International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ASSESSMENT OF HEAT SHOCK PROTEINS (HSPs) AND THEIR POTENTIAL ROLE IN THE DEVELOPMENT OF PROSTATE CANCER

KHUDIJA KIRAN[4], AAISHA QADIR[2], HAMID HASSAN[3], GULSHIN PARVEEN[1], QURBAN ALI[1], ARIF MALIK[1*]

1-Institite of Molecular Biology and Biotechnology, The University of Lahore-Pakistan

2-Pak Red Crescent Medical and Dental College Lahore-Pakistan

3-Department of Physiology, Nishtar Medical University-Multan

4-Department of Physiology, Gujranwala Medical College, Gujranwala-Pakistan

*Corresponding author: Arif Malik: Email: arifuaf@yahoo.com; Cell: 0321-8448196

ABSTRACT

INTRODUCTION: Cancer the most deadly disease nowadays and out of all types of cancers, prostate cancer (PCa) is the second most commonly occurring cancer after skin cancer worldwide and it is the sixth leading cause of death in men. It is mostly developed in the age over fifty and the rate of PCa in Pakistan is 5.3 per 1 lac persons per year. Prostate cancer is the cancer of prostate gland, which resides under the urinary bladder, and in anterior of the rectum and its size changes with the growing age. It develops quickly during the puberty and pumped by the increase in androgens. These hormones include testosterone and dihydrotestosterone (DHT).There are several risk factors involved in prostate cancer but this does not necessarily means that a person would develop cancer if he were having one of the risk factors.

MATERIALS AND METHODS: Total hundred (100) samples were collected for present study design and equally distributed into two groups like group A (control) consist of 50 samples of normal healthy individuals but group B (subject) consist of fifty (50) samples of prostate cancer suffering individuals. All of these samples were taken to evaluate the role of oxidative,

inflammatory biomarkers as well as heat shock protein (HSPs) in prostate suffering individuals. All of above-mentioned biomarkers were performed through respective protocols. Like isoprostane, 8-OHdG, IL-6, TNF-α, MMP-9, TGF-β, HSP-90, 70, and 27 were performed through commercially available ELIZA kits as well as MDA were evaluate through spectrophotometry method.

RESULTS: As shown in results the levels of MDA (nmol/ml) were high in subjects (5.19±0.99) as compare to normal healthy individuals (0.97±0.085). Levels of oxidative biomarker like Isoprostane (pg/ml) (84.29±4.19), 8-OHdG (pg/ml) (1.02±0.035), 4-HNE (μmol/ml) (12.39±2.19)were also higher in subjects Vs control group (32.19±4.19), (0.18±0.019) and (1.22±0.19) respectively. Levels of HSPs were also disturbed in positive control group like HSP-70 (36.29±5.19), HSP-90 (21.59±2.88), and HSP-27 (17.89±3.08) as compare to negative control group (6.99±1.99), (8.56±1.08) and (5.19±1.17) respective. Same above mention variables, the levels of inflammatory biomarkers were also elevated in subjects.

CONCLUSION: The present study depicts that antioxidant status is decrease in prostate cancer patients. The resulting oxidant-antioxidant imbalance proposes that patients with prostate disorders are vulnerable to a lot of oxidative stress, which may be one of the factors responsible for the development and progression of prostate cancer. Assessing the antioxidant levels in the patients with these prostatic disorders may help in proper management and decreasing disease morbidity.

Keywords: Prostate cancer, oxidative biomarkers, inflammatory biomarkers, Heat shock proteins, matrix metalloproteases

INTRODUCTION

About 6 in 10 cases are found in men who are above 65-year-old [1]. The reasons for ethical differences are not clear but still it has been reported that this cancer is most frequent in Caribbean men and in American-African men. However, occurrence less frequently in Asian men and in Hispanic/Latinos men as compared to nonhispanic whites. It has also been reported that prostate cancer seems to run in families through might be involvement of genetic factors. It has been observed that men are at higher risk if they have one or more relatives with the history of prostate cancer [2]. There are several risk factors involved in prostate cancer but this does not necessarily means that a person would develop cancer if he is having one of the risk factors. These risk

factors involve age in which prostate cancer may develop after the age of 40 and rises swiftly when the age crosses 50. Genetic factors involves GSTP1 and NK3.1 gene silencing and PTEN gene silencing which increases the risk of prostate cancer [3]. The relation of diet with this cancer is still not very clear but some observations linked its relation like some men who eat high-fat dairy products and red meat and also eat fewer fruits and vegetables are seen to be at higher risk of developing prostate cancer [4]. Although prostate cancer is the glandular cancer but it can also invade other organs like rectum, bones, bladder, lymph nodes and lower ureters. The course of bone is believed to be through vein. Urinary function is directly affected when changes in prostate gland occurs because the prostate gland surrounds the prostatic urethra. It is also involved in sexual dysfunction which could lead to erection problems or painful ejaculations [5]. Prostate cancer is metastatic and can cause additional symptoms in which the commonest is the bone pain particularly in vertebrae region, pelvis and ribs area. If the prostate cancer invades the spine hen it can also compress the spinal cord which could cause tingling, weakness in leg and fecal and urinary incontinence [6]. Prostate gland is a type of organ that accumulates zinc

and produces citrate. ZIP1 protein is accountable for the vigorous transportation of zinc into the prostate cells. One of the zinc's critical parts is to change the digestion system of the cell keeping in mind the end goal to create citrate, a vital segment of semen. The procedure of zinc gathering, modification of digestion system, and citrate creation is energy inefficient and the prostate cells have to sacrifice huge amounts of energy in the form of ATP for this purpose [7].

There are several ways of diagnosing prostate cancer but the most accurate is by taking biopsy in which small pieces of prostate are removed so that they could be examined using a microscope. Other methods include cystoscopy, Digital Rectal Examination (DRE), trans rectal ultrasonography, ultrasound and MRI [8]. After taking a biopsy, the specimen is observed using a microscope to check either the tumor cells are present or not and to further assess the microscopic characteristics of any cancer if found. This is done through Gleason score in which the cancer stage is determined by looking at the morphology of cancer cells. The more deformed cells will be at the least stage. Prostate specific antigen is overexpressed in prostate cancer tissues. It is a transmembrane carboxypeptidase and

displays folate hydrolase activity which is associated with high Gleason score [9]. There has been evidence which supports that prostate cancer could be prevented by taking vegetarian diet. It could also be prevented by intake of foods which contains lycopenes and selenium. Foods that are rich in soy, beans, cruciferous vegetables and other legumes may also be associated with a decreased rate of prostate cancer. Physical activity is also thought to be associated with it. Men who do regular exercise are at lower risk as compared to men who does not do regular exercise [10]. Oxidative stress is considered to be the most noticeable characteristic of various chronic and acute diseases which includes cancer, lung disease, neurodegenerative disease, cardiovascular disease and the process of aging. It is categorized by difference between an increased level of free radicals and antioxidant defenses. Free radicals are mostly derived from oxygen. Antioxidants are composed of both small molecular weights antioxidants which includes glutathione and also composed of antioxidant enzymes which includes superoxide dismutase. There are two sources of free radicals production. One is the endogenous source like mitochondria; oxidative burst during activation of phagocytes. Second is the exogenous source

which includes environmental toxins and cigarette smoke. Free radicals cause direct damage to vital biomolecules like DNA, lipids and proteins. These free radicals include ROS and RNS [11]. Lipids are the chief goal of this attack which is persuades lipid peroxidation. It is a self-progressing phenomenon which is stopped by antioxidants. There has been an increasing interest in micronutrients nowadays because of their role as antioxidants and controlling levels of potentially damaging reactive oxygen, nitrogen and chlorine species. Micronutrients are substances that are required throughout life in small quantities to organize a whole range of physiological functions. They include vitamins and minerals such as iron, chromium, copper, manganese, selenium and zinc. They act as essential cofactors of enzymes and as coordinators of the molecular structures of the cell. Many of them are crucial for the differentiation, activation and performance of various functions of immune cells [12]. This type of peroxidation can be very injurious because due to this biophysical property of the membrane gets modified which includes the grade of fluidity, and it can also lead to the inactivation of membrane-bound receptors or enzymes, which accordingly might damage cell's function. Furthermore,

generation of isoketals from isoprostane pathway and 4-hydroxynonenal can contribute to and intensify cellular damage because of their ability to covalently transform acute biomolecules. These isoketals are extremely reactive secondary aldehyde products of lipid peroxidation. Therefore, assessment of products of lipid peroxidation has been frequently used to evaluate oxidative stress or injury [13].

MATERIALS AND METHODS

SOURCE OF DATA

The present study was designed to investigate the role of antioxidants, Matrix metalloproteinase and isoprostanes in the development of prostate cancer. All the selected patients were screened at Inmol Hospital Lahore. Fifty male patients in the age group of 50-75 years were eligible for inclusion in the study. Fiftyclinically apparently healthy individuals were also included as controls. The Research Ethical Committee of The University of Lahore approved the experimental protocol. Five ml of venous blood sample were taken from the anticubital vein of each participant. The sample bottles were centrifuged within one hour of collection, after which the serum were separated and stored at -70°C until assayed.The subjects with the history of taking drugs (including alcohol and cigarette), pre-diagnosis medications (e.g. antiparkinsonian/ antipsychotic), were excluded from this study.

CHEMICALS

All chemical reagents of analytical grades were purchased from sigma/Invitrogen Chemical Co. (St. Louis, Mo, USA).

ESTIMATION OF OXIDATIVE BIOMARKERS

Blood GSH was estimated according to the method of Moron method (14).Lipid peroxidation in blood samples was estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al* [15]. Superoxide dismutase (SOD) activity was determined by the method of Kakkar method [16]. Glutathione reductase was evaluated by using method of David and Richard method [17].

DETERMINATION OF AOPPs

AOPP were determined in the plasma using the semi-automated method previously devised in our laboratory. Briefly, AOPP were measured by spectrophotometry on a microplate reader (model MR 5000, Dynatech, Paris, France) and were calibrated with chloramine-T (Sigma, St. Louis, MO) solutions that in the presence of potassium iodide absorb at 340 nm.

ESTIMATION OF MMP-9 AND IL-6

The levels of MMP-9 and Interleukin-6 (IL-6) were detected by Human matrix metalloproteinase 9 (MMP-9) and Interleukin-6 ELISA Kits.

EVOLUTION OF HEAT SHOCK PROTEINS

The levels of heat shock proteins (HSP-90, 27, 70) were also identified through commercially available human ELIZA kit method

RESULTS

Age have significant effect on the health of patient e.g. 4 patient with 90 plus age have diabetes and the values differ through the consumption of alcohol and through smoking. Similarly BMI also have positive correlation with disease, in crease of BMI risk of disease is also higher. In consistence with many other observations present study table revealed a significant elevation (P=0.016) of MDA plasma levels (5.19±0.99) among the prostate cancer subject as compared to control group

(0.97±0.085). Levels of oxidative biomarker like Isoprostane (pg/ml) (84.29±4.19), 8-OHdG (pg/ml) (1.02±0.035), 4-HNE (µmol/ml) (12.39±2.19) were also higher in subjects Vs control group (32.19±4.19), (0.18±0.019) and (1.22±0.19) respectively. Levels of HSPs were also disturbed in positive control group like HSP-70 (36.29±5.19), HSP-90 (21.59±2.88), and HSP-27 (17.89±3.08) as compare to negative control group (6.99±1.99), (8.56±1.08) and (5.19±1.17) respective. Same above mention variables levels of inflammatory biomarkers were also elevated in subjects like IL-6 (pg/ml) (7.48±1.29), TNF-α (pg/ml) (22.19±2.18), TGF-β(ng/ml) (18.59±4.19) and MMP-9 (ng/ml) (129.26±3.29) in prostate cancer suffering subjects as compare to control group (4.03±0.861), (6.59±1.19), (4.19±4.16) and (40.19±4.19) respectively. The P values were more significant (0.039), (0.000), (0.008) and (0.017) respectively.

Table 1: Comparison of Clinic Pathological Characteristics Between Patients And Healthy Controls

	Cancer (n=50)	Controls (n=50)	p-value
Age, years	96.32±7.16	65.26±10.26	0.095
Sex (male)	50	50	0.393
BMI, kg/m^2	25.26±3.88	24.26±2.88	0.135
Diabetes	4	0	0.878
Smoking	27	16	0.891
Alcohol consumption	6	3	0.869
Tumour size			
T1	6		
T2	2		
T3	29		
T4	13		

Data are presented as n or mean±standard deviation. BMI, body mass index

Table 2: Profile Of Different Variables Having Potential Role In The Development Of Prostate Cancer

VARIABLES	CONTROL (n=50)	SUBJECT (n=50)	*P*- VALUE
MDA (nmol/ml)	0.97±0.085	5.19±0.99	0.016
Isoprostanes (pg/ml)	32.19±4.19	84.29±4.19	0.026
8-OHdG (pg/ml)	0.18±0.019	1.02±0.035	0.037
4-HNE (μmol/ml)	1.22±0.19	12.39±2.19	0.001
IL-6 (pg/ml)	4.03±0.861	7.48±1.29	0.039
TNF-α (pg/ml)	6.59±1.19	22.19±2.18	0.000
MMP-9 (ng/ml)	40.19±4.19	129.26±3.29	0.017
TGF-*β* (ng/ml)	4.19±4.16	18.59±4.19	0.008
HSP-70	6.99±1.99	36.29±5.19	0.000
HSP-90	8.56±1.08	21.59±2.88	0.035
HSP-27	5.19±1.17	17.89±3.08	0.001

DISCUSSION

The prostate is involved in producing a fluid that safeguards and fosters sperm cells which are present in semen so that it can become more liquid [18]. There are various kinds of cells on prostate gland but nearly every type of prostate cancer develops from cells of gland which is known as adenocarcinoma [20]. There are some precancerous conditions of prostate which includes Prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA). The former has two possibilities which includes low grade PIN in which morphology of prostatic cells appears nearly typical and High-grade PIN in which morphology of prostate cells appear abnormal.Prostate cancer is the glandular cancer but it can also invade other organs like rectum, bones, bladder, lymph nodes and lower ureters. There is various causative factor of cancer, which believed to cause by an increase in oxidative stress. Oxidative stress is an imbalance between the levels of oxidants and antioxidants [4, 9].

Usually oxidative stress is increased in cancer due to the levels of antioxidants get affected. This oxidative stress leads towards various phenomenon like inflammation, lipid peroxidation etc which have deleterious effects on cells. Oxidative stress is increased due to the generation of free radicals like reactive oxygen species and reactive nitrogen species. Like all other cancers, oxidative stress is also increased in the prostate cancer due to which the levels of antioxidants like GSH, CAT, GSH-Px, SOD (Cu-SOD, Mn-SOD and Zn-SOD), GR, Vitamins A, E and C get affected. When the oxidative stress is increased further, proteins undergo oxidation and glycation due to AOPPs and AGEs are produced which in turn increase the production of free radicals. This increased quantity of free radicals lead towards lipid peroxidation and inflammation which causes an increase in the levels of MDA and

isoprostanes which are specific and reliable biomarkers of lipid peroxidation. Other inflammatory markers include Interlerkin-6 and TNF-α. The oxidative markers are glutathione (GSH), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) which includes Mn-SOD, Cu-SOD and Zn-SOD. Glutathione (GSH) is the chief soluble antioxidant which is found abundantly in all cell compartments [20]. The ratio of GSH and GSSG is a key factor of oxidative stress. It displays its antioxidants in various forms. It detoxifies H_2O_2 and lipid peroxides through the action of Glutathione peroxidase. GSH gives one of the electrons to hydrogen peroxide to reduce it into water and oxygen. In present study, the levels of glutathione were decreased as the stage of the cancer is progressed in comparison to controls. The levels were lowest at stage III which indicates that as the stage progresses the oxidative stress is increased due to the levels of glutathione decreases and along with this it showed a highly significant inverse correlation with MDA (GSH Vs MDA, r=-0.512**). This depicts that if the levels of GSH are decreased then the antioxidative activity would also decrease which would in turn increase the process of inflammation and hence the production of

MDA. A highly significant correlation was observed between GSH and catalase, GPx, GR and SOD (GSH Vs catalase, r=0.529***, GSH Vs GPx, r=0.523**, GSH Vs GR, r=0.670* and GSH Vs SOD, r=0.341* respectively). This indicates that all these antioxidants might act in coordination with each other to combat against oxidative stress. However, a significant inverse correlation was found out between GSH, MMP-9 and isoprostanes (GSH Vs MMP-9, r=-0.511*** and GSH Vs isoprostanes, r=-0.602** respectively). This study was in accordance with the studies performed by Zima *et al* [21, 22] who reported a rise in the levels if glutathione in PCa patients. However, the present study contradicts with the findings of Kotrikato *et al* [23] who reported a down fall in the levels of glutathione in PCa patients. H_2O_2 which is generated through the activity of superoxide dismutases or by oxidases such as xanthin oxidase is diminished to H_2O via the action of glutathione peroxidase and catalase. Degradation of the hydrogen peroxide is achieved through a change among the two adaptations of catalase-ferricatalase (Fe attached to H_2O) and compound II (Fe attached to an O_2 particle). Catalase likewise ties NADPH as a reducing correspondent to inhibit oxidative inactivation of enzymes (formation of

compound II by hydrogen peroxide as it is reduced to H_2O. Interleukin-6 is a pro-inflammatory cytokine and it acts in the cross talking between inflammatory cells and cells of prostate cancer and it also stimulates malignant processes, inducing angiogenesis and apoptosis. Moreover, it has been reported that this cytokine increases propagation and operates as an endurance molecule for various cell lines of prostate tumor like LNCaP, PC3 and DU145 [11, 18].

Tumor necrosis factor-α is another proinflammatory cytokine which is known to act as a key participant in many tumor's growth. It has been reported that serum containing high levels of TNF-α and interleukin-6 are related with the advanced metastatic condition and the survival rate is also decreased in these patients. The levels of TNF-α were increased in prostate cancer patients as compared to healthy individuals which depicts that there is high survival rate of tumor cells with more proliferation and advance metastasis. The levels of IL-6 were also higher in prostate cancer patients who indicate more tumor growth and angiogenesis in the patients. The results of the present study shows that IL-6 significantly correlated with MDA (IL-6 Vs MDA, r=0.454**) shows that by the increasing levels of IL-6 as inflammatory markers. Inflammatory process

during disease progression causes a decreasing trend in the levels of vitamin E that would eventually cause an up rise in the levels of IL-6. GSH-Pxs are the proteins which are included in the redox cycle and are incharge of the reduction of hydrogen peroxide and lipid hydroperoxides that are created as an alter effect of membrane lipid peroxidation. The levels of catalase and glutathione peroxidase were higher in control group and lower in the prostate cancer patients who indicate that the levels got decreased due to the increase of oxidative stress and with the progression of disease the levels got even lower. Along with this, GPx and GR depicted a significant high correlation with each other (GPx Vs GR, r=0.405**). This data was consistent with the findings of Romando *et al* [24] and who reported a decrease in the levels of catalase and GPx in patients suffering from prostate cancer. Glutathione reductase reduces GSSG into glutathione by utilizing NAD(P)H as the donor of electron. The levels were lowered in control group and were raised in the patients. At stage I and II the levels were raised which indicate that the antioxidant activity was at its peak to combat against oxidative stress then got decreased at stage III as the disease progressed. This result was contradictory with the findings of Iynem *et al* [25] who

reported a rise in the levels of GR in patients with prostate cancer. Superoxide is the major type of ROS and its removal by SOD is of prime importance. The oxidative biomarker superoxide dismutase (SOD) eradicates superoxide by converting it to hydrogen peroxide which is then converted to water by other antioxidants. Micronutrients like Cu, Mn, Fe and Zn act as co-factor for SOD. All three types of SOD which are Mn-SOD, CuZn-SOD and EC-SOD broadly circulate in lungs. Generally, superoxide radicals are removed by these three types because they operate as hunters for these radicals [26]. The levels of SOD were higher in control group and lower in prostate cancer patients. There was a rise and fall in the levels at different stages of cancer which shows that to eradicate the free radicals the antioxidants must be in greater quantity [27].

CONCLUSION

The present study depicts that antioxidant status is decreased in prostate cancer patients. The resulting oxidant-antioxidant imbalance proposes that patients with prostate disorders are vulnerable to a lot of oxidative stress, which may be one of the factors responsible for the development and progression of prostate cancer. Assessing the antioxidant levels in the patients with these prostatic disorders may help in proper management and decreasing disease morbidity. Thus, there may be a need for antioxidants supplementation in the management of these patients since antioxidant deficiency may related with more cellular degeneration, cancer progression and poor prognosis. Along with this, inhibitory strategies could be designed against IL-6 or MMP-9 release because they are involve in inflammation and metastasis of cancer respectively. By inhibiting these, the metastasis of this cancer could be prevented somehow.

REFERENCES

[1] Zeegers MP, A Jellema and H Ostrer. Empiric risk of prostate carcinoma for relatives of patients with prostate carcinoma: a meta-analysis. Cancer j. (2003); **97(8): 1894-903**.

[2] Hoffman RM, FD Gilliland , JW Eley , LC Harlan , RA Stephenson , JL Stanford, PC Albertson , AS Hamilton , WC Hunt and AL Potosky. Racial and ethnic differences in advanced-stage prostate cancer: the Prostate Cancer Outcomes Study. J. Natl. Cancer Inst. (2001); **93(5): 388-95**.

[3] Thomas G, KB Jacobs , M Yeager , P Kraft , S Wacholder , N Orr , K Yu , N Chatterjee , R Welch , A

Hutchinson , A Crenshaw , G Cancel-Tassin , BJ Staats , Z Wang , J Gonzalez-Bosquet , J Fang , X Deng , SI Berndt , EE Calle,, HS Feigelson , MJ Thun , C Rodriguez , D Albanes , J Virtamo , S Weinstein , FR Schumacher , E Giovannucci , WC Willett, O Cussenot, A Valeri, GL Andriole, ED Crawford, M Tucker, DS Gerhard, JF Fraumeni, R Hoover, RB Hayes, DJ Hunter and SJ Chanock. Multiple loci identified in a genome-wide association study of prostate cancer. Nature Genetics. (2008); **40(3): 310-315**.

[4] Alexander DD, PJ Mink, CA Cushing and B Sceurman. A review and meta-analysis of prospective studies of red and processed meat intake and prostate cancer. Nutrition Journal. (2010); **9:50**.

[5] Miller DC, KS Hafez K, A Stewart, JE Montie and JT Wei. Prostate carcinoma presentation, diagnosis, and staging: an update from the National Cancer Data Base. Cancer. (2003); **98(6): 1169-78**.

[6] Van der Cruijsen-Koeter IW, Vis AN, Roobol MJ, Wildhagen MF, de Koning HJ, van der Kwast TH, Schröder FH. Comparison of screen

detected and clinically diagnosed prostate cancer in the European randomized study of screening for prostate cancer, section Rotterdam. Urol. (2005); **174(1):121-5**.

[7] Verhamme KM, JP Dieleman, GS Bleumink. Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic hyperplasia in primary care-the Triumph project. Eur. Urol. (2002); **42(4): 323-328**.

[8] Bonekamp D, MA Jacobs, R El-Khouli, D Stoianovici and KJ Macura. Advancements in MR Imaging of the Prostate: From Diagnosis to Interventions. Radio graphics. (2011); **31(3): 677-703**.

[9] Figueiredo JC, MV Grau , RW Haile , RS Sandler , RW Summers , RS Bresalier , CA Burke , GE McKeown-Eyssen and JA Baron. Folic Acid and Risk of Prostate Cancer: Results from a Randomized Clinical Trial. J. Natl. Cancer Inst. (2009); **101 (6): 432-5**.

[10] Wilt TJ, R MacDonald, K Hagerty, P Schell hammer and BS Kramer. Five-alpha-reductase Inhibitors for prostate cancer prevention.

Cochrane Database Syst Rev. (2008); **(2).**

[11] Roberts L. J. and J.D. Morrow. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. Free Radic. Biol. Med. (2000); **2: 505-513**.

[12] Shariat S.F., B. Andrews, M.W Kattan, J. Kim, T.M. Wheeler and K.M. Slawin. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. Urology. (2001); **58: 1008-1015**.

[13] Wiggle DT, MC Turner, J Gomes and ME Parent. Role of hormonal and other factors in human prostate cancer. Journal of toxicology and Environmental health. Part B, Critical Reviews. (2008); **11(3-4): 242-259**.

[14] Moron MS, JW Depierre and B Mannervik. Levels of glutathione reductase and glutathione S-transferase in rat lung and liver. Biochem Biophysic Acta. (1979); **582: 67-68**.

[15] Ohkawa H, N Ohishi and K Yagi. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. J Anal Biochem. (1979); **95: 351-358**.

[16] Kakkar PB, P Das and PN Viswanathan. A modified spectrophotometer assay of superoxide dismutase. Ind J Biochem Bio. (1984); **21: 130-132**.

[17] David M and JS Richard. Methods of enzymatic analysis. (1983); **258-265**.

[18] Myers and P Robert. Structure of the adult prostate from a clinician's standpoint. Clinical Anatomy. (2000). **13(3): 214-215**.

[19] Verhamme KM, JP Dieleman, GS Bleumink. Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic hyperplasia in primary care-the Triumph project. Eur. Urol. (2002); **42(4): 323-328**.

[20] Watanabe SI, Y Miyata, S Kanda, T Iwata, T Hayashi, H Kanetake and H Sakai. Expression of X-linked inhibitor of apoptosis protein in human prostate cancer specimens with and without neo-adjuvant hormonal therapy. J Cancer Res Clin Oncol. (2009); **136 (5): 787-793.**

[21] Zima, T., I. Spicka, S. Stipek, J. Crkovska, J. Platenik, M. Merta and V. Tesar. Antioxidant enzymes and lipid peroxidation in patients with multiple myeloma. Neoplasma. (1996); **43: 69-73**.

[22] Guven M., B. Ozturk, A. Sayal, A. Ozeturk and T. Ulutin. Lipid peroxidation and antioxidant system in the blood of cancerous patients with metastasis. Cancer Biochem. Biophys. (1999); **17: 155-162**.

[23] Kotrikadze N, M Alibegashvili, M Zibzibadze, N Abashidze, T Chigogidze, L Managadzeand K Artsivadze. Activity and content of antioxidant enzymes in prostate tumors. ExpOncol. (2008); 30(3): 244-247.

[24] Romanda D, N Obioma and M Ignatius. Oxidative Stress Indicators in Patients with Prostate Disorders in Enugu, South-East Nigeria. BioMed Research International (2014).

[25] Iynem AH, AZ Alademir, C Obek, AR Kural, D Konukoğlu and T Akçay. The effect of prostate cancer and antiandrogenic therapy on lipid peroxidation and antioxidant systems. IntUrolNephrol. (2004); **36(1): 57-62**.

[26] Zelko IN, TJ Mariani, RJ Folz. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free RadicBiol Med. (2002); **33: 337-349**.

[27] Farmer EE and C Davoine. Reactive electrophile species. CurrOpin Plant Biol. (2007); **10(4): 380-386.**