (H_2O_2) [21]. GPX acts further in the extracellular environment to transform H_2O_2 into O_2 and H_2O [7, 22]. Along with catalase, a cellular active enzyme, SOD, and

GPX represent the main markers of antioxidant defense [23]. MDA, a thiobarbituric acid reactive breakdown product, results from the peroxidation of polyunsaturated fatty acids and from the metabolism of arachidonic acid and it has been used intensely as a lipid peroxidation marker [15, 24].

In patients with active intestinal inflammation, we found increased values of peripheral antioxidant enzymes (GPX and SOD) with a very similar pattern. This points towards an intense production of ROS due to mucosal injury. Conflicting data are available up to the present, with many previous studies having similar findings [13–15], but there were also authors who observed no difference [25, 26].

In contrast, the patients in clinical and biological remission not only had significant lower values of SOD and GPX than the active patients but they also presented a statistical relevant decrease of these antioxidant enzymes, when compared to the control group. The similar distribution of both markers supports our conclusion. There are a few authors who also found significant lower values of SOD and/or GPX in the serum of patients with IBD in remission [26, 27], with the majority of studies having documented no significant differences [14, 15]. However, there have been a few studies examining the antioxidant enzymatic system in different activity phases of IBD [14], but the number of patients enrolled in most studies was relatively small. GPX is a selenium-dependent enzyme, and therefore the low levels of plasma selenium found in IBD patients could explain our findings [27, 28]. Moreover, when examining the antioxidant defense in the colon mucosa, many authors revealed decreased levels of these enzymes [9, 29, 30].

A possible explanation of the low GPX and SOD in remission patients could be the consumption of antioxidants during the active phases. Another and more tempting hypothesis is that the patients suffering from IBD, even if they are in remission, have a low antioxidant defense. This depletion could be present even before the onset of disease. In this way, lower antioxidant enzymatic capacity could represent a risk factor for the development of ulcerative colitis and Crohn's disease. Many patients in remission do not reach mucosal healing and have continuous intestinal inflammation [31]. Thus, a poor antioxidant defense could explain the continuing cellular damage.

Ulcerative colitis and Crohn's disease patients had very similar values of GPX activity. However, SOD activity had an intense variation during the active and non-active phases in Crohn's disease patients, with a marked increase in active disease and significant decrease in remission. Those findings were less evident in ulcerative colitis. Considering the systemic nature of Crohn's disease and the fact that SOD is the first enzyme intervening in the antioxidant chain, our findings could be justified. Still, the small

number of CD subjects should call for caution in interpretation.

Along with the antioxidant enzymes, lipid peroxidation, as tested by the serum level of MDA, was significantly increased in patients with active IBD. With an elevated number of ROS as demonstrated through high SOD and GPX values, the tissue injury due to structural lipids alteration could be a logical consequence, as shown by other work groups [13, 14, 24]. There are also studies which did not indicate any difference in peripheral MDA levels between active disease and control groups [15, 32]. We also found that remission patients had a higher MDA serum level, but the difference was not statistical significant.

As the volume of evidence regarding the involvement of oxidative stress in IBD has been growing, a lot of interest has been paid to antioxidant treatment. Various antioxidants were proposed as supplements for patients with inflammatory colitis (vitamins, unsaturated fatty acids, N-acetyl-L-cysteine). The results in increasing total antioxidant capacity are mostly positive [27, 33]. A lecithinized superoxide dismutase was used with good results in improving the inflammation in ulcerative colitis [34]. There is still little or no evidence of the antioxidant influence on preventing the relapses or complications, or influencing the course of the disease.

There are of course several limitations to our study which include the relatively small size of the groups we used and also the fact that the specific medication was not stopped when performing the study. Even though the percentage of smokers has not statistically influenced the study, it is preferable to avoid enrolling active smokers when investigating oxidative stress.

Future studies should definitely consider taking into account all the factors that may cause oxidative stress to increase homogeneity of the treated groups. Also, studies investigating effects of antioxidant supplementation on the evolution of IBD seem warranted.

To conclude, in this study, we have demonstrated an increased oxidative stress and lipid peroxidation in active IBD patients. We have also shown that patients with non-active disease have a lower antioxidant defense than healthy people. This could be a consequence of the disease but can also be interpreted as a pre-existing condition. In this last hypothesis, dysfunction in antioxidant capacity may be considered a predisposing factor for developing IBD and thus can be an argument for the etiological theory of oxidative stress in IBD.

References

1. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 4th ed. New York: Oxford University Press; 2007.
2. Scarpa M, Stylianou E. Epigenetics: concepts and relevance to IBD pathogenesis. *Inflamm Bowel Dis*. 2012;18:1982–1996.
3. Glocker E, Grimbacher B. Inflammatory bowel disease: is it a primary immunodeficiency?. *Cell Mol Life Sci*. 2012;69:41–48.
4. Harris ML, Schiller HJ, Reilly PM, et al. Free radicals and other reactive oxygen metabolites in inflammatory bowel disease. Cause, consequence or epiphenoma? *Pharmacol Ther*. 1992;53:375–408.
5. Rezaie A, Parker RD, Abdollahi M. Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause? *Dig Dis Sci*. 2007;52:2015–2021.
6. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474:307–317.
7. Kruidenier L, Verspaget HW. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease—radicals or ridiculous? *Aliment Pharmacol Ther*. 2002;16:1997–2015.
8. Hendrickson BA, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev*. 2002;15:79–94.
9. Lih-Brody L, Powell SR, Collier KP, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci*. 1996;41:2078–2086.
10. Koutroubakis IE, Malliaraki N, Dimoulios PD, Karmiris K, Castanas E, Kouroumalis EA. Decreased total and corrected antioxidant capacity in patients with inflammatory bowel disease. *Dig Dis Sci*. 2004;49:1433–1437.
11. Kruidenier L, Kuiper I, Van Duijn W, et al. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. *J Pathol*. 2003;201:17–27.
12. Durak I, Yasa MH, Bektas A, et al. Mucosal antioxidant defense is not impaired in ulcerative colitis. *Hepatogastroenterology*. 2000;47:1015–1017.
13. Tüzün A, Erdil A, Inal V, et al. Oxidative stress and antioxidant capacity in patients with inflammatory bowel disease. *ClinBiochem*. 2002;35:569–572.
14. Maor I, Rainis T, Lanir A, Lavy A. Oxidative stress, inflammation and neutrophil superoxide release in patients with Crohn's disease: distinction between active and non-active disease. *Dig Dis Sci*. 2008;53:2208–2214.
15. Beltrán B, Nos P, Dasí F, et al. Mitochondrial dysfunction, persistent oxidative damage, and catalase inhibition in immune cells of naïve and treated Crohn's disease. *Inflamm Bowel Dis*. 2010;16:76–86.
16. Freeman HJ. Use of the Crohn's disease activity index in clinical trials of biological agents. *World J Gastroenterol*. 2008;14:4127–4130.
17. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm Bowel Dis*. 2008;14:1660–1666.
18. Hritcu L, Ciobica A, Gorgan L. Nicotine-induced memory impairment by increasing brain oxidative stress. *Cent Eur J Biol*. 2009;4:335–342.
19. Padurariu M, Ciobica A, Dobrin I, Stefanescu C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. *Neurosci Lett*. 2010;479:317–320.
20. Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neurosci Lett*. 2010;469:6–10.
21. Bild W, Ciobica A, Padurariu M, Bild V. The interdependence of the reactive species of oxygen, nitrogen and carbon. *J Physiol Biochem*. 2012;. doi:10.1007/s13105-012-0162-2.
22. Pietarinen-Runtti P, Lakari E, Raivio KO, Kinnula VL. Expression of antioxidant enzymes in human inflammatory cells. *Am J Physiol Cell Physiol*. 2000;278:C118–C125.
23. Hata Y, Kawabe T, Hiraishi H, et al. Antioxidant defenses of cultured colonic epithelial cells against reactive oxygen metabolites. *Eur J Pharmacol*. 1997;321:113–119.
24. Alzoghaibi MA, Al Mofleh IA, Al-Jebreen AM. Lipid peroxides in patients with inflammatory bowel disease. *Saudi J Gastroenterol*. 2007;13:187–190.
25. Akman T, Akarsu M, Akpinar H, Resmi H, Sezer E. Erythrocyte deformability and oxidative stress in inflammatory bowel disease. *Dig Dis Sci*. 2012;57:458–464.
26. Reimund JM, Hirth C, Koehl C, Baumann R, Duclos B. Antioxidant and immune status in active Crohn's disease: a possible relationship. *Clin Nutr*. 2000;19:43–48.
27. Geerling BJ, Badart-Smook A, van Deursen C, et al. Nutritional supplementation with N-3 fatty acids and antioxidants in patients with Crohn's disease in remission: effects on antioxidant status and fatty acid profile. *Inflamm Bowel Dis*. 2000;6:77–84.
28. Hinks LJ, Inwards KD, Lloyd B, Clayton B. Reduced concentrations of selenium in mild Crohn's disease. *J Clin Pathol*. 1988;41:198–201.
29. Dagli U, Balk M, Yucel D, et al. The role of reactive oxygen metabolites in ulcerative colitis. *Inflamm Bowel Dis*. 1997;3:260–264.
30. Tsunada S, Iwakiri R, Ootani H, Aw TY, Fujimoto K. Redox imbalance in the colonic mucosa of ulcerative colitis. *Scand J Gastroenterol*. 2003;38:1002–1003.
31. Lichtenstein GR, Rutgeerts P. Importance of mucosal healing in ulcerative colitis. *Inflam Bowel Dis*. 2010;16:338–346.
32. Barbosa DS, Cecchini R, El Kadri MZ, Rodríguez MA, Burini RC, Dichi I. Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty-acids. *Nutrition*. 2003;19:837–842.
33. Aghdassi E, Wendland BE, Steinhart AH, Wolman SL, Jeejeebhoy K, Allard JP. Antioxidant vitamin supplementation in Crohn's disease decreases oxidative stress: a randomized controlled trial. *Am J Gastroenterol*. 2003;98:348–353.
34. Suzuki Y, Matsumoto T, Okamoto S, Hibi T. A lecithinized superoxide dismutase (PC-SOD) improves ulcerative colitis. *Colorectal Dis*. 2008;10:931–934.