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Lipid peroxidation and antioxidant status in prostate cancer patients

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Abstract

Objective: To assess the oxidative stress in plasma and erythrocytes of prostate cancer patients by measuring the levels of lipid peroxidation and antioxidants. *Patients & methods*: 20 newly diagnosed prostate cancer patients and an equal number of age between 55-70 years and sex matched normal subjects from Guntur, AP, India were chosen for the study. Biochemical parameters like TBARS, enzymatic antioxidants, non enzymatic antioxidants were analyzed and monitored in plasma and erythrocyte membranes of normal and prostate cancer patients. *Results*: The subjects were ranging in age from 55-70 years. Changes observed from baseline expressed as mean ±SD in plasma of normal and prostate cancer patients are TBARS 3.8±0.2, 6.9±0.52(P<0.001), vitamin E 1.4±0.06,1.28±0.09,vitamin C 1.39±0.07, 1.29±0.06, reduced glutathione 52.7±4.2, 42.8±2.9 (p<0.01), glutathione peroxidase 189.8±23.4, 160±12.7(p<0.01), catalase 0.76±0.07, 0.56±0.04 (p<0.01)respectively and TBARS 0.33±0.04, 0.92±0.07(P<0.001)reduced glutathione 54.9±3.8, 44.8±2.7(p<0.01) vitamin E 2.31±0.19, 1.76±0.09(p<0.01) respectively in erythrocyte membrane. TBARS 4.3±0.51, 5.7±0.42(P<0.001) superoxide dismutase 4.71±0.52, 4.32±0.34, catalase 1.7±0.09, glutathione peroxidase 22.2±1.7, 20.6±1.7(p<0.01) in erythrocyte of normal and prostate cancer patients. *Conclusion:* The antioxidants and lipid peroxides levels in prostate cancer patients were altered when compared to normal patients. The picture of prostate cancer in India is potentially underestimated owing to scant data.

Keywords: Oxidative stress, lipid peroxidation, antioxidants, prostate cancer.

Introduction

Prostate cancer is the fourth most common male malignancy worldwide and is the commonest malignancy of older age (Ray *et al*., 2002). The prevalence of prostate cancer increases with rise in age (Irwin & Donald, 1981). It is the major cause of morbidity and mortality in male older than 65 years of age worldwide (Russel *et al*., 2000). Autopsy studies have shown that every man at age of 90 almost have prostate cancer. Prostate cancer has the lowest number of life years lost of all major cancers in men and women. It is the leading cancer diagnosed and is the second most common causes of cancer related death in men in United States (Irwin & Donald 1981). African-American men have the highest incidence of prostate cancer in the United States (Walsh *et al*., 1996) and also Asian-American men have lower prostate cancer incidence than white or African-American men (Landis *et al*., 1998). The picture of prostate cancer in India is potentially underestimated owing to scant data.

Although the specific etiological factors of cancer are not yet known, considerable evidence indicate that both genetic and environment play a role in the evolution of prostate cancer. Diet may not initiate prostate cancer but rather may promote its progression. Diets high in vegetables have been reported to decrease the risk and high fat, saturated fat and animal fat to increase the risk (Lemarehand *et al*., 1994). Fat consumption has long been suspected to be a risk factor for prostate cancer. High levels of dietary fat can stimulate proliferation of prostate cancer cells both *in vitro* and *in vivo* (Giovanucci *et al*., 1993). Several epidemiological studies have shown that a correlation between dietary fat and prostate cancer risk. Men who consumed a high fat diet had an increased risk of developing advanced prostate cancer. Previous studies have also confirmed the association between linoleic acid and prostate cancer (Gann *et al*., 1994).

Oxidative stress and cancer

Active oxygen species are known to be mutagenic and therefore playing an important role in cancer formation. The mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA (Scully *et al*., 1993). Reactive oxygen species induce membrane damage by peroxidising lipid moiety with a chain reaction known as lipid peroxidation (Klauning *et al*., 1998). The initial reaction generates a second radical, which in turn can react with a second macromolecule to continue the chain reaction. Among the more susceptible targets are polyunsaturated fatty acids. A newly formed free radical reacts with next lipid molecule and destroying thereby, propagating the lipid peroxidation process with the continuous formation of new free radical. The process is also terminated by free radical scavengers such as enzymatic and non enzymatic anti-oxidants (Sies *et al*., 1994).

Minerals and antioxidants

Both calcium and zinc have been associated with an increased risk of prostate cancer in prospective studies

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and case control studies, although their exact role has yet to be determined (Bosland *et al*., 1999). Numbers of studies have found an association between high calcium consumption and increased prostate cancer risk. Chan *et al*. (1998) performed a case- control study of Swedish men with and without prostate cancer. They found that calcium intake was an independent predictor of prostate cancer risk. High levels of calcium may down regulate vitamin D production there by promoting cell proliferation (Giovanucci *et al*., 1998). Lycopene is a potent antioxidant and has been studied extensively as a potential negative risk factor for cancer (Di Mascio *et al*., 1989). Studies showed that high lycopene intake was associated with a 21% lower risk of prostate cancer and the mechanism by which lycopene may decrease prostate cancer risk are not known (Giovanucci *et al*., 1995). Vitamin E (α-tocopherol) is an antioxidant which protects cell membranes from free-radical damage. *In vitro* studies have shown a pro-apoptotic and anti-proliferative effect of vitamin E on prostate cancer cells (Sigounas *et al*., 1997).

Table 1. Estimations carried out in blood samples of normal and prostate cancer patients

	Plasma	Erythrocytes	Erythrocyte lysate	Erythrocyte membrane
Parameters	TBARS Vitamin C Vitamin E Reduced glutathione Glutathione peroxidase	TBARS Reduced glutathione	Glutathione peroxidase Superoxide dismutase Catalase	TBARS Vitamin E

Patients and methods

20 newly diagnosed prostate cancer patients from Guntur, India were chosen for the study. An equal number of age and sex matched normal subjects were also investigated. Patients and normal subjects were males ranging in age between 50-70 years. Blood samples were obtained by venous arm puncture in heparinised tubes and the plasma was separated by centrifugation at 3000 rpm for 15 minutes. After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocyte was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 10,000rpm for 15minutes at 20°c. The erythrocyte membrane was isolated according to the procedure of Dodge *et al*. (1968) modified by Quist (1980). Aliquots from these preparations were used for biochemical estimations (Table 1).

Thiobarbituric acid reactive substances (TBARS) in plasma was assayed by the method of Yagi (1976) and in erythrocyte and erythrocyte membrane was estimated by the method of Donnan (1950), vitamin E by the method of Desai (1984), vitamin C by the method of Omaye *et al*. (1979), reduced glutathione by Buetler and Kelley (1963), glutathione peroxidase (GSH-Px) by Rotruck *et al*. (1973) and superoxide dismutase (SOD) and catalase were estimated by the methods of Kakkar *et al*. (1984) and Sinha (1972), respectively.

Statistical analysis

The values are expressed as mean/ SD. Statistical comparisons were done by students t-test. The null hypothesis was rejected for $p<0.05$ (Table 2 & 3).

Results and discussion

The present study has investigated the levels of lipid peroxidation and antioxidants, in 20 prostate cancer patients and an equal number of age and sex matched healthy subjects. The subjects were ranging in age, from 55-70 years. The levels of TBARS significantly increased in plasma, erythrocytes and erythrocyte membranes of prostate cancer patients as compared to normal subjects. Although levels of vitamin E and C were decreased in prostate cancer patients, the values were not statistically significant. The level of plasma glutathione was a moderately decreased in prostate cancer patient as compared to normal subjects. Activities of enzymatic antioxidants like catalase, superoxide dismutase and glutathione peroxidase were significantly decreased in prostate cancer patients compared to normal subjects. Oxidative stress plays an important role for the initiation of DNA damage. The oxidative stress can be assessed by measuring the plasma vitamin E and C reduced glutathione and enzymatic antioxidants such as SOD, CAT and GPx (Homma *et al*., 2004).

The levels of vitamin E and glutathione were decreased in erythrocyte of prostate cancer patients as compared to normal subjects. Vitamin E inhibition of the high fat diet promoted growth of established human prostate tumors in nude mice (Fleshner *et al*., 1999) has been reported. The dietary fat and prostate cancer linkage is supported by epidemiological evidence of animal studies and prospective trials (Statland, 1992). Current evidence does suggest that vitamin E may have a role in prostate cancer chemoprevention (Djavan *et al*., 2004). Increased intake of vitamin E has been suggested to be protective against prostate cancer in men, but the effects of vitamin E on prostate growth and function remain poorly defined (Wilson *et al*., 2003).

A positive relationship between antioxidants such as vitamin E, C and lipid peroxides has been reported (Woodson *et al*., 2003). The increased levels of TBARS are due to depletion in these antioxidants or excessive generation of lipid peroxides in erythrocyte membranes with consequent leakage into plasma or as a result of excessive and diffusion of lipid peroxides from prostate tumor. In the present study, we have observed an increase in TBARS levels, decrease in non enzymatic anti oxidant levels and decreased activities of enzymatic antioxidants in plasma and erythrocytes of prostate cancer patients compared to normal subjects. Decreased levels of non enzymatic anti-oxidants and decreased

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activities of enzymatic antioxidants can be correlated to enhanced lipid peroxidation and subsequent neoplastic transformation. Antioxidant enzymes which catalyze the conversion of reactive oxygen species to water include catalase (CAT), manganese containing superoxide dismutase (Mn-SOD) and copper and zinc containing superoxide dismutase (Cu Zn Mn SOD), a mitochondrial enzyme that plays a key role in protecting the cell from oxidative damage (Woodsaon *et al*., 2003). Cellular levels of CAT, Mn SOD, and Cu Zn SOD in prostate adenocarcinoma reveal that many tumors appear to have decreased levels of expression. Several reports indicate that the antioxidant enzymes activities are decreased in cancerous patients as compared to normal subjects (Eaton *et al*., 1991). Three cell lines of human hormone-independent prostate cancer (were examined for activities of superoxide dismutase, catalase, and glutathione peroxidase, and for levels of protein and nonprotein thiols such as metallothionein, glutathione, and thioredoxin and the results suggest that enhanced ability in scavenging free radicals by antioxidant enzymes and thiol compounds may, at least in part, contribute to the resistance of bone metastatic prostate cancer during chemotherapy (Yashumoto *et al*., 2000). The roles of glutathione (GSH), cysteine, vitamin C., liposome-encapsulated superoxide dismutase (L-SOD) and vitamin E in preventing oxidative DNA damage and cytotoxicity in the rat kidney after administration of potassium bromate ($KBrO_3$) to male F344 rats were investigated by measuring 8-hydroxydeoxyguanosine (8-OH-dG), an oxidative DNA product, lipid peroxidation (LPO) levels and relative kidney weight (RKW). The results suggest that intracellular GSH plays an essential protective role against renal oxidative DNA damage and nephrotoxicity caused by $KBrO_3$ (Kimic *et al*., 1983). In a similar study, the activities of GSH-Px and SOD and the levels of copper, zinc, and malondialdehyde were determined and compared with healthy subjects acting as controls. The MDA levels were higher and the antioxidant activity and Zn levels lower in the prostate cancer groups than in the healthy control. These results confirm the value of therapies aimed at increasing the antioxidant capacity and encourage the use of plasma and erythrocyte Zn levels in the differential diagnosis of BPO (Benign prostatic obstruction) and prostate cancer (Yilmaz *et al*., 2004). Our results lend credibility to these observations.

Thus, in the present study we have demonstrated the status of lipid peroxides and antioxidants in plasma and erythrocytes of prostate cancer patients in comparison with normal subjects. Thus, we feel that the overproduction of free radicals by the inflammatory processes of prostate cancer causes potential oxidative injury to erythrocytes and erythrocyte membranes and damage their antioxidant defense system in prostate cancer patients.

Table 2. Investigations carried out in plasma of normal and prostate cancer patients

Samples	Plasma	
Parameters	Normal subjects	Prostate cancer patients
Age	55-70	55-70
No	20	20
Sex	Male	Male
Habits	---	Smoking and alcohol consumption
Clinical diagnosis	Normal	Prostate cancer
TBARS(moles/ml)	3.8 ± 0.2	6.9 ± 0.52*[2]
Vitamin E(mg/dl)	1.4 ± 0.06	1.28 ± 0.09[NS]
Vitamin C(mg/dl)	1.39 ± 0.007	1.29 ± 0.06[NS]
Reduced glutathione(mg/dl)	52.7 ± 4.2	42.8 ± 2.9*[1]
Glutathione peroxidase(u[a/]l)	189.8 ± 23.4	160.1 ± 12.7*[1]
Catalase (u[b/]ml)	0.76 ± 0.07	0.56 ± 0.04*[1]

*Values are expressed as mean ± SD; n =20; *[1] Significantly different from normal subjects p<0.01; *[2] Significantly different from normal subjects p<0.001; [a] µ 50% inhibition of NBT reduction; [b] µ moles of hydrogen peroxide consumed /minute; NS - Not Significant*

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Table 3. Investigations carried out in erythrocytes and erythrocyte membrane of normal and prostate cancer patients

Sample	Erythrocyte		Erythrocyte		
Parameters	Normal	Prostate cancer patients	Parameters	Normal	Prostate cancer patients
TBARS (n moles/ mg protein)	0.33±0.04	5.7± 0.42*[2]	Superoxide dismutase (U[a]/mg Hb)	4.71±0.52	4.32±0.34*[1]
Reduced glutathione	54.9±3.8	44.8± 2.7*[1]	TBARS (P moles/mg	4.3±0.51	5.7±0.42*[2]
Vitamin E (µg/mg protein)	2.31±0.09	1.76± 0.09*[1]	Catalase (U[b]/mg Hb)	1.7±0.09	1.3±0.07*[1]
			Glutathione peroxidase (U[c]/g Hb)	22.2±1.7	20.6±1.7*[1]

*Values are expressed as mean± SD; n=20; *[1] significantly different from normal subjects p<0.01 *[2] Significantly different from normal subjects p<0.001; [a] µ 50% inhibition of NBT reductions [b] µ moles of hydrogen peroxide consumed /minute; [c] µ mole of reduced glutathione utilized/minute*

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