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Oxidative Stress and Antioxidant Markers Interaction in Prostate Cancer Patients in Southern Nigeria

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Abstract

Prostate cancer is the most diagnosed solid malignant tumor and second leading cause of cancer-related deaths in developing countries. This study examined the following parameters; the Total Antioxidant Capacity (TAC), Malonaldehyde (MDA) and trace metals (Copper, Zinc, Magnesium and Manganese) among prostate cancer patients. Sixty patients were recruited from the urology clinics of University of Benin Teaching Hospital Benin City. Prostate cancer patients (CAP) were grouped according to International Society of Urological Pathology (ISUP) into grade II, IV and V. Some essential trace metals, TAC and MDA were assayed for using Inductively Coupled Plasma Mass Spectrophotometer. Higher mean age ($p < 0.05$) of CAP patients (63.63 ± 1.68) were observed when compared with BPH (57.76 ± 1.24) and apparently healthy participants (49.50 ± 1.05). Significantly lower mean levels ($p < 0.05$) of TAC (6.33 ± 0.15), Cu (100.87 ± 1.16), Zn (87.75 ± 1.77), Mg (1.49 ± 0.07) and Mn (9.28 ± 0.29) was also observed for CAP patients when compared other groups. Higher mean levels ($p < 0.05$) of MDA (44.17 ± 1.27) was observed for CAP patients when compared with other groups. Grade V CAP revealed lower levels ($p < 0.05$) of TAC, Cu, Zn, Mg, Mn and significantly higher level of MDA when compared with other grades of CAP. Our results indicated trace metal deficiency and oxidant-antioxidant imbalance was observed in more severe CAP malignancy.

Keywords: Lipid Peroxidation, Trace Metals, prostate cancer, International society of Urological pathology

Introduction

Prostate cancer is the most diagnosed solid malignant tumor and second leading cause of cancer related deaths in both developed and developing countries (Siegel *et al.*, 2015). It is the fourth most common cancer overall worldwide with about 70% of the registered cases occurring in developed regions (759 000 cases) (GLOBOCAN, 2012). Mortality rates are generally high in predominantly Black populations (Caribbean, 29 per 100,000 and sub-Saharan Africa, age standardized rates (ASRs) 19-24 per 100,000), very low in Asia (ASR 2.9 per 100,000 in Eastern Asia for example) and intermediate in Europe and Oceania (GLOBOCAN, 2012).

Prostate cancer is classified as an adenocarcinoma which begins when normal prostate gland cells (Figure 1) mutate into cancer cells (Humphrey, 2003). Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN) (Bostwick and Cheng, 2012).

Samaratunga, *et al.* (2016) reviewed the classification of prostate cancer from Gleason to International Society of Urological Pathology (ISUP) grading of prostate cancer. This system consists of five grades: grade 1 (Gleason Score $\leq 3+3$), grade 2 (Gleason Score 3+4), grade 3 (Gleason Score 4+3), grade 4 (Gleason Score 4+3, 3+5, 5+3) and grade 5 (Gleason Score 4+5, 5+4, 5+5).

Over time, these cancer cells begin to multiply

and spread to the surrounding prostate tissue forming a tumor. Eventually, the tumor may grow large enough to invade nearby organs. It commonly metastasizes to the bones, lymph nodes, and may invade rectum, bladder and lower ureters after local progression (Bostwick and Cheng, 2012).

Oxidative stress refers to an imbalance between oxidants and reductants within the body due to excess production of peroxides and free radicals called reactive oxygen species (Finkel, 2011). This imbalance results in oxidative DNA damage that constitutes a major mutagenic and carcinogenic factor in cancer pathogenesis (Shazia *et al.*, 2012). Oxidative stress results from environmental pollutants, toxins, high dietary fat intake or high androgens levels and these may act as risk factors in the pathogenesis

and metastasis of prostate cancer (Pathak *et al.*, 2005). Ramanujam (2004) established a link between nutrition and oxidative stress and opined that this link may play key role in the progression from benign prostatic hyperplasia to metastatic cancer of the prostate. Also, essential trace metals which are key components of antioxidant enzymes play essential roles in Deoxyribonucleic acid maintenance and repair. To the best of our knowledge there is paucity of data on studies that examined oxidative stress biomarker and the essential trace elements interactions (Cu, Zn, Mg and Mg) in different grades of prostate cancer and BPH patients in a selected group in Southern Nigeria.

This study is designed to assess the serum level interactions of Cu, Zn, Mg, Mn and MDA levels in different stages of prostate cancer patients.

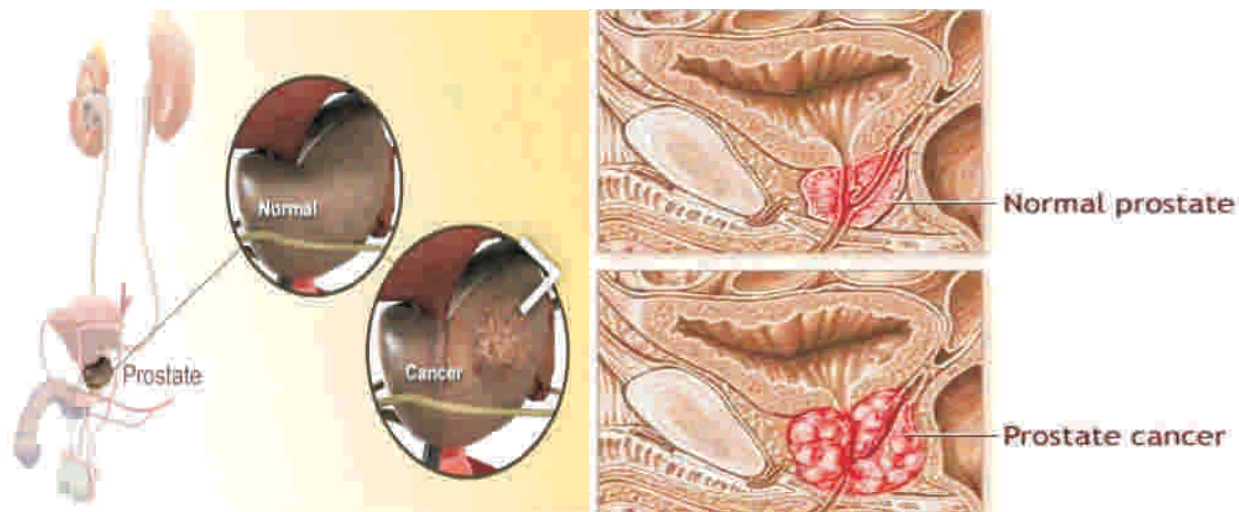


Figure 1: Normal and cancerous cells of the prostate

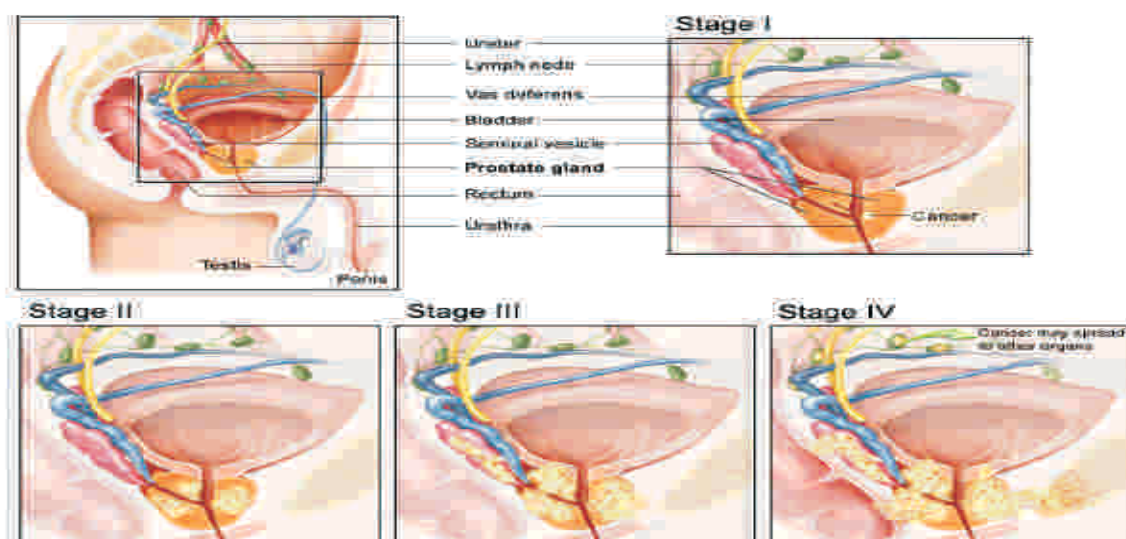


Figure 2: Stages of prostate cancer

Materials and Methods

This study included males aged between 40-79 years old (suspected and confirmed to suffer from prostate cancer and benign prostatic hyperplasia) visiting University of Benin Teaching Hospital urology outpatient clinics. other forms of cancer and gave their consent were recruited in this study.

Patients with clinical evidence of prostate cancer and benign prostatic hyperplasia with no other histories of reproductive and endocrine system disorders, underlying disease or other forms of cancer and gave their consent were recruited in this study. Patients having urinary tract infection, prostatitis, history of treatment for prostate cancer, history of previous prostate surgery or treatments with drugs were excluded from the study.

Participants were grouped into A, B and C; Prostate cancer patients were classified as Group A (positive rectal examination, PSA level of >4ng/ml and a positive prostate biopsy), Group B: Benign Prosthetic Hyperplasia (positive rectal examination, PSA level >4ng/ml and a negative prostate biopsy), and Group C (negative rectal examination, PSA level <4ng/ml).

Ethical Clearance

Ethical approval (UBTH/ADM/ E 22/A/VOL. VII/14707) was obtained from the **Ethics and Research Committee of University of Benin Teaching Hospital (UBTH), Benin, Nigeria.**

Method

Ten milliliters (10ml) of venous blood samples was obtained from participant's vein using vacutainer and needles and then dispensed into two (2) anticoagulant free specimen bottles (5 ml) and properly labelled. Samples in the anticoagulant free specimen bottles plain tube

were allowed to clot and after 30 minutes when the serum has retracted, it was centrifuged at 4,000 rpm for 10 minutes and the serum collected and stored in another anticoagulant free specimen bottle (Duru *et al.*, 2014). The blood serum samples were stored at 2-8 °C until they were analyzed.

Essential Trace metals (Cu, Zn, Mg and Mn) were determined using Inductively Coupled Plasma Mass Spectrophotometer based on standard methods described by Fong *et al.* (2007).

Malondialdehyde (MDA) level in the serum was measured spectrophotometrically at 532 nm according to the method described by Varshney and Kale (1990). MDA produced from the peroxidation of the membrane fatty acids under acidic conditions reacted with 2-thiobarbituric acid (TBA) to yield a pink coloured complex. This was determined by the improved spectrophotometric method described by Re *et al.* (1999) based on the ability to scavenge 2, 2'-Azinobis- (3-ethylbenzothiazoline-6-sulphonic acid)ABTS⁺ radical cation.

Statistical Analysis

Statistical package for Social Sciences (SPSS) version 20 was used to compare the means of analytes of different groups. Results with p-values less than 0.05 were considered significant.

Results

Gleason score and International Society of Urological Pathological (ISUP) grade of prostate cancer patients is illustrated in Table 1. The mean age of prostate cancer patients was significantly higher (P<0.05) when compared with Benign Prostatic Hyperplasia Patients and the apparently healthy participants (Table 2).

Table 1: Gleason scores and the International Society of Urological Pathological (ISUP) Grade of Prostate Cancer Patients (Group A)

S/N	GLEASON SCORE	ISUP GRADE
1	3+4	II
2	3+4	II
3	3+4	II
4	3+4	II
5	3+4	II
6	3+4	II
7	3+4	II
8	3+4	II
9	3+4	II
10	3+4	II
11	4+4	IV
12	4+4	IV
13	4+4	IV
14	4+4	IV
15	4+4	IV
16	4+4	IV
17	4+4	IV
18	4+4	IV
19	4+4	IV
20	4+4	IV
21	4+5	V
22	4+5	V
23	4+5	V
24	4+5	V
25	4+5	V
26	5+4	V
27	5+4	V
28	5+4	V
29	5+4	V
30	5+4	V

Table 2: Mean Ages ($X \pm SEM$) of Prostate Cancer Patients (Group A), Benign Prostatic Hyperplasia Patients (Group B) and the apparently healthy participants (Group C)

Participants	Mean Ages
Group A (N=30)	63.63 \pm 1.68
Group B (N=30)	57.76 \pm 1.24
Group C (N=30)	49.50 \pm 1.05
F value	27.381
P value	0.000
<u>Post Hoc Analysis</u>	
Group A Vs Group B	0.003
Group A Vs Group C	0.003
Group B Vs Group C	0.000

Significant at $p \leq 0.05$

Key

X = Mean value

SEM = Standard error of mean

N = Number of values

Table 3 illustrates the levels ($X \pm SEM$) of essential metals (Cu, Zn, Mg and Mn) in prostate cancer patients (Group A), benign prostatic hyperplasia patients (Group B) and the apparently healthy participants (Group C). The mean levels of Cu, Zn, Mg and Mn were observed to be significantly lower ($p \leq 0.05$) in prostate cancer (Pca) and Benign Prostatic Hyperplasia Patients (BPH) when compared with the controls.

Table 4 illustrates the mean levels ($X \pm SEM$) of total antioxidant capacity (TAC) and lipid peroxidation (MDA) in prostate cancer patients (Group A), benign prostatic hyperplasia patients (Group B) and the apparently healthy participants (Group C). The mean levels TAC was significantly lower ($p < 0.05$) in prostate cancer (Pca) and benign prostatic hyperplasia patients (BPH) when compared with the controls. However, the level of lipid peroxidation was significantly higher in prostate cancer patients when compared with other groups.

Table 5 illustrates levels ($X \pm SEM$) of essential trace metals (Cu, Zn, Mg, Mn), malondialdehyde production and TAC in the different grades of CAP. The mean levels of Cu, Zn, Mg, Mn and TAC were observed to be significantly lower ($p \leq 0.05$) in grade V prostate cancer compared to grade II and IV. The level of lipid peroxidation on the other hand, was also significantly higher ($p \leq 0.05$) in grade V Pca compared to grade II and IV.

Table 3: Levels ($X \pm SEM$) of essential metals (Cu, Zn, Mg and Mn) in prostate cancer patients (Group A), benign prostatic hyperplasia patients (Group B) and the apparently healthy participants (Group C)

Participants	Cu ($\mu\text{g/dL}$)	Zn ($\mu\text{g/dL}$)	Mg ($\mu\text{g/dL}$)	Mn ($\mu\text{g/dL}$)
Group A (N=30)	100.87 \pm 1.16	87.75 \pm 1.77	1.49 \pm 0.07	9.28 \pm 0.29
Group B (N=30)	117.37 \pm 2.59	99.33 \pm 2.57	1.67 \pm 0.03	10.56 \pm 0.42
Group C (N=30)	127.37 \pm 1.45	108.05 \pm 1.56	1.80 \pm 0.05	11.98 \pm 0.36
F- value	52.980	25.657	9.421	14.000
p- value	0.000	0.000	0.000	0.000
<u>Post Hoc Analysis</u>				
Group A Vs Group B	0.000	0.000	0.015	0.014
Group A Vs Group C	0.000	0.000	0.000	0.000
Group B Vs Group C	0.000	0.003	0.067	0.007

Significant at $p < 0.05$

Key

X = Mean value

SEM = Standard error of mean

N = Number of values

Table 4: Mean Levels ($X \pm SEM$) of Total antioxidant capacity and lipid peroxidation (MDA) in prostate cancer patients (Group A), benign prostatic hyperplasia patients (Group B) and the apparently healthy participants (Group C)

Participants	TAC ($\mu\text{mol Trolox equiv/L}$)	MDA (nmol/dL)
Group A (N=30)	6.33 \pm 0.15	44.17 \pm 1.27
Group B (N=30)	7.12 \pm 0.14	34.99 \pm 1.99
Group C (N=30)	8.61 \pm 0.33	30.74 \pm 1.46
F- value	26.247	18.350
p- value	0.000	0.000
<u>Post Hoc Analysis</u>		
Group A Vs Group B	0.015	0.000
Group A Vs Group C	0.015	0.000
Group B Vs Group C	0.000	0.064

Significant at $p < 0.05$

Key

X = Mean value

SEM = Standard error of mean

N = Number of values

Table 5: Levels (X ± SEM) of essential trace metals (Cu, Zn, Mg, Mn), malondialdehyde production and total antioxidant capacity in different grades of prostate cancer patients

ISUP CANCER GRADE	Cu	Zn	Mg	Mn	MDA	TAC
I (N=10)	102.83 ± 2.66	97.55 ± 1.22	1.64 ± 0.12	11.03 ± 0.17	36.11 ± 1.51	7.00 ± 0.18
IV (N=10)	103.80 ± 0.69	89.48 ± 2.33	1.66 ± 0.06	8.97 ± 0.31	46.39 ± 1.08	6.39 ± 0.21
V (N=10)	96.73 ± 1.06	78.77 ± 1.56	1.24 ± 0.11	8.14 ± 0.30	49.99 ± 0.81	5.59 ± 0.19
F-value	5.034	28.338	5.157	29.995	37.487	12.618
p-value	0.014	0.000	0.013	0.000	0.000	0.000
<u>Post Hoc Analysis</u>						
II vs IV	0.690	0.003	0.881	0.000	0.000	0.041
IV vs II	0.690	0.003	0.881	0.000	0.000	0.041
V vs II	0.018	0.000	0.021	0.000	0.000	0.000

Significant at $p < 0.05$

Key

X = Mean value

SEM = Standard error of mean

N = Number of values

Discussion

Cancer cells which have distorted metabolism and high energy demand usually relying on the host's metabolic machinery by utilizing its nutritional supplies for its growth and proliferation (Ferreira, 2010). The resultant consequence is deficiency of a number of micronutrients implicated in the pathogenesis of a number of malignancies (Akiibinu *et al.*, 2011). Micronutrients are essential constituents of the antioxidant system and play key roles in the overall metabolic activities of the human body (Ruffin and Rock, 2001). The deficiencies of these micronutrients can lead to altered metabolic function and cancer development. Zn, Cu and Mn are integral parts of superoxide dismutase (Ruffin and Rock, 2001). Trace metals play key roles in the catalytic activities of major antioxidant enzymes and DNA repair as they short circuit the generation of free radicals, tumor growth and cancer spread (Xia *et al.*, 1999). The deficiencies of trace metals have been reported to contribute to various metabolic dysfunctions and cancer development (Xia *et al.*,

1999).

In our study the mean levels of Cu were observed to be significantly lower ($p < 0.05$) in prostate cancer (Pca) and Benign Prostatic Hyperplasia Patients (BPH) when compared with the controls. Akiibinu *et al.* (2011) reported that the levels of cu decreased significantly in Pca patients than when compared with the controls.

Zn is an essential trace element possessing antioxidant properties and function as component of many proteins involved in physiological regulation of apoptosis, transcription and cellular differentiation. It has an anti-carcinogenic role through structural stabilization of genetic materials and has a protective effect against free radical injury by acting as a cofactor of superoxide dismutase (Wu *et al.*, 2004). This study observed the serum level of Zn in CAP patients was significantly lower than the BHP and control participants. Park and his team (2013) while working on levels of some metals in serum of prostate cancer patients by polarography observed that serum Zn

concentration between cases and control did not differ significantly. This is very different from our study. However, in a study conducted in Ozmen it was reported that Zn levels were significantly lower in patients with Pca than in the controls (Mehran *et al.*, 2015). This variation could have been due to the ethnicity and difference in the methodologies used.

Zn and Cu are essential metals that may be toxic at high levels in the serum (Klaasse *et al.*, 2007). Magnesium is the second most abundant cation that plays major roles in a number of biological functions in the body. Its deficiency has been linked with chronic inflammation which may play a key role in the progression to prostate cancer (Dsi, 2011). This supports our findings of significantly lower levels of Mg in Pca and BPH patients. Mg plays a key role in many essential cellular processes. It is recognized that connection between mg concentration in body fluids and cancer occurrence is still unclear, however Mg⁺ are crucial co-factors for enzymes involved in DNA repair; mg deficiency may result in genomic instability and consequently cause cancer (Wojciech *et al.*, 2015).

Zinc, Copper and Manganese are integral parts of antioxidant enzymes such as superoxide dismutase hence play key roles in maintaining antioxidant balance against free radical generation (Ruffin and Rock, 2001). The significantly lower levels of Mn observed in CAP and BPH patients when compared with control group suggest increased cancer risk.

Over the years, MDA has been a major marker of lipid peroxidation (Akiibinu *et al.*, 2011). We observed MDA levels to be significantly higher in PCa and BPH patients than in the controls. From our understanding, increased lipid peroxidation as observed in our study indicates an imbalance in the oxidant-antioxidant status as it results in increased production of free radicals resulting in oxidative stress. Oxidative stress has been reported to be a mechanism of cancer development, resulting from genetic mutation (Shazia *et al.*, 2012).

Oh *et al.* (2015) in a review on oxidative stress in prostate cancer patients reported MDA levels

were significantly higher for Pca patients than controls. This is in agreement to the findings of our study. They further confirmed in their review that markers of OS are increased in PCa patients compared with healthy controls, with the strongest and most consistent circulating biomarker being MDA.

Total antioxidant capacity is an index for measuring the body's antioxidant status. In our study, significantly lower levels ($p \leq 0.05$) of TAC were observed in Pca and BPH patients. The mean levels of TAC, Cu, Zn, Mg, Mn also showed significantly lower levels ($p < 0.05$) in grade V CAP patients compared with grade II and IV. There were also increased significant higher levels of MDA in grade V CAP patients compared with grade II and IV.

There have been documented reports that low risk of cancer is associated with sufficient plasma levels of antioxidants (Simopoulos, 2004). The significantly lower mean levels of TAC and higher mean levels of MDA observed in higher grade (V) of CAP could account for the development and progression of prostate malignancy.

Conclusion

Trace metals deficiency and increased lipid peroxidation are evidence of prostate cancer malignancy. Micronutrient supplementation may be required as key component in the therapeutic management of prostate cancer.

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