

ADVANCED OXIDATION PROTEIN PRODUCTS AND TOTAL ANTIOXIDANT ACTIVITY IN COLORECTAL CARCINOMA

AVINASH S. S., ANITHA M., VINODCHANDRAN,
GAYATHRI M. RAO, SUDHA K. AND
BEENA V. SHETTY*

*Department of Biochemistry,
Centre for Basic Sciences,
Kasturba Medical College,
Bejai, Mangalore – 575 004*

(Received on January 21, 2009)

Abstract : The present study was designed to assess the levels of advanced oxidation protein products (AOPP) and percent hemolysis (that indirectly indicates the degree of membrane damage secondary to lipid peroxidation) in colorectal carcinoma. Glutathione (GSH), total thiols and albumin were measured to determine the antioxidant status. Considering the dynamic interaction between various antioxidants in the body, we measured the total antioxidant activity (AOA). Globulin was measured to assess the inflammatory response secondary to oxidative stress. Investigations were conducted in 45 cases of recently diagnosed primary colorectal adenocarcinoma. As control, 45 age and sex matched healthy persons were chosen. GSH was estimated in whole blood, percent hemolysis in RBC suspension and other parameters in plasma. We observed a very high significant increase ($P<0.001$) in AOPP, percent hemolysis and a highly significant increase ($P<0.01$) in globulin in colorectal carcinoma. We observed a very high significant decrease ($P<0.001$) in whole blood GSH, total thiols, albumin, AOA and a significant decrease ($P<0.05$) in plasma GSH in colorectal carcinoma. A very high significant negative correlation between percent hemolysis and AOA and an apparent negative correlation between total thiols and AOPP was seen in colorectal carcinoma. This demonstrated oxidative stress, decreased antioxidant status and secondary inflammatory response in colorectal carcinoma.

Key words : AOPP AOA colorectal carcinoma GSH

INTRODUCTION

Reactive oxygen species (ROS) could be important causative agents of a number of human diseases, including carcinoma (1). Thus, antioxidants, which determine the

degree of oxidative stress, represent a major line of defense protecting overall health (1).

The antioxidant status of human plasma is dynamic and can be affected by various factors, including diet, physical exercise,

*Corresponding Author

injury and disease. The relationship between total antioxidant activity of plasma, oxidized protein, lipid hydroperoxides, total thiol groups and GSH levels reflects the status of oxidative stress and over all health.

Colorectal carcinoma remains today an important cause of death (2). To evaluate the oxidative damage AOPP and lipid hydroperoxide levels (measured indirectly by determining their capacity to induce membrane damage to erythrocytes) were estimated in the blood. The antioxidant status was evaluated by analysis of total thiol groups, GSH levels, albumin levels and total antioxidant activity in plasma of subjects.

Globulins increase secondary to any inflammatory response. In carcinoma the oxidative stress due to free radical injury can induce inflammatory response. Levels of globulins were estimated to evaluate this inflammatory response secondary to oxidative stress in colorectal carcinoma.

MATERIALS AND METHODS

This study was approved by institutional ethical review committee. Informed consent was obtained from each subject before sample collection. The samples were collected from KMC Hospital, Jyothi Circle, KMC Hospital, Attavar and Government Wenlock Hospital.

Blood was collected from 45 patients aged between 30 to 70 years, both male and female, who were diagnosed to have colorectal adenocarcinoma on clinical and histopathological basis and who had not undergone any treatment by surgery,

chemotherapy or radiotherapy. Control group comprised of 45 healthy individuals. 5 ml of venous blood was collected in vacutainers under aseptic conditions from both patients and controls. All the parameters were estimated by using suitable standards in a Systronics spectrophotometer.

GSH estimation in whole blood and plasma was done by Ernest Beutler method (3). Percent hemolysis was estimated by modified method of Kartha and Krishnamurthy (4). The other parameters were estimated in plasma. Total thiols were estimated by G.L. Ellman method (5). Total protein (TP) and albumin (A) were estimated by Biuret method (6). Globulins were calculated by TP-A. Total antioxidant activity (AOA) was estimated by Koracevic method (7). Concentration of AOPP was estimated by modified Witko method (8).

The statistical analysis was done using SPSS/PC+ (version 11.5) software. Mann Whitney U test and students unpaired t test were done to determine the significance of difference between means, Spearman's test was done to determine the correlation between two variables.

RESULTS

The mean±standard deviation (SD) levels of parameters analysed are given in respective tables. The increase in percent hemolysis and plasma AOPP, the markers of oxidative damage were significantly increased in colorectal carcinoma patients. Percent hemolysis level and plasma AOPP levels showed a significant increase ($P<0.001$) in colorectal carcinoma patients when compared to controls (Table I).

TABLE I: Markers of oxidative damage.

	<i>Plasma AOPP (mmol/L)</i>	<i>% Hemolysis</i>
Control	0.07±0.02 (n=40)	0.26±0.16 (n=40)
Colorectal Carcinoma	0.18±0.18 (n=42)	1.33±1.02 (n=45)
P value	0.001	0.001

n=number of sample.
P=probability of chance of significance of difference between two means.

Whole blood GSH, plasma total thiol, plasma albumin and AOA levels decreased significantly (P<0.001) in colorectal carcinoma patients when compared to controls (Table II). There was also a significant decrease (P<0.05) in plasma GSH in colorectal carcinoma patients when compared to controls (Table II).

The plasma globulin level showed significant increase (P<0.01) in colorectal carcinoma patients when compared to controls (Table III).

There was an apparent negative correlation (r = -0.189) between plasma total thiols and AOPP in the study group patients. (Table IV).

TABLE II: Non enzymatic antioxidant metabolites and AOA.

	<i>Whole blood GSH (mmol/L)</i>	<i>Plasma GSH (mmol/L)</i>	<i>Plasma total thiols (mmol/L)</i>	<i>Plasma albumin (g/dl)</i>	<i>Plasma AOA (mmol/L)</i>
Control	1.36±0.33 (n=40)	0.23±0.16 (n=40)	0.45±0.05 (n=40)	4.83±1.08 (n=45)	1.03±0.26 (n=40)
Colorectal Carcinoma	1±0.33 (n=45)	0.21±0.23 (n=45)	0.28±0.11 (n=45)	3.6±1.24 (n=45)	0.84±0.11 (n=45)
P value	0.001	0.001	0.001	0.001	0.001

n=number of sample.
P=probability of chance of significance of difference between two means.

TABLE III: Plasma globulin.

	<i>Plasma globulin (mmol/L)</i>
Control	3.6±1.5 (n=45)
Colorectal Carcinoma	4±0.9 (n=45)
P value	0.09

n=number of sample.
P=probability of chance of significance of difference between two means.

Further, the plasma AOA and percent hemolysis showed a statistically significant (P<0.001) negative correlation (r = -0.641) between them in the patients. (Table IV).

TABLE IV: Correlation in colorectal carcinoma.

	<i>% Hemolysis</i>	<i>Plasma AOPP</i>
Plasma AOA	r -0.64 n 45 P 0.001	Plasma r -0.19 Total Thiols n 42 P 0.23

r=spearman's correlation coefficient.
n=number of sample.
P=probability of chance of significance of difference between two means.

DISCUSSION

Free radicals are highly reactive species that are involved in cellular damage and by inducing DNA damage can contribute to the conversion of normal cells to malignant ones (1). ROS have been known to play an important role in the initiation and promotion of multi-step carcinogenesis (1). The increase in percent hemolysis in colorectal carcinoma patients could be due to the free radical induced lipid peroxidation of erythrocyte membrane lipids. Lipid peroxidation induces an efflux of oxidized glutathione, decreasing red blood cell glutathione, leading to a reduced life span for the erythrocyte (9, 10). Thus, the enhanced erythrocyte lipid peroxidation and decreased non enzymatic antioxidant activities observed in colorectal carcinoma patients indicate the potential for oxidative injury to erythrocytes and their membranes.

Total plasma thiol compounds include free amino thiols such as glutathione (GSH), cysteine and homocysteine and protein bound thiols, which are a natural reservoir of the reductive capacity of the cell. The most significant of the multifarious roles played by thiols *in vivo* is their function as components of the intracellular and extracellular redox buffer (11). Thiol groups (-SH) play a prominent role in antioxidant reactions and also in reactions of catalysis, regulation, electron transport and those preserving the correct structure of proteins (12). The redox status of plasma thiols can be a diagnostic indicator of different pathological states including carcinoma (11, 13).

The present study demonstrates in colorectal carcinoma patients, a very highly significant decrease in total plasma thiols

and intracellular GSH. Plasma GSH was also found to be significantly decreased. This decrease in free thiol (plasma GSH) and intracellular GSH hampers the S-thiolation of protein bound thiols, leading to uninhibited, increased irreversible oxidation of protein bound -SH groups.

The dityrosine-containing protein cross-linking products formed due to oxidation of amino acids by ROS in the plasma are designated as advanced oxidation protein products (AOPP). AOPP are predominantly aggregates of albumin damaged by oxidative stress (14). The plasma AOPP level increased significantly in colorectal carcinoma. The negative correlation between plasma total thiols and AOPP observed in colorectal carcinoma indicates that the protein oxidation and hence AOPP increases as the protective antioxidant activity of total thiols decrease.

Serum albumin acts as a cytoprotective antioxidant of potential relevance to circulating chronic lymphocytic leukemia cells, lowers the oxidative stress and inhibits spontaneous and reactive oxidant-induced apoptosis in cancer (15). Albumin degradation secondary to protein oxidation plays an important role in the hypoalbuminemia of the cancer patient (16, 17). Studies of electrophoresis have shown that the AOPP peak is attributable to albumin (18). The significant decrease in level of albumin in colorectal carcinoma could be, along with cancer cachexia (19, 20), due to the increased rate of degradation of such oxidised and conformationally altered plasma proteins and albumin, secondary to increased oxidative stress (14, 21, 22), leading to increased AOPP. The significant rise of globulins observed may be secondary to systemic inflammatory response observed in cancer.

It also can occur as a part of compensatory increased synthesis (23).

The AOA is a dynamic equilibrium that is influenced by the interactions between each serum antioxidative constituent (7). The cooperation of antioxidants in human serum provides greater protection against attacks by free radicals than any antioxidant alone (7). The observed decrease in plasma AOA in colorectal carcinoma could be due to reduced activity of enzymatic antioxidant and their interactions with the measured non-enzymatic antioxidants. There was significant negative correlation between AOA and % hemolysis in colorectal carcinoma. This indicated the effect of dynamic interaction between enzymatic and non-enzymatic

antioxidants in preventing the protein and membrane lipid damage.

Thus the results of the present study shows elevated oxidative damage and reduced antioxidant protection in colorectal carcinoma. It can be clearly concluded that there is an elevated oxidative stress and increased inflammatory response in colorectal carcinoma. This opens a new avenue to study the prognostic and diagnostic implication of these parameters in the evaluation and management of colorectal carcinoma. Further studies are needed to assess the effect of progression of colorectal carcinoma, its stages, histopathological grades, surgery, chemotherapy and radiotherapy on the above parameters.

REFERENCES

- Halliwell B, Gutteridge JMC. Free radicals, ageing and disease. In: Halliwell B, Gutteridge JMC, eds. *Free Radicals in Biology and Medicine* edn 2. New York, Oxford University Press 1989; 416–508.
- Colin DM, Cynthia BP, Alan DL, Christopher JLM. Cancer incidence, mortality and survival by site for 14 regions of the world. www.who.int/entity/healthinfo/paper13.pdf. *World Health Organization* 2001.
- Beutler E, Ogla D, Barbara MK. Improved method for determination of glutathione. *J Lab and Din Med* 1963; 62(5): 117–131.
- Kartha VN, Krishnamurthy S. Effect of hypervitaminosis A on hemolysis and lipid peroxidation in the rats. *J Lipid Research* 1978; 19: 332–335.
- Ellman GL. Measurement of total thiols in plasma. *Arch Biochem Biophysics* 1959; 82: 70.
- Harold V, Alan HW, Maurice B. The plasma proteins. In: Harold V, Alan HW, Maurice B, eds. *Practical clinical biochemistry* edn 5. Great Britain, The Whitefriars Press 1984; 1: 545–547.
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001; 54: 356–361.
- Witko V, Nguyen AT, Descamp S, Latscha B. Microtitre plate assay for phagocyte derived taurine chloramines. *J Clin Lab Annals* 1992; 6: 47–53.
- Hebel RP. Erythrocyte antioxidants and membrane vulnerability. *J Lab Clin Med* 1986; 107: 401–405.
- Srivastava SK, Beutler E. The transport of oxidized glutathione from human erythrocytes. *J Biol Chem* 1969; 244: 9–12.
- Malgorzata I, Grazyna C, Elzbieta LK, Edward B, Lidia W. Plasma levels of total, free and protein bound thiols as well as sulfane sulfur in different age groups of rats. *Acta Biochemica Polonica* 2004; 51(3): 815–824.
- Rokutan K, Johnston RB Jr, Kawai K. Oxidative stress induces S-thiolation of specific proteins in cultured gastric mucosal cells. *Am J Physiol* 1994; 266: 247–254.
- Heinecke JW, Li W, Daehnke HL, Goldstein JA. Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. *J Biol Chem* 1993; 268: 4069.
- Witko SV, Friedlander M, Capeillere BC et al. AOPP as a novel marker of oxidative stress in uremia. *Kidney Int* 1996; 49(5): 1304–1313.
- Elizabeth CM, Aura SK, John CC, Andrew RP. Cytoprotective antioxidant activity of serum albumin and autocrine catalase in chronic lymphocytic leukaemia. *British Journal of Haematology* 2002; 116(2): 316–328.
- Fearon CH, Falconer JS, Slater C et al. Albumin synthesis rates are not decreased in hypoalbuminaemic cachectic cancer patients with an ongoing acute phase protein response. *Ann Surg* 1998; 227: 249–254.