JMB 2010; 29 (2) DOI: 10.2478/v10011-010-0013-z

UDK 577.1 : 61 **ISSN 1452-8258**

JMB 29: 102–106, 2010

Original paper
Originalni naučni rad

OXIDATIVE PRODUCTS OF PROTEINS AND ANTIOXIDANT POTENTIAL OF THIOLS IN GASTRIC CARCINOMA PATIENTS

PROIZVODI OKSIDACIJE PROTEINA I ANTIOKSIDATIVNI POTENCIJAL TIOLA KOD OBOLELIH OD GASTRIČNOG KARCINOMA

Anita Mukthapura[1], Avinash Sidhuraju Shimogga[2], Vinodchandran Sudha K[3], Beena V Shetty[3], Gayathri M Rao[3]

[1]Department of Biochemistry, Banglore Medical College, Bangalore, India
[2]Fr. Muller's Medical College
[3]Kasturba Medical College, Mangalore, India

Summary: It has been suggested that oxidative stress defined as a shift in antioxidant/oxidant balance towards oxidants is associated with the pathogenesis of many diseases, including carcinogenesis. Reactive oxygen species can induce carcinogenesis via injury to macromolecules such as DNA, carbohydrates and proteins. Forty primary gastric carcinoma patients and 40 healthy controls were included in the study. Advanced oxidation protein products, total thiols, total protein, albumin in plasma, % hemolysis in RBC suspension and glutathione in both whole blood and plasma were estimated. Our studies demonstrated a significant increase in advanced oxidation protein products, % hemolysis (p=0.033), A:G ratio (p=0.003) and a highly significant decrease in blood glutathione (p=0.036), total thiols (p=0.001), plasma thiols other than glutathione and total antioxidant activity. The findings suggest that gastric carcinoma is associated with oxygen derived free radicals accumulation, and depletion of total antioxidant activity has lead to oxidative stress and advancement of oxidative-antioxidative disorders followed by progression of gastric cancer.

Keywords: gastric cancer, oxidative stress, advanced oxidation protein products, total thiols, total protein, albumin, total antioxidant activity

Kratak sadržaj: Postoje podaci o tome da je oksidativni stres, koji se definiše kao promena u ravnoteži između antioksidanata i oksidanata u korist oksidanata, povezan sa patogenezom mnogih bolesti, uključujući karcinome. Reaktivne vrste kiseonika mogu izazvati nastanak karcinoma putem oštećivanja makromolekula kao što su DNK, ugljeni hidrati i proteini. Studija je obuhvatila četrdeset pacijenata sa primarnim gastričnim karcinomom i 40 zdravih osoba. Određeni su proizvodi uznapredovale oksidacije proteina, ukupni tiol, ukupni protein, albumin u plazmi, procenat hemolize u suspenziji eritrocita i glutation u punoj krvi i plazmi. Istraživanje je ukazalo na značajan porast proizvoda uznapredovale oksidacije proteina, procenta hemolize (p=0,033), odnosa A:G (p=0,033) i veoma značajan pad glutationa u krvi (p=0,036), ukupnih tiola (p=0,001), tiola u plazmi osim glutationa i ukupne antioksidantne aktivnosti. Na osnovu rezultata se može zaključiti da postoji veza između gastričnog karcinoma i akumulacije slobodnih radikala iz kiseonika i da smanjenje ukupne antioksidantne aktivnosti dovodi do oksidativnog stresa i napredovanja oksidativnih/antioksidativnih poremećaja koje prati progresija gastričnog kancera.

Ključne reči: gastrični karcinom, oksidativni stres, proizvodi uznapredovale oksidacije proteina, ukupni tioli, ukupni protein, albumin, ukupna antioksidantna aktivnost

Introduction

Gastric cancer is a multifactorial disease and has multietiopathogenic factors. It is widely accepted that a major underlying factor of this disorder is the generation of free radicals. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of various tissues. Potential sources of free radicals may be the activated inflammatory cells, the hypoxanthine-xanthine oxida-

Address for correspondence:
Dr. Gayathri M Rao
Associate professor
Department of Biochemistry
Centre for Basic Sciences
Kasturba Medical College
Bejai, Mangalore-575004
e-mail: gayatrimrao@yahoo.com

se system, disrupted electron transport system, metabolism of arachidonate via lipoxygenase pathway or vascular endothelial cells. The lipoxygenase pathway and the activated inflammatory cells could be involved in the pathogenesis of mucosal damage. Inflammation could be activated by *Helicobacter pylori*. In *H. pylori* infected gastric mucosa and duodenal gastric metaplasia, active inflammation with infiltration of neutrophils in the acute stage of the infection and of macrophages/monocytes, lymphocytes and plasma cells in the chronic stage is observed in the lamina propria of the stomach. These may act as a source of a large quantity of oxygen derived free radicals that could cause cell damage and ultimately lead to mucosal injury (1).

Since free radicals cause damage to lipids, proteins and DNA, their oxidative products level in the plasma or serum may be increased in diseased conditions. The generation of superoxide anion as a mechanism of damage is well established in different models of acute and chronic injury, which in turn might lead to gastric mucosal damage. The chronic inflammation again leads to proliferation of the epithelial cells of the stomach. Much of the chemistry of the products of protein oxidation has detected that formation of carbonyls and the »peptide alpha-amidation« scheme for oxidative breakage of polypeptide back bones, seems to be a common component of chain fragmentation reactions (2).

Several studies suggest that the pathogenesis of disease is increased due to the oxidation or nitration of plasma proteins. Hence we made an attempt to study the oxidant-antioxidant relationship by estimating the levels of protein oxidation products and a few antioxidant parameters in gastric cancer subjects.

Subjects and Methods

Blood samples were collected from 40 subjects, both male and female diagnosed with gastric carcinoma on clinical basis, who have not undergone surgery, chemotherapy or radiotherapy and age and sex matched 40 healthy individuals devoid of disease conditions like hypertension, diabetes mellitus, epilepsy and psychiatric disorders.

Five mL of venous blood was collected in heparinised tubes, from which 0.2 mL of whole blood was used for the estimation of reduced glutathione by the method of Ernest Beutler (3). The remaining volume of blood was centrifuged at 3000 rpm and the plasma was separated into sterile containers and refrigerated until use. Plasma advanced oxidation protein products (AOPP) was quantitated by a modified Witko's method (4), where an aliquot of suitably diluted plasma was made to react with 1.16 mol/L KI followed by the addition of acetic acid. Optical density was read at 340 nm against a blank containing KI and acetic acid. Total thiols of the plasma were measured by the reaction of sulfhydryl groups with dithionitro benzoic acid, by Ellman's procedure (5). The method adopted to measure total antioxidants of plasma was developed by Koracevic (6) based on the measurement of thiobarbituric acid reactive substances and reduced glutathione by the method of Ernest Beutler (3).

Statistical analysis was done by Mann Whitney U test.

The work has been approved by the Human Ethics Committee of the institution, consent was obtained from each patient/subject after full explanation of the purpose.

Result

In the gastric cancer subjects, whole blood GSH was decreased very significantly and plasma GSH levels remained in the normal range as indicated in *Table I*. Total thiol in plasma, another antioxidant group of plasma, was highly significantly decreased in the study group. The decrease in the plasma thiols (total thiols – plasma GSH) was highly significant ($p = 0.008$) compared to the normal group, as represented in *Table I*. The total antioxidant activity also showed a significant decrease in the study group compared to the control group. Statistical analysis showed a significant decrease in total protein along with albumin levels, however the

Table I Comparison of antioxidants (Mean ± SD).

	Control (n=40)	Gastric cancer (n=40)	p value
Blood GSH (mmol/L)	1.35 ± 0.38	1.00 ± 0.39	0.003
Plasma GSH (mmol/L)	0.21 ± 0.16	0.19 ± 0.20	0.789
Total thiols (mmol/L)	0.45 ± 0.05	0.24 ± 0.09	0.001
Plasma thiols (mmol/L) (Total thiols – GSH)	0.28 ± 0.12	0.15 ± 0.11	0.008
TAO (mmol/L)	1.00 ± 0.24	0.84 ± 0.23	0.014
Total protein (g/L)	8.19 ± 1.43	6.91 ± 2.31	0.014
Albumin (g/L)	4.84 ± 1.07	3.37 ± 1.29	0.001
Globulin (g/L)	3.35 ± 1.39	3.53 ± 1.59	0.946
A:G	1.77 ± 1.01	1.06 ± 0.47	0.003

Table II Markers of oxidative damage (Mean ± SD)

	Control (n=40)	Gastric cancer (n=40)	p value
AOPP (mmol/L)	0.074 ± 0.022	0.097 ± 0.041	0.033
% Hemolysis	0.31 ± 0.19	1.36 ± 0.54	0.001

mean value remained in the normal range. A significant increase in AOPP ($p=0.033$) was observed in the study group when compared with controls. The % hemolysis showed a highly significant increase in gastric cancer ($p=0.001$) subjects compared to normal group (see *Table II*).

Discussion

Several recent studies done by Ebubekir et al. (7) and Batcioglu et al. (8), point out the role of oxidative stress in gastric cancer. It is known that free radicals cause protein damage and degradation (2). Until recently radical-induced damage to proteins was considered to be mainly a chain terminating process and it was thought that the products of damage produced on the protein were relatively inert. It has been demonstrated that two types of protein bound reducing moieties (3, 4) such as dihydrophenylalanine and protein peroxide are capable of initiating further chemical reactions. The protein bound reducing moieties have been shown to be able to reduce transition metals resulting in redox cycling of these species. The protein peroxides have been demonstrated to consume important cellular reductants such as ascorbate and GSH.

Advanced oxidation protein products (AOPP) were proposed as one of the possible markers of oxidative injury, which originates under oxidative and carbonyl stress and increases the inflammatory activity. Recent studies done on acute coronary syndrome suggest that AOPP is not only a mediator of oxidative stress, but also acts as an inflammatory mediator (9).

The free radicals cause oxidation or nitration of plasma proteins such as ceruloplasmin, transferrin, fibrinogen and albumin which explains the biochemical basis of pathogenesis (10). The myeloperoxidase (MPO) catalysed reactions could contribute to AOPP generation in plasma (11, 12). The importance of neutrophil activation, as the main source of oxidative stress through protein oxidation (AOPP) was also found to be responsible for the pathogenesis of the disease (12) and may be one of the underlying causes for the observed increase in the levels of plasma AOPP in the present study. Percentage hemolysis showed a highly significant increase in the patients compared to the controls, indicative of membrane damage secondary to membrane lipid peroxidation and serves as a marker of lipid peroxidation. Lipid peroxidation induces an efflux of oxidized glutathione, decreasing the erythrocyte reduced glutathione and in turn the lifespan of RBC (13).

Thiols such as glutathione interfere with the complex carcinogenic process. Under stress conditions, they scavenge harmful molecules. Glutathione conjugation of electrophilic carcinogens may prevent tumor initiation and reduced thiols defend against oxidative stress. The studies on erythrocyte GSH conducted in various conditions showed a significant decrease (1, 14) compared to the controls. Depletion in the levels of GSH either in plasma or gastrointestinal mucosa may result in the accumulation of free radicals that can initiate membrane damage due to oxidation, which may be a causative factor for ulcer, chronic gastritis and in turn gastric cancer (15). The sulfhydryl group of GSH can be used to reduce peroxides formed during oxygen transport. Many conditions alter the GSH level via changes in GCS activity (gamma - glutamyl cysteine synthetase, rate-limiting enzyme of GSH synthesis) GCS gene expression. Both transcriptional and post-transcriptional mechanisms modulate the activity of this critical cellular enzyme (17). This may be the cause for the observed decrease in the erythrocyte GSH levels.

Thiols are extraordinarily efficient antioxidants and the redox states of thiols play a critical role in the reactions such as catalysis, regulation, electron transport and those preserving the correct structure of proteins. The levels and mutual relations between different redox forms of thiols in plasma are decisive for the plasma redox capacity which determines its proper function. The present study also shows a significant decrease in total thiols as compared to studies done on colon cancer (18), liver and renal diseases (19). This may be due to the autooxidation or formation of mixed disulphides (20).

The present study is in agreement with the studies done on gastric cancer antioxidant status by Batciogluk et al (8), Arivazhagan et al. (10), Dincer (21, 22) and also studies done on inflammatory bowel disease (23), colorectal cancer (24, 25) and bladder cancer (26). Thus, the results of the present study are in accord with those of previous studies. Therefore it can be understood that antioxidants are often described as »mopping up« free radicals, meaning they neutralize the electrical charge, preventing free radical from taking electrons from other molecules.

Evidence is provided for the generation of a wide variety of protein derived reactive radical species, which may play an important role in the initiation and propagation of radical chain reactions in vivo and the occurrence of a novel chain fragmentation process of glutamate derived radicals, which are suggested to be of importance in modifying protein structure and recognition (27).

Total protein (TP) and albumin (TA) showed a significant decrease compared to the controls. The main role in the evolution of hypoproteinemia was played by albumin. Albumin, which is considered a »sacrificial« antioxidant, via its thiol groups, provides quantitatively almost tenfold greater antioxidant protection against hypochlorous acid in human plasma (28) and is responsible for capturing free radicals (29). The population with carcinoma of GIT showed an incidence of hypoproteiemia suggesting malnutrition,

hemorrhage and the GIT protein losing phenomenon. The hypoproteinemia in cancer may be an expression of cachexia, representing homeostatic derangement in which the utilization and destruction of albumin by the tumor cannot be compensated by the organism, especially by the liver. A significant positive correlation is also seen between total thiols and albumin which are effective antioxidants in the plasma. The observed negative correlation between total thiols and % hemolysis, and blood glutathione and AOPP indicates the antioxidant activity of thiols. This shows an imbalance between antioxidants and oxidative markers with a shift towards oxidative markers.

From the above data we can come to the conclusion that increased free radical production causes oxidative stress, which shifts the oxidant/antioxidant balance towards oxidants leading to pathogenesis of the disease. Direct damage to DNA is probably one of the key events but alone is insufficient to produce cancer, suggesting the ability of reactive species to suppress apoptosis and to promote proliferation, invasiveness and metastasis. Thus, cancer preventive strategies involve enhancement of the antioxidant or conjugating capacity by increasing the levels of thiols, particularly glutathione through precursor application or synthesis stimulation and by inducing the corresponding enzymes. The antioxidant potential of thiols is, however, a part of a more general capacity to regulate redox status even in the absence of unequivocal stress conditions. Redox status controls the activities of various cellular signalling proteins through oxidation or reduction of particular sensor structures that are also mostly thiols. The development of feasible chemotherapeutic strategies on the basis of this complex system of redox-sensitive messenger proteins can be a goal in future research.

References

1. Süleyman D, Mustafa Y, Mehmet K, Nalan A, Diler A. Role of free radicals in peptic ulcer and gastritis. The Turkish Journal of Gastroenterology 2003; 4 (1): 39–43.
2. Roger T, Shanlin FU, Roland S, Michael JD. Biochemistry and pathology of radical-mediated protein oxidation. Biochem Journal 1997; 324: 1–18.
3. Beutler E, Duron O, Mikus KB. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 883–7.
4. Witko SV, Nguyen AT, Descamps LB. Microtitre plate assay for phagocyte derived taurine chioramines. J Clin Lab Anal 1992; 6: 47–53.
5. Ellman GL. Measurement of total thiols in plasma. Arch Biochem Biophys 1959; 82 (70).
6. Koraćević D, Koraćević G, Đorđević V, Andrejević S, Ćosić V. Method for the measurement of antioxidant activity in human fluids. J Cli Pathology 2001; 54: 356–61.
7. Ebubekir B, Seyithan T, Fevzi P, Sabahattin D, Zuhal U, Nuri B, Metehan G. Nitric Oxide Levels and Lipid Peroxidation in Plasma of Patients with Gastric Cancer. Japanese Journal of Clinical Oncology 2002; 32: 162–6.
8. Batcioglu K, Mehmet N, Ozturk IC. Lipid peroxidation and antioxidant status in stomach cancer. Cancer Invest 2006; 24: 18–21.
9. Škvařilová MA, Bulava D, Stejskal S, Adamovská JB. Increased level of advanced oxidation products (AOPP) as a marker of oxidative stress in patients with acute coronary syndrome. Biomed Papers 2005; 149: 83–7.
10. Arivazhagan S, Kavitha K, Nagini S. Erythrocyte lipid peroxidation and antioxidant status in gastric cancer patients. Cell Biochem Function 1997; 15: 8–15.
11. Yazici C, Kose K, Calis M, Demler M. Increased advanced oxidation protein products in Behcets disease: a new activity marker. Br J Dermatol 2004; 151: 105–11.
12. Capeillere BC, Gausson V, Descamps LB, Witko SV. Biochemical and spectrophotometric significance of advanced oxidized protein products. Biochim Biophys Acta 2004; 28: 91–102
13. Sharmila U, Subramanya U, S. Krishna Mohan S, Vanajakshamma K, Mamatha K, Seema Mathias. Oxidant-Antioxidant status in colorectal cancer patients before and after treatment. Indian Journal of Clinical Biochemistry 2004; 19 (2): 80–83.
14. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione Metabolism and Its Implications for Health. Journal of Nutrition 2004; 134: 489–92.
15. Skrzydlewska E, Sulkowski S, Koda M, Zalewski B, K-Koda L, Mariola S. Lipid peroxidation and antioxidant status in colorectal cancer. World J Gastroenterol Jan 2005; 11: 403–6.
16. Shelly CLU. Regulation of hepatic glutathione synthesis: current concepts and controversies. The Faseb journal 1999; 13: 1169–80.
17. Yazici C, Kose K, Calis M, Demler M. Increased advanced oxidation protein products in Behcets disease: a new activity marker. Br J Dermatol 2004; 151: 105–11.
18. Di Giacomo C, Acquaviva R, Lanteri R, Licata F. Non-proteic antioxidant status in plasma of subjects with colon cancer. Exp Biol Med 2003; 228: 525–8.
19. Andersson A, Lindgren A, Arnadottir M, Prutz H, Hultberg B. Thiols as a measure of plasma redox status in healthy subjects and in patients with renal or liver failure. Clinical Chemistry 1999; 45: 1084–7.
20. Kleinman WA, Richie JP. Status of glutathione and other thiols and disulfides in human plasma. J Biochem Pharmacol 2000; 60: 19–29.

21. Dincer Y, Tülay A , Osman T, Gülen D. Nitric Oxide and Antioxidant Defense in patients with Gastric Cancer. Dig Dis Sci 2006; 26: 1367–70.

22. Dincer Y, Himmetoglu S, Akcay T, Ersoy EY, Gunes KN, Tortum O. Prognostic significance of oxidative DNA damage evaluated by 8-hydroxy-deoxyguanosine and antioxidant enzymes in patients undergoing resection of gastric and colon carcinoma. Neoplasma 2007; 54: 131–6.

23. Dincer Y, Yusuf E, Solen H, Kezban G, Kadir B. Oxidative DNA Damage and Antioxidant Activity in Patients with Inflammatory Bowel Disease. Dig Dis Sci 2007; 5: 1636–41.

24. Saygili EI, Konukoglu D, Papila C, Akcay T. Levels of plasma vitamin E, vitamin C, TBARS, and cholesterol in male patients with colorectal tumors. Biochemistry (Mosc) 2003; 68: 325–8.

25. Eyüp IS, Tülay A, Dildar K, Ci Dem P. Glutathione and glutathione-related enzymes in colorectal cancer patients. J Toxicol Environ Health 2003; 66: 411–5.

26. Arikan S, Akcay T, Konukoglu D, Obek C, Kural AR. The relationship between antioxidant enzymes and bladder cancer. Neoplasma 2005; 52: 314–317.

27. Davies M, Shanlin FU, Roger T. Protein hydroperoxides can give rise to reactive free radicals. Biochemical Journal 1995; 305: 643–9.

28. Akinori S, Naoki M, Rio K, Takako O, Kazuhito F, Akira Y, Toshihiko N, Seiichi E. Alteration of redox state of human serum albumin before and after hemodialysis. Blood Purification 2004; 22: 525–9.

29. Małgorzata I, Grażyna C, Elżbieta L, Edward B. Plasma levels of total, free and protein bound thiols as well as sulfane sulfur in different age groups of rats. Acta Biochimica Polonica 2004; 51: 815–24.

Received: March 4, 2010

Accepted: April 13, 2010