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SERUM MALONDIALDEHYDE, GAMMA GLUTAMYL TRANSFERASE AND PROSTATE SPECIFIC ANTIGEN AS MARKERS OF CANCER OF THE PROSTATE

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ABSTRACT

OBJECTIVE: Prostate specific antigen (PSA) has been found not to be specific for the screening of cancer of the prostate (CaP). We determined serum levels of prostate specific antigen, malondialdehyde (MDA) and gamma glutamyl transferase in patients with newly diagnosed CaP and benign prostatic hyperplasia (BPH) DESIGN: A cross sectional study SETTING: It was carried out in the urology clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria SUBJECTS: A total of 67, 50 and 70 men for patients with BPH, CaP and healthy age matched control respectively were recruited. RESULTS: Serum MDA(µmol/l) was observed to be significantly higher in CaP(2.05±0.64) than in BPH (1.68±0.41); p<0.05. There were also a significant higher value of serum MDA in CaP(p<0.01) and BPH(p<0.05) when compared with control (0.06±0.09). Significant higher serum levels of PSA (ng/ml) was observed in patients with CaP (70.25±50.40) than patients with BPH (22.77±10.17); p<0.01. Serum PSA(ng/ml) also was found to be significantlly higher in CaP(p<0.01) as well as BPH (p<0.01) when compared with control (0.76±0.54). There was a significant positive correlation between serum MDA and PSA in patients with CaP(r=0.701;p<0.05) and BPH (r=0.651;p<0.05). There were no significant differences in serum GGT among the study groups CONCLUSSION: Serum level of malonaldehyde may be used as a marker in screening for prostate cancer as a compliment to PSA. Gamma glutamyl transferase may have no place in prostatic cancer detection

Keywords: Prostate specific antigen (PSA), Gamma glutamyl transferase (GGT), Malondialdehyde (MDA), Cancer of the prostate (CaP), Benign prostatic hyperplasia (BPH)

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INTRODUCTION

Prostate cancer has been on the increase (Ries *et al*., 1973). The incidence is higher among Africans and African-Americans (Hoffman *et al*., 2001; American Cancer Society, 2002). It is now regarded as number one cancer in Nigerian men (Ogunbiyi and Shittu, 1999). Majority of patients are unoticed untill when the disease is in advanced stage. Prostatic biopsy for histology still remains the only way for the definative diagnosis (Kumar *et al*., 2006)

Attempt to diagnose differrent type of prostatic lesions using minimally invasive technique has be on for decades now. Two main prostatic lesions are commonly encountered namely; prostatic cancer and benign prostatic hyperplasia (BPH). Prostate specific antigen (PSA) has been in use to monitor and to suspect either of these prostatic lesions depending on the reference cut off for the age (American Cancer Society, 2002; Catalona, 2007). Serum PSA, overtime has withnessed a lot of criticism regarding its specificity and reliability (Guven *et al*., 1999). However, a modification into the use of PSA has been introduced; PSA density (Rodríguez *et al*., 2008) and PSA velocity (Benecchi, 2006; Rodríguez *et al*., 2008). A prostatic volume by altrasound is needed in determining PSA density and serum PSA has to be done on more than one occasions in determining PSA velocity. Considering the lenght of time to do these as well as the cost, it may be difficult to achieve in most centres.

This gives room for a search of another maker that could be more specific and sensitive to the monitoring and diagnosis of BPH as well as cancer of the prostate. Free radical injury has been linked with the pathogenesis of prostate cancer (Mittal and Scrivastava, 2005) and BPH (Mittal and Scrivastava, 2005; Aryal *et al*., 2007; Savas *et al*., 2009). Free radicals cause attack on polyunsaturated membrane lipid (lipid peroxidation) generating a product called malondialdehyde (MDA) (De Zwart *et al*., 1999). Serum levels of malondialdehyde however may be elevated in any of the prostatic lesions (Mittal and Scrivastava, 2005). Gamma glutamyl transferase(GGT) has also been obsereved to be secreted from the prostate (June and Junanita, 2006). This is evident by report that GGT is 50% higher in the sera of male than female (Rosalki, 1976). It has also been reported in America that GGT could be used as a marker of oxidative stress (Anton *et al*., 2002). However, other tissues like liver, kidney also produce GGT, infact it is commonly used as a biological marker for alcohol consumption (Anton *et al*., 2002) In view of the morbidity and mortality associated with prostatic cancer as well as suspected reliability of PSA in its diagnosis and treatment monitoring. There is need for early detection using alternative biomarkers in addition to serum PSA. This study was therefore designed to ccompare serum levels of PSA and MDA as well as GGT in patients with newly diagnosed prostatic cancer and benign prostatic hyperplasia. This is to determine if the values of MDA or GGT or both can be complimentary to PSA level in screening for cancer of the prostate.

MATERIALS AND METHODS

This study was conducted in Urology Clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo. Osogbo is a city of about 500,000 people located in the heart of

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southern Nigeria. A total of 67, 50 and 70 men for patients with BPH, prostate cancer and healthy control respectively were recruited into the study. Subjects with BPH and prostate cancer were either histologically or cytologically confirmed. Controls were age matched and sellected from volounteered general population. They were all confirmed of not having signs and symptoms of either BPH or protate cancer. Subjects and controls showed no evidence of bacground liver pathology and they are non-alcholics .Thier plasma ALT, AST and GGT were not elevated except for only one control and he was excluded from the study. Patients with chronic diseases like hypertension and diabetes as well as patients already on management for BPH and Prostate cancer were also excluded.

Blood Sample Collection

About 5mls of blood sample was taken from each subject and control into the screwed cap plain specimen bottle and was left to retrract for about 30mins. Each set of samples were then centrifuged at 3000rpm for 5mins after which serum (supernatant) was seperated into another screwed cap plain specimen bottle. The serum was later kept frozen at -20 ^{o}C till analysis. Analysis of MDA, PSA and GGT were done from this serum sample

Laboratory Analysis of Biochemical Parameters

Aspartate Aminotransferase (AST) and alanine aminotransferase were analyzed using commercial manufacturred kit from Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim NT41 IQS, United Kingdom. AST was based on the principle that oxaloacetate formed from the reaction of α- oxoglutamate and L-aspartate reduces $NADH^{+}$ by the help of malate dehydrogenase. The reduced $NADH^{+}$ is measured spectrophotometrically at 340nm (Bergmeyer *et al*., 1986). Alanine aminotransferase was measured also spectrophometrically from reduced $NADH^{+}$ formed from the reaction of α- oxoglutarate and L-alanine (Bergmeyer *et al*., 1986). This reaction is catalyzed by alanine aminotransferase. Gamma glutamuy transferase(GGT) was analyzed based on the principle that GGT catalyzes the transfer of gamma-glutamyl group from the donor substrate (L gamma-glutamyl-3-carboxy-4-nitroanilide) to the glycylglycine acceptor to yield 3-carboxy-4-nitroaniline. The rate of the absorbance increases at 412 nm is directly proportional to the activities of GGT in the sample (Theodorsen and Strømme, 1976). Malondialdehyde (MDA) was estimated using method of Satoh (1978) using thiobarbituric acid reacting substance. After the initial precipitation by trichloroacetic acid (TCA), the reaction of MDA with thiobarbituric acid gives a red coloured complex that is read spectrophotometrically at 532nm. Serum PSA was determined using ready to use Enzyme Immunoassay commercial manufactured kit by Teco Diagostic Laboratory, USA. This is based on the principle that PSA molecule is sandwiched between solid phase (rabbit anti-PSA antibody) and enzyme linked antibodies (momoclonal anti-PSA conjugated to Horse raddish peroxidase). After removing the unbound-labelled antibodies, TMB was added as substrate for the conjugated enzyme to digest

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resulting into colour complex that is proportional to the concentration of PSA in the serum (Stowell *et al*., 1991).

Statistical Analysis

Categorical variables were compared using chi-square. Student's t-test was used to evaluate the significance of the difference between the mean value of the measured parameters in the subjects and the control group. Correlation was done using Pearson correlation tests. These were done using SPSS 18 taking level of significance to be $p<0.05$

Ethical Approval

Ethical approval was obtained from ethical committee of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria

RESULTS

There was no statistical significant difference in mean age of controls(68.23±5.66) when compared with patients with BPH (66.67±5.78;p=0.111) and CaP (74.50±5.66;p=121). Body Mass Index when compared among the study group showed 23.67±2.00, 24.35±3.01, and 22.40±1.41 for BPH, CaP and control groups respectively. These differences were therefore not statistically significant (p=0.210) Serum MDA(µmol/l) was observed to be significantly higher in CaP(2.05±0.64) than in BPH (1.68±0.41); $p<0.05$. There were also a significant higher value of serum MDA in CaP($p<0.01$) and BPH($p<0.05$) when compared with healthy control (0.06±0.09). The significant higher serum levels of PSA was observed in patients with CaP (70.25±25.20) than patients with BPH (22.77±10.17); $p<0.01$. Serum PSA(ng/ml) also was found to be statistically significantlly higher when compared between control (0.76±0.54) and CaP($p<0.01$) as well as with patients with BPH ($p<0.01$). There was no statistical significant difference(p=0.103) in serum GGT(U/L) when compared among study groups. Values of 33.81±6.17, 35.53±14.92 and 25.50±5.32 for CaP, BPH and control were observed respectively

There was a significant positive correlation between serum MDA and PSA in patients with CaP($r=0.701;p<0.05$) and BPH ($r=0.651;p<0.05$). Although positive correlation was also observed in control group but this was not statistically signifcant. There was no significant correlation between MDA and GGT as well as between PSA and GGT among study groups.

DISCUSSION

The age range of the study groups was similar. The mean age of healthy control was not statistically significant when compared to other two study groups (patients with cancer of the prostate and BPH). In the present study, mean body mass index in the study groups falls within

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the same range; these give an averagely good statistical comparison of the selected biochemical parameters. Obesity as well as ageing have been found to influence some biochemical parameters like malondialdehyde (MDA) and prostate specific antigen (PSA) (Brawer *et al*., 1993). Furthermore, our findings in biochemical parameters were not influenced by age as well as obesity.

This study looked into possibilty of using MDA or GGT as an alternative marker for monitoring or diagnosing prostatic lesions; cancer of the prostate and benign prostatic hyperplasia. This was neccessary because of criticism that serum PSA has witness in the resent times (Guven *et al*., 1999). However, in this study serum PSA was found to be significantly higher in patients with cancer of the prostate and patient with BPH when compared with the control. This is consistent with existing reports from around the world (Guven *et al*., 1999; American Cancer Society, 2002), hence its choice as a marker of prostatic lesions. Infact its serum levels was higher in patients with cancer of the prostate than patients with benign prostatic hyperplasia, also consistent with previous studies (Guven *et al*., 1999; American Cancer Society, 2002). However, serum PSA has been found to be normal for age in patients finally diagnosed to having prostatic cancer (Brawer *et al*., 1993), also it has been found to be secreted by some other tissues in the body like in breast cancer (Black *et al*., 2000). Although its serum level in breast cancer was low but there was a statistical significant difference when compared with women without diagnosed breast cancer (Black *et al*., 2000). Hence the word prostate specific antigen may be confusing.

Free radicals are unstable in nature; they appear transiently in the system, thus their assay is difficult. However, before its disappearance, it ensures some degree of damage to cellular organelles. Its tendency to attack membrane polyunsaturated lipid leading to its peroxidation remains the evidence of free radical injury. In the course of this reaction, a product known as malondialdehyde is produced and this is currently being used as an index of free radical injury because it is more stable in the system. This study observed higher serum levels of MDA in pateints with cancer of the prostate as well as in patients with BPH when compared with control. This finding is similar to the finding observed by Mittal and Scrivastava (2005) and Savas *et al*. (2009) as well as Ozmen *et al* in 2006. When serum MDA was compared between patients with cancer of the prostate and that of BPH, there was a significant higher value in cancer of the prostate than BPH. A similar finding has be reported in the previous study (Strasak *et al*., 2008). However, it is not clear whether this is a cause-effect relationship with regards to increased free radical levels leading to the developement of cancer or vice versa. A study in this regard is desirable in future There is a report that 50% of circulating GGT in men comes from the prostate (Rosalki, 1976), then there is tendency that the disease of the prostate may present with an abnormal serum elevation. Infact elevated serum gamma glutamyl transferase (GGT) with other predisposing factors has been associated with prostate cancer (Rodríguez *et al*., 2008). However, this is not the case in this study, there was no significant difference in serum GGT when

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compared among the study groups. This is contrary to the work done in 1976 by Rosalki *et al.* There is no resent study to further support or against this. This study also observed significant positive correlation between MDA and PSA. Increasing serum level of PSA shows severity of the prostatic lesion and this is used to monitor treatment outcome (Guven *et al.*, 1999; American Cancer Society, 2002). In this study PSA increased along with MDA and this study has also shown that MDA is higher in patients with cancer of the prostate than in the patients with BPH. It shows, like PSA that MDA increases with the disease severity.

CONCLUSSION

Despite all that have been said about specificity and reliability of serum PSA in screening for prostatic lesions (Benign Prostatic Hyperplasia and Cancer of the prostate), it is still a relevant consideration. Serum level of malondialdehyde may be used as a marker in screening for prostate cancer as a compliment to PSA. Gamma glutamyl transferase may have no place in prostatic cancer detection

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Table-1. Comparison of Mean ±2SD of parameters in Subjects and Controls

Parameters	BPH N= 67	Prostate Cancer N= 50	Control N= 70	P-value
Age (yr)	66.67±5.78	74.50±5.16	68.23±5.66	0.231
Weight (kg)	60.90±5.09	63.83±3.55	61.43±3.46	0.123
Height (m)	1.60±0.05	1.58±00.03	1.66±0.04	<0.05
BMI (kg/m^2)	23.67±2.00	24.35±3.01	22.40±1.41	0.210
MDA (µmol/l)	1.68±0.41	2.05±0.64	0.06±0.09	<0.05
GGT(U/L)	35.53±14.92	33.81±6.17	25.50±5.32	0.103
PSA (ng/ml)	22.77±11.18	70.25±25.20	0.75±0.54	<0.01

- Signifiant at p<0.05

Table-2. Comparison of Mean ±2SD of Age and BMI Among Study Groups

Variables	Study groups	Mean Values	P-values
Age (Yr)	BPH Vs Control	66.67±5.89Vs 68.23±5.66	0.111
	CaP Vs Control	74.50±5.16 Vs 68.23±5.66	0.121
	BPH Vs CaP	66.67±5.89 Vs 74.50±5.16	<0.05
BMI (kg/m^2)	BPH Vs Control	23.67±2.00 Vs 22.40±1.41	1.41
	CaP Vs Control	24.35±3.01 Vs 22.40±1.41	1.54
	BPH Vs CaP	23.67±2.00 Vs 24.35 ±3.01	0.48

- **Signifiant at p<0.05**

Table-3. Comparison of Mean ±2SD of Biochemical Variables

Variables	Study groups	Mean Values	P-Values
MDA (µmol/l)	BPH Vs Control	1.68±0.41 Vs 0.06±0.09	<0.05
	CaP Vs Control	2.05±0.64 Vs 0.06±0.09	<0.01
	BPH Vs CaP	1.68±0.41 Vs 2.05±0.64	<0.05
GGT (U/L)	BPH Vs Control	35.53±14.94 Vs 25.50±5.32	0.145
	CaP Vs Control	33.81±6.17 Vs 25.50±5.32	0.321
	BPH Vs CaP	35.53±14.94 Vs 33.81±6.17	0.212
PSA (ng/ml)	BPH Vs Control	22.77±14.16 Vs 0.76±0.54	<0.01
	CaP Vs Control	70.25±25.20 Vs 0.76±0.54	<0.01
	BPH Vs CaP	22.77±14.16 Vs 70.25±25.20	<0.01

- **Signifiant at p<0.05**

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Table-4. Pearson Correlation Within Biochemical Parameters Among Subjects and Controls

Variables	CaP	BPH	Control
MDA Vs GGT	R=-0.41 P=0.72	R=-0.06 P=0.75	R=-0.03 P=0.873
MDA Vs PSA	R=0.701* P=0.042	R=0.651* P=0.04	R=0.22 P=0.250
GGT Vs PSA	R=0.05 P=0.983	R=0.11 P=0.547	R=0.14 P=0.448

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