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FREE RADICAL INJURY AND ANTIOXIDANT STATUS IN PATIENTS WITH BENIGN PROSTATE HYPERPLASIA AND PROSTATE CANCER

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ABSTRACT

Reactive oxygen species and other free radicals are known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. There are some primary antioxidants such as glutathione peroxidase (GPx), glutathione S-transferases (GSTs) and reduced glutathione, which protect against cellular and molecular damage caused by the reactive oxygen metabolites (ROMs). The present study was conducted to determine the level of malondialdehyde (MDA), as an index of lipid peroxidation, along with the GPx, GSTs activities and level of reduced glutathione in 45 prostate cancer (PC) patients, 55 benign prostate hyperplasia (BPH) patients as compared to the controls. Significant higher levels of MDA and GSTs activities in the serum, ($P<0.005$) and significant lower levels of reduced GSH concentration and GPx activity in blood haemolysates ($P<0.05$) of PC and BPH patients were observed as compared to the controls. The relatively higher GSTs activity and low level of reduced GSH may be due to the response of increased reactive oxygen metabolites production in the blood. The higher MDA and lower GPx activities may be inadequate to detoxify high levels of H_2O_2 into H_2O leading to the formation of the *OH radical followed by MDA. This result hypothesizes that oxidant-antioxidant imbalance may be one of the major factor responsible for the development of prostate cancer and benign prostate hyperplasia.

KEY WORDS

Antioxidant enzymes, Benign prostate hyperplasia, Prostate cancer, Reactive oxygen species.

INTRODUCTION

Both enzymatic and non-enzymatic pathways leading to formation of reactive compounds by one electron reduction or oxidation generate free radicals. Role of free radicals has been proposed in the pathogenesis of many diseases involving different organs such as breast, gastric, colon, multiple myeloma, ovarian and oral cancer (1-6). Various natural antioxidants available in the cells like glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase, catalase, vitamin E and reduced glutathione (GSH) etc. prevents free radical chain reaction. When these antioxidant defence system get exhausted or generation of free radicals exceed to their scavenging capacity, free radical mediated damage results (7).

Reactive oxygen species play an important role in carcinogenesis. It has been reported that changes in MDA level and glutathione peroxidase were associated with the pathogenesis of breast cancer whereas increase in MDA level but decrease in GPx activity in blood was linked to metastasis (8). Recent study in Egyptian population showed that total GST activity was higher in ovarian cancer as compared to the controls (6). Zima *et al.* (1996) hypothesized that free radicals were known to be mediators of phenotypic and genotypic changes that lead from mutation to neoplasia (4).

To the best of our knowledge there has been no report so far on free radical parameters like GPx, glutathione S-transferase (GST) activity, GSH and lipid peroxidation in serum or haemolysates of prostate cancer and BPH patients in Indian population Therefore the present study was undertaken to determine MDA levels and GST activity in serum and GPx activity and total reduced glutathione in haemolysates of controls and patients to investigate whether pro-oxidant and antioxidant were associated in the etiology of prostate cancer.

MATERIAL AND METHOD

Forty-seven prostate cancer patients with mean age (61.9±11.4) and 55 Benign Prostate hyperplasia (BPH)

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patients mean age (59.6±8.4) were seen in the department of Urology at Sanjay Gandhi PGI. These studies were conducted over a period of March 2002 to December 2003. Diagnosis of each patient was made on clinical, biochemical and histological criteria. The control group comprised of 25 healthy individuals matched for age (60.5±14.3). The chemicals were of pure grade purchased either from Sigma St.Louis USA or Sisco BRL India.

Five ml blood was collected in a clean tube, 2.5 ml was used for serum for estimation of MDA and GST activity and remaining 2.5 ml was used for estimation of reduced GSH and GPx activity. Lipid profile was measured by estimating malondialdehyde (MDA) levels by previously described method, from blood serum, expressed in nmol/ml (9). Briefly, 0.2 ml of serum was mixed with 1 ml of 20% trichloroacetic acid. To the mixture 0.4 ml of 0.67% thiobarbituric acid (TBA) was added, shaken and kept at 3 min in a boiling water bath. After cooling to room temperature 1.6 ml of butanol was added and the mixture was shaken. Organic mixture was separated by centrifugation and its absorbancy was measured at 531 nm. The breakdown product of 1,1,3,3 tetramethoxypropane was used as standard. The GST activity was assayed spectrophotometrically at 340 nm with standard substrate (1-chloro-2, 4-dinitrobenzoic acid, CDNB) and co-substrate (reduced glutathione, GSH) as described by Habig *et al.* (1974) and expressed in U/ml/ min (10) The total glutathione estimation was done in human peripheral blood by using previously described method, and expressed in mg % of haemolysates (11). GPx activity was estimated by kits purchased from Priman (UK.) Pvt.Ltd. and expressed in U/L

Statistical analysis was done using SPSS software programme. One-Way ANOVA assessed significance of oxidative stress parameters between case and control groups. Linear Regression model were used to describe the strength of association.

RESULT

Lipids peroxidation, which was measured as the endogenous MDA levels in the serum, was found to be statistically significant in prostate cancer and BPH patients (P=<0.001) as compared to normal individuals; and serum GST was observed to be higher in prostate cancer and BPH patients as compared to the controls (P=<0.005; P=<0.001) respectively. However, in blood haemolysates, GSH concentration was found to be significantly low in prostate cancer (P<0.001) and BPH (P<0.05) patients. GPx activity was also observed to be significantly low in prostate cancer (P<0.005) as well as BPH patients (P<0.05) in comparison to controls (Table 1).

We also tried to correlate lipid peroxidation (MDA) with other antioxidant parameters. In BPH patients, MDA though positively correlated with GST, GSH and GPx statistical significance was observed for GST activity (P =0.049) only. In case of prostate cancer patients, MDA was negatively correlated with GST, GSH and GPx.and statistically insignificant (P >0.05) (Table 2).

DISCUSSION

Prostate cancer is the second most common malignancy in the world .The present study shows an increased level of serum lipid peroxide in patients group, indicating its involvement in the pathogenesis of the disease. Activity of antioxidants (GSH, GPx) except GST was observed to be lower in patients than controls as shown in Table I. This indicated a greater extent of oxygen free radical scavenging action of the antioxidants.

Positive correlation between serum MDA level with GSH, GST, and GPx in BPH patients suggested a cause and effect relationship, i.e. if oxidative stress developed, then increase in level of antioxidant try to nullify the effect. Negative correlation between serum MDA level and GSH, GST, and GPx in prostate cancer

Table 1. Pro-oxidant and antioxidant markers in normal, prostate cancer and BPH patients

Values are Mean±SD

Groups	No of Subjects	Serum		Haemolysates	
		MDA (nmol/ml)	GST (U/ml.min)	GPx (U/L)	GSH (mg % of GSH)
Controls	25	4.45 ±1.65	307±151	675±163	42.73±3.3
Prostate Cancer	45	16.98±6.66** *	410±174**	540±158**	36.75±3.9***
BPH Patients	55	11.79±5.5***	456±104***	597±84*	39.23±7.4*

*P<0.05; **P<0.005; *** P<0.001

Table 2. Association of MDA with other parameters

Patients (No.)	Parameters	Regression coefficient (β)	p-value
Prostate Cancer (45)	MDA vs GSH	-0.079	0.597
	MDA vs GST	-0.192	0.197
	MDA vs GPx	-0.001	0.994
BPH Patient (55)	MDA vs GSH	0.213	0.118
	MDA vs GST	0.266	0.049
	MDA vs GPx	0.078	0.572

patients indicated generation of more free radicals that may result in destruction of protein structure or formation of DNA adducts. These cascades of events may lead to reduced expression of the detoxifying enzymes or protein, which may result in development of prostate cancer. GSTs have an important role in conjugating GSH to the products of endogenous lipid peroxidation. Lee *et al.* (1994) proposed that early loss of GSTP1 expression could lead to increased susceptibility to carcinogens and promoting mutation and cancer development. It is also possible that loss of expression is a bystander effect of some other critical event in prostate cancer development such as methylation of broader chromosomal region or loss of transcriptional factor, which is necessary for maintenance for GSTP1 expression (15).

Glutathione S-transferase (GST) is a polymorphic super family of multifunctional enzymes, which exist as homodimer and heterodimers. These enzymes are potentially crucial in regulating susceptibility to cancer, due to their ability in metabolizing reactive metabolites of carcinogens. Among them pi-and alpha-GST have attracted interest mainly because of high expression in different pathologies such as cancer, liver and kidney diseases (12-14). The decrease in the levels of GSH in blood haemolysates observed in our study may be due to the increased utilization of GSH by glutathione S-transferases (GST) in detoxification of endogenous or exogenous exposed carcinogens. Ghalia *et al.* (2000) also reported that GST activity was higher in malignant tissue as compared to non-malignant tissue (6). The decrease of GPx and GSH, and the increase of MDA levels give the evidence of significant alteration of pro-oxidant and antioxidant of blood haemolysates of BPH and cancer patients .Our observations are in agreement with the study of Zima *et al.* (1996) in patient with multiple myeloma (4). A study reported earlier in Turkish population showed increase in serum MDA and decrease of GPx in cancer patient with metastasis (8). This could be hypothesized that impaired antioxidant system favors accumulation of free radical, which may affect the process of metastasis.

In conclusion, the present study highlights that oxidant-antioxidant imbalance may be one of the major factors responsible for the development of prostate cancer and benign prostate hyperplasia. The generation of free radicals as reflected by increased GST activity and the oxidative damage caused by increased MDA levels in Prostate cancer and BPH patients could be one of the cause leading to the development of cancer. Whether the observed correlations between serum MDA levels and activities of serum GPx and GSH could be used to predict prognosis in patients with prostate cancer and BPH is a question which requires further, larger studies for better management of disease for such patients.

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