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Full Length Research Paper

Trace metals and oxidative metabolic changes in malignant prostate cancer patients

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Metabolic derangement commonly associated with prostate cancer (PCa) has not been well researched in Nigerian malignant PCa patients yet. The present study was designed to assess the levels of selected trace metals (Zn, Cu, Cr, Fe, Co, and Se) and markers of oxidative stress, such as total plasma peroxide (TPP), total antioxidant potential (TAP), oxidative stress index (OSI) and free malondialdehyde (MDA), in Nigerian patients with malignant (PCa). Twenty three newly diagnosed patients with malignant PCa participated in this study. Inclusion criteria included prostate-specific antigen (PSA) levels in blood >20 µg/ml and clinical presentation. Thirty apparently healthy staffs of University College Hospital, Ibadan, Nigeria served as controls. There were significantly ($p<0.05$) lower plasma levels of Zn, Fe, Cu, Co, Se and TAP in malignant PCa patients when compared with the controls. In contrast, there was no significant change in the plasma level of Cr when compared with the controls. Meanwhile, significantly ($p<0.05$) higher levels of TPP, MDA and OSI were observed in malignant PCa patients when compared with the controls. Our results indicate trace metal deficiency and oxidative stress in malignant PCa patients. Since micronutrients' deficiencies play critical roles in oxidative stress, micronutrients supplementation might be used to overcome complications in malignant PCa patients.

Key words: Trace metals, oxidative metabolites, malignant prostate cancer.

INTRODUCTION

Prostate cancer (PCa) is the consequence of chromosomal aberration and pathological proliferation of cells of the prostate tissue (Anderson, 1985). It is the most common neoplasm in men and the second cause of cancer death worldwide (Segev and Native, 2006). Various factors associated with the chromosomal aberration include nutritional factor, genetics, immunologic exhaustion and infection (Nelson et al., 2004; Pathak et al., 2005).

Recent data revealed that chronic inflammation of the prostate gland and high free radical load contribute to DNA damage and genomic instability, which may facilitate subsequent progression of cancer cells (Nelson et al., 2004). It is also evident that oxidative stress provoked by toxins, dietary fat consumption, or high level of androgens is important etiologic factors in the development and progression of prostate cancer (Pathak et al., 2005). Increased free radical generation has been reported in cancer cells when compared with normal cells (Kumar et al., 2008). The free radicals generated further attack the DNA of other cells and increase the mutation rates, genome instability and apoptosis evasion (Sawa and Ohshima, 2006; Szabo and Ohshima, 1997).

The effect of free radicals on the DNA has been

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Abbreviations: PCa, prostate cancer; **TPP,** total plasma peroxide; **TAP,** total antioxidant potential; **OSI,** oxidative stress index; **MDA,** malondialdehyde; **PSA,** prostate-specific antigen.

Table 1. Levels (Mean ± SD) of Cu, Zn, Co, Se, Fe Cr, in malignant PCa patients and controls.

	N	Cu (µg/dL)	Co (µg/dL)	Zn (µg/dL)	Se (µg/L)	Fe (µg/dL)	Cr (µg/dL)
Pca patients	23	36.8 ±9.4	51.2±4.0	100.5±11.6	39.4 ± 7.8	61.5±10.0	29.6± 4.4
Controls	30	46.4±10.2	60.0±5.7	120.2±20.5	57.2±10.8	79.2±9.2	27.9±3.4
t, p values		2.4,<0.05*	6.6,< 0.01*	3.0,<0.02*	8.2, <0.01*	4.1,<0.01*	1.4,>0.05

N=number of subjects. *=significantly different from controls.

implicated in the pathophysiology of many cancers (Sawa and Ohshima, 2006). Ramanujam (2004) reported that the DNA of a cell undergoes about 10,000 free radical attacks each day; the effect of which may cause gene mutation in the absence of efficient antioxidant system. High free radical load can cause fragmentation, cross-linking, aggregation and ultimately denaturation of protein molecules, such as hormones and enzymes (Ramanujam, 2004). The synergistic effect of nutritional factors and oxidative metabolic changes may therefore play prominent roles in the progression of benign to malignant prostate cancer (Ramanujam, 2004). Ruffin and Rock (2001) stressed that dietary antioxidants such as carotenoids, vitamins C, E and Se play significant roles in DNA and cell maintenance and repair. Therefore, the present study was designed to assess the plasma levels of Zn, Fe, Cu, Co, Se, Cr, total antioxidant potential, total plasma peroxide, malondialdehyde and oxidative stress index in malignant PCa patients.

HUMAN SUBJECTS AND METHODS

Volunteer human subjects

A total number of twenty three (23) PCa patients volunteered to participate in this study. The PCa patients were recruited at the Medical Out Patient Department, University College Hospital (UCH), Ibadan, Nigeria. Recruitment criteria included the plasma levels of prostate specific antigen (PSA)≥20.0 µg/ml and the clinical assessment by the consultant in charge of the patients. Another 30 apparently healthy individuals (PSA ≤ 4.0 µg/ml), who were staffs of University College Hospital, Ibadan, served as controls.

Determination of trace metals

Trace metals' levels were determined by atomic absorption spectrophotometer (AAS), as described by Kaneko (1999).

Estimation of MDA

Level of lipid peroxidation was determined by measuring the formation MDA using the method of Varshney and Kale (1990). This procedure is based on the fact that malondiahydehyde (MDA) produced from the peroxidation of membrane fatty acid reacts with the chromogenic reagent 2-thiobarbituric acid (TBA) under acidic conditions to yield a pink–coloured complex measured spectrophotometrically at 532 nm. 1, 1, 3, 3-tetramethoxylpropane was used as standard.

Estimation of TAP

TAP was determined using the ferric reducing/ antioxidant power (FRAP) assay (Benzie and Strain, 1996; Harma et al., 2003). 1.5 ml of working pre-wormed 37°C FRAP reagent (300 mM acetate buffer - pH 3.6, 10 mM 2,4,6- tripyridyl-s-triazine in 40 mM HCl and 20 mM $FeCl_3$ at ratio 10:1:1) was vortex mixed with 50 µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv./ L.

Estimation of TPP

Determination of total plasma peroxide levels made use of the reaction of ferrous-butylated hydroxytoluene-xylenol orange complex (F0X-2 reagent) with plasma hydrogen peroxide, which yields a colour complex that was measured spectrophotometrically at 560 mm. H_2O_2 was used as standard. 1.8 ml of F0X-2 reagent was mixed with 200 µ1 of plasma. This was incubated at room temperature for 30 m. 100 µM H_2O_2 was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560 nm (Harma et al., 2003).

Determination of oxidative stress index (OSI)

OSI, an indicator of the degree of oxidative stress, is the percent ratio of the TPP to the TAP values (Harma et al., 2003).

Statistical analysis

The data are presented as Means ± Standard deviation. Student (t) test was used for comparison between groups. Results were considered significant when the p-values were less than 0.05.

RESULTS

As shown in Table 1, the mean levels of Cu, Zn, Co, Se and Fe decreased significantly ($p<0.05$) in malignant PCa patients when compared with the controls. However, plasma levels of Cr in malignant PCa patients ($p>0.05$) were similar to controls. Table 2 shows a significantly ($p<0.05$) lower level of TAP with significantly ($p<0.05$) higher levels of TPP, MDA and OSI in malignant PCa patients when compared with the controls.

DISCUSSION AND PERSPECTIVES

Cancer cells depend on the host for their nutrition, blood

Table 2. Levels (Mean ± SD) of markers of oxidative stress in malignant PCa patients and controls.

	N	TAP (µmol Trolox equiv/ L)	TPP (µmolH_2O_2/L)	OSI (%)	MDA (nMol/ml)
Pca patients	23	1025 ± 450	28.1 ± 8.0	2.7 ± 1.7	10.7 ± 1.6
Controls	30	1480 ± 610	12.4 ± 5.0	0.8 ± 0.6	6.2 ± 1.5
t, p values		3.1, <0.02*	6.9, <0.01*	5.1, <0.01*	10.5, <0.01*

N=number of subjects. *=significantly different from controls.

supply and supporting stroma. Due to their altered metabolism and high energetic demands, cancer cells ultimately override the entire system by diverting the body's nutritional materials for their own use (Bongaerts et al., 2006; Ferreira, 2010). Exhaustion of nutritional materials causes the deficiencies of several micro-nutrients commonly implicated in many cancers. In the present study, significantly lower plasma levels of more trace metals (Cu, Co, Zn, Se and Fe) were observed in malignant PCa patients. These significantly lower levels of the trace metals could be due to increased demand by the cancerous tissues and other associated metabolic dysfunctions commonly encountered in cancers.

Since micronutrients play important roles in the general metabolic activities in man, their deficiencies may have profound effect in various metabolic dysfunctions and cancer formation (Xia et al., 1999). Deficiencies of certain trace metals (that is, Zn and Fe) cause general low cellular immune response and decreased secretion of interferon-γ, tumor necrotic factor–α and interleukin–2 (Hopkins and Failla, 1999). Cobalt, in the presence of other factors has profound influence on erythropoiesis. It is an essential trace metal linking the four pyrrol rings of cobalamin for effective synthesis of red blood cells (Hall and Malia, 1984). Deficiency of cobalt, in the presence of other factors may therefore contribute to anaemia commonly encountered in malignant prostate cancer patients.

Trace metals are also important in the catalytic activities of major antioxidant enzymes and DNA repair, short-circuiting the generation of malignancy, tumor growth and cancer spread (Xia et al., 1999). Low cancer risk has been associated with adequate plasma levels of vitamins A, E and C, Cu, Zn, and Se (Simopoulos, 2004). The deficiencies of trace metals therefore contribute to various metabolic dysfunctions and cancer development (Xia et al., 1999). Zn is the most abundant trace metal in the cells, and increasing evidences emphasize its role in genetic stability and function. Zn is a component of many DNA repair proteins and it is also involved in a variety of general cellular functions, including cell signal trans-duction, transcription and replication. Xia et al., (1999) stressed that the loss of Zn from biological membranes could increase the susceptibility of such cells to oxidative damage and impaired cell functions. Ozmen et al. (2006) reported significantly lower levels of Se and Zn in plasma of PCa patients. Arinola and Marbel (2008) also reported significantly lower level of Se in all categories of PCA patients.

Micronutrients are essential constituents of antioxidant system (Ruffin and Rock, 2001). Certain vitamins (vitamins C, E and A) exert direct antioxidant effects while trace metals are integral parts of antioxidant enzymes. Zn, Cu and Mn are integral parts of superoxide dismutase. Se is an integral part of glutathione peroxidase and Fe is an integral part of catalase (Ruffin and Rock, 2001). The TAP is therefore an index of all classes of antioxidants. The malignant PCa patients recruited for this study demonstrated significantly lower level of TAP. Since low risk of cancers have been associated with adequate plasma levels of antioxidants (Simopoulos, 2004), lower level of TAP in our PCa patients could account for the development and progression of prostate malignancy. Also, in the report of Clark et al., (1996), significantly lower incidences of lung, colorectal and prostate cancers were observed in the group of people given Se therapy. The present result corroborates that of Akinloye et al. (2009) who reported significantly lower level of antioxidant vitamins in all categories of PCa patients. Since Cu, Zn, Se and Fe are integral parts of antioxidant enzymes, their significantly lower levels observed in this study could account for the lower level of TAP in the malignant PCa patients.

In this study, significantly higher levels of TPP, MDA and OSI were observed in malignant PCa patients, when compared with the controls. This result corroborates the findings of some previous workers who implicated oxidative stress in the oncogenesis and pathogenesis of several cancer cases. Thalmann et al. (2000) reported that in all the free radicals, expressing higher levels of hydrogen peroxide (H_2O_2) are more tumorigenic and metastatic. In another study, it was reported that higher level of H_2O_2 could be generated from cells that have mitochondrial DNA mutation during tumor development and progression (Bianchi et al., 2001; Mandavilli et al., 2002). These reports therefore support the present study where higher level of TPP was found in the malignant PCa patients. Kumar et al. (2008) observed increased free radical generation in cancer cells compared with normal cells. The free radicals generated attack the DNA and increase the mutation rates, genome instability and loss of apoptosis in cancer cells (Sawa et al., 2006; Szabo and Ohshima, 1997). Increased free radicals in prostate cancer cells and tissues correlated with increased potential of the prostate cancer cells and tissues (Lim et al., 2005). The high free radical load and

oxidative stress are associated with the gelatinous transformation of the bone marrow which causes anaemia (Ghaffari, 2008) commonly found in malignant PCa patients. The elevated level of TPP and exhaustion of TAP observed in this study may account for the oxidant-antioxidant imbalance that caused the increased level of OSI in our PCa patients.

MDA is an index of lipid peroxidation, which was found significantly higher in the malignant PCa patients used for this study. Since excessive free radical generation causes macromolecular damage through lipid peroxidation and protein fragmentation, the significantly higher level of MDA observed in this study could be associated with the higher level of TPP in the patients. Our findings corroborate that of Osmen et al. (2006) who reported significantly higher level of MDA in patients with prostate cancer. Caliskan-can et al. (2008) also reported increased oxidative stress and higher levels of products of lipid peroxidation in lung cancer patients.

In conclusion, trace metal deficiency and oxidative stress are features of malignant PCa. Therefore, micronutrient supplements may be required as adjuvant therapy in the management of malignant PCa patients.

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