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Arsenic, Selenium, Zinc, Copper, Hormonal changes, and oxidative stress in AL-Basra and Missan provinces patients with prostate tumors

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Abstract:

Prostate cancer (PCa) and benign prostatic hyperplasia (BPH), were investigated in both AL-Basra and Missan provinces–Iraq. The study provides data relating serum selenium (Se), arsenic(As), zinc(Zn), and copper(Cu) levels to activity of Glutathione–S– transferase (GST), Malondialdehyde (MDA); and prostate specific antigen (PSA) . sixty patients (30 patients with BPH mean age 56.40 ± 9.74 years and 30 patients with PCa mean age 57.55 ± 9.15 years) and thirty healthy controls mean age (54.150 ± 7.856) years were participated in the study. The results revealed a highly significant difference ($P<0.001$) in MDA, PSA, and Se levels; significant different in GST, Zn, and Cu/Zn ratio levels. No statistical significant difference was found in mean age, As, Cu, and E2 in BPH as compared to control group. Also, there was no statistical significant difference in mean age, and E2 level in PCa patients when compared to control group, while there was a highly significant difference ($p<0.001$) in MDA, PSA, GST, Se, As, Zn, and Cu/Zn ratio levels; and significant difference in Cu and testosterone levels were detected in PCa patients as compared to control group. Correlations studies indicated a significant correlation between MDA and) PSA, GST, Se, As ,Zn ,Cu, Cu/Zn, Testosterone, and E2) in BPH and PCa patients. In conclusion, trace elements which used in this study (Zn, As, Se, and Cu) can be used as a bioindicator for prostate illness. Serum MDA may be considered a marker for predicting prostate cancer as a compliment to PSA.

Keywords: *trace elements, prostate tumor, malondialdehyde, glutathione-S-transferase.*

Introduction:

Prostate cancer (PCa) is one of the most common non coetaneous malignancy and the famous leading cause of cancer death in men in worldwide (Jemal et al., 2006). Epidemiological data suggested that age, smoking, dietary habits, genetics and many other factors may be involved in PCa development (Malik et al., 2015). Also, the most common prostate disease is benign prostatic hyperplasia (BPH), an immune inflammatory disease and chronic inflammation were implicated. Usually starts after the age of 40 years and is more common in older men (Merendino et al., 2003). Prostate specific antigen (PSA), a serine protease synthesized by benign and malignant prostatic epithelium, is a sensitive serum marker for prostatic hypertrophy and cancer. In fact, increased PSA levels are often seen in carcinomas of the prostate, but have also been reported in benign inflammatory disorders of the prostate (Merendino et al., 2003).

The 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 as well evident that Increased free radicals generation were reported in cancer cells when compared with normal cells (Kumar et al., 2008).The oxidative stress was provoked by toxins, dietary fat consumption, or high level of androgens is important etiologic factors in the development and progression of PCa (Pathak et al., 2005). Reactive Oxygen Species (ROS) initiate autocatalytic lipid peroxidation, which generates a large variety of potential genotoxic breakdown products, including alkoxyl radicals (LO.), peroxyl radicals (LOO.), and aldehydes, such as malondialdehyde (MDA) (Tandon et al., 2012). Dillioglugil et al., (2012) reported that common oxidants are nitric oxide (NO) and malondialdehyde (MDA) the marker of Lipid peroxidation, and the common antioxidants are glutathione and superoxide dismutase. Glutathione–S–transferases (GSTs), one of the major phase II detoxification enzymes are involved in the metabolism of xenobiotics and

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play an important role in cellular protection against oxidative stress (van Bladeren, 2000). Some trace elements have major role in cancer biology; however, there is still a gap in our understanding regarding relationship between trace elements functions and initiation, advancement and inhibition of carcinogenic process in prostatic gland (Banas et al., 2010). Thus, there is a need for trace element analysis in human tissues with or without cancer that can show relationship between cancer and these elements (Kaba et al., 2014). A particularly high concentration of Zn is present in healthy prostate , and it is required for important prostatic functions, like citrate production and sperm health (Costello et al., 2006). Clinical trials showed that selenium (Se) also protect from PCa (Rosen and Liu 2009). Copper (Cu) acts as a vascular endothelial growth factor and induces tumor cell growth by angiogenesis. Lowering Cu levels offer a safe and effective way to stabilize the growth of advanced and metastatic cancers (Shobeiri et al., 2011).

Estrogens regulate the development and function of prostate at several stages of a man's life by indirect and direct mechanisms, also they considered as one of hormonal risk factors in association of development of BPH and PCa (Harkonen and Makela 2004). There is relationship between circulating sex hormones and influence the risk of PCa (Roddam et al., 2008). Although only reduction in testosterone levels will not, in most occasions, permanently heal PCa, it causes its regression and significantly delays further progression of PCa. The aim of this study is to estimate the serum levels of some trace elements, GST, PSA, and hormones level in PCa and BPH patients, also to find the correlation between lipid peroxidation, given by MDA level's with other parameters used in this study.

Materials and Methods:

All chemicals and reagents of analytical grade were purchased from Fluka unless indicated otherwise.

Patients samples

This study consisted of 30 patients with BPH mean age (56.40 ± 9.74) years, 30 patients with PCa mean age (57.55 ± 9.15) years, who were diagnosed confirmed by histopathological examination and 30 volunteers healthy males mean aged (54.15 ± 7.85) years served as control. All patients in this study were not received any chemotherapy or radiotherapy treatment. Eight milliliters of blood sample were collected from patients and control, 3 mL were placed into plain tubes and centrifugation at 1500 × rpm for 5 min, the serum were removed and refrigerated at -20°C until analysis of the trace elements and MDA. The remain blood placed into EDTA tubes and centrifugation at 1500 × rpm for 10 min. The plasma was separated and refrigerated at -20°C until analysis of PSA and hormones levels. Erythrocytes were washed three times with cold saline to determine GST enzyme activity. All samples were collected from patients treated in Al–Basra teaching hospital and Al–Shifaa centre in AL-Basra and Missan province–Iraq respectively (the south provinces of Iraq), between April to September 2015.

Laboratory assessments

Serum trace elements (Cu, Zn, Se, and As) levels were measured by flame and hydride generation atomic absorption spectrometry (Model 951 dual – channel atomic absorption spectrometry equipped with a single–slot burner head, Instrumentation Laboratories) (Hershey and Oostdyk 1988). Lipid peroxidation level was measured by the method of Buege and Aust (Buege,1978). GST activity was assayed by the procedure of Carrmagnole (Carrmagnole et al., 1981). Serum of PSA, testosterone, and E2 levels was measured by mini–VIDIS assay using kit supplied by Bio Merieux – France.

Statistical analysis

All data were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed using least significant difference (LSD), considering $p < 0.05$ as the lowest limit of significance. Statistical analysis was performed using a software program (SPSS 21 for Windows, USA). One-way analysis of variance (ANOVA) was used to compare means with LSD, and Pearson's correlation coefficient was applied to observe the correlation between MDA and different biochemical parameters in patients with BPH and PCa patients.

Results:

Tables 1 depicts all the biochemical parameters which used in this study in patients of BPH and control group. There was 40 % nonsmokers and 60 % smokers of BPH patients . There was non significance differences ($P>0.05$) in (mean± SD) age, As, Cu, and E2 of BPH patients when compared with control group. There was a highly significant difference ($p<0.001$) in MDA, PSA, and Se levels. While there was a significant differences ($p< 0.05$) in GST, Zn, and Cu/Zn ratio levels between BPH and control group as shown in Table 1.

Table 1: Comparison of different biochemical parameters in BPH patients and controls group.

Parameters		BPH (N= 30) Mean ± SD	Controls (N= 30) Mean ± SD	P value
Age (years)		56.400 ± 9.740	54.150 ± 7.856	0.393
Smoking %	Never	12 (40 %)	21 (70 %)	
	Current	18 (60 %)	9 (30 %)	
MDA(µmol/L)		5.536± 1.173	0.557± 0.235	0.001
PSA (ng/ml)		4.918 ± 1.655	0.490 ± 0.187	0.001
GST (U/gH)		0.109± 0.131	0.852± 0.283	0.016
Se (µg/L)		59.118 ± 23.693	380.701 ± 193.348	0.001
As (µg/L)		1.523 ± 1.249	1.728 ± 1.280	0.489
Zn (µg/dL)		123.2± 59.9	176.1 ± 86.4	0.004
Cu (µg/dL)		130.2 ± 44.6	117.3 ± 50.3	0.839
Cu/Zn ratio		0.759 ± 0.358	0.869 ± 0.589	0.042
Testosterone (ng/mL)		3.940 ± 0.787	2.825 ± 1.022	0.107
E2 (pg/mL)		41.100 ± 11.521	35.600 ± 11.997	0.528

The mean difference is significant at the $p < 0.05$ level.

Table 2 shows that 50 % of PCa patients were smokers. There was non significant differences ($P>0.05$) in (mean± SD) ages, and E2 levels in PCa patients when compared to control group. A highly significant differences ($p<0.001$) in MDA, SPA, GST, testosterone, Se, As, Zn, and Cu/Zn ratio levels; while a significant increased in Cu level was detected in PCa patients than control group.

Table 2: Comparison of different biochemical parameters in PCa patients and control group.

Parameters		PCa (N= 30) Mean ± SD	Controls (N= 30) Mean ± SD	P value
Age (years)		57.550 ± 9.151	54.150 ± 7.856	0.601
Smoking	Never	15 (50 %)	21 (70 %)	_____
	Current	15 (50 %)	9 (30 %)	_____
MDA(µmoL/L)		8.536± 1.916	0.557± 0.235	0.001
PSA (ng/mL)		24.413±6.306	0.491 ± 0.187	0.001
GST (U/Hb)		0.078± 0.107	0.852± 0.283	0.001
Se (µg/L)		11.453 ± 3.312	380.701 ± 193.347	0.001
As (µg/L)		9.695 ± 4.128	1.728 ± 1.28	0.001
Zn (µg/dL)		41.8± 12.8	176.1 ± 86.4	0.001
Cu (µg/dL)		246.9 ± 86.3	117.3 ± 50.3	0.013
Cu/Zn ratio		5.905 ± 4.013	0.869 ± 0.589	0.001
Testosterone (ng/mL)		17.640 ± 4.021	2.825 ± 1.022	0.001
E2 (pg/mL)		71.400 ± 10.128	35.600 ± 11.997	0.307

The mean difference is significant at the $p < 0.05$ level.

The comparison between three groups (PCa, BPH and control) using ANOVA test was presented in Table 3. No significant difference (p>0.05) was observed in (mean± SD) in age for both PCa and BPH patients. A highly significant increases (p<0.001) was noticed in all parameters in PCa and BPH patients.

Table 3: Comparison of mean ± SD of parameters in patients and controls.

Parameters	BPH N= 30	PCa N= 30	Control N= 30	P value
Age (years)	56.400 ± 9.741	57.550 ± 9.151	54.150 ± 7.856	0.479
MDA(µmol/L)	5.725 ± 1.401	8.033 ± 1.929	0.644 ± 0.241	0.0001
PSA (ng/ml)	4.918 ± 1.655	24.414 ± 6.306	0.490 ± 0.187	0.0001
GST (U/gH)	0.109 ± 0.137	0.033 ± 0.012	0.801 ± 0.242	0.0001
Se (ppm)	59.118 ± 23.693	11.453 ± 3.312	380.701 ± 193.347	0.0001
As (ppm)	1.523 ± 1.249	9.695 ± 4.128	1.728 ± 1.28	0.0001
Zn (µg/dL)	1.9301± 0.599	0.418 ± 0.129	1.762± 0.865	0.0001
Cu (µg/dL)	1.302 ± 0.446	2.469 ± 0.863	1.1730 ± 0.503	0.0001
Cu/Zn ratio	0.759 ± 0.358	5.905 ± 4.013	0.869 ± 0.589	0.0001
Testosterone(ng/ml)	3.940 ± 0.787	17.64 ± 4.021	2.825 ± 1.022	0.0001
E2 (pg/ml)	41.100 ± 11.521	71.40 ± 10.128	35.600 ± 11.997	0.0001

.The mean difference is significant at the p < 0.001 level

Table 4: Correlation coefficients and the significance levels of different chemical components in patients with BPH and PCa patients.

Component Vs. MDA	BPH					PCa				
	R^2	r	slope	Intercept	P value	R^2	r	slope	Intercept	P value
Age (years)	0.0017	0.0412**	-0.0059	6.0567	0.005	0.181	0.425	0.081	2.872	0.287
PSA (ng/ml)	0.0405	0.2012**	0.1704	4.8874	0.0001	0.0005	0.022**	0.007	7.864	0.0004
GST (U/gH)	0.0007	0.0264*	3.9059	5.5071	0.0132	0.093	0.306*	48.548	6.452	0.038
Se (ppm)	0.035	0.1870*	0.0111	5.0712	0.0106	0.009	0.092**	0.054	7.419	0.0002
As (ppm)	0.068	0.2607	0.2924	5.2797	0.0791	0.068	0.261**	-0.122	9.215	0.001
Zn (ng/ml)	0.0326	0.1805**	-0.4223	6.5403	0.0078	0.041	0.201**	3.013	6.772	0.0002
Cu (ng/ml)	0.1202	0.3466**	1.088	4.3085	0.0003	0.002	0.039**	-0.087	8.247	0.008
Cu/Zn	0.1305	0.3612*	1.4132	4.6518	0.0106	0.064	0.254*	-0.122	8.752	0.018
Testosterone (ng/ml)	0.1525	0.3905*	-0.6952	8.4641	0.0229	0.082	0.286*	-0.138	10.461	0.029
Estradiol (pg/ml)	0.011	0.1*	-0.0126	6.2437	0.0458	0.077	0.277**	-0.053	11.817	0.001

*Correlation is significant at the 0.05 level, **correlation is significant at the 0.01 level.

Table 4 shows the results of correlation between oxidative stress index (represented by MDA level) and all the component which used in this study, in PCa and BPH patients. In BPH patients, a highly significant correlations was noticed between MDA and each of PSA (P = 0.0001) and Cu (P = 0.0003). Also, a highly significant correlation was observed between MDA and Age, GST, Se, Zn, Cu/Zn ratio (P < 0.01), while there is a significant correlation between MDA and testosterone and E2 (P < 0.05). Non significance correlation was noticed between MDA and As. In PCa patients, a highly significant correlations was observed between MDA and PSA, Se, and Zn (P < 0.001). A similar trend of significant was noticed in the levels of As, Cu, and E2 with MDA (p< 0.01). A significant correlation between MDA and GST, Cu/Zn, and testosterone (p< 0.05). the results show non significant correlation between MDA and age.

Discussion:

Oxidative stress is commonly considered a biologic marker of aging and of the metabolic syndrome both contributing to the development of the stochastic disorders frequently observed in the elderly population including men affected by BPH and PCa. The results in Table 3 show that serum MDA level was higher in PCa than BPH patients (Pace et al., 2010). Rise in MDA could be due to increased generation of ROS due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids (Surapaneni and Ramana, 2006). A similar finding was reported in the previous studies (Mittal and Srivastava, 2005, Ozmen et al., 2006, Strasak et al., 2008, and Savas et al., 2009). It is not clear whether this is a cause – effect relationship with regards to increased free radical levels leading to the development of cancer or vice versa (Oparinde et al., 2013). A study observed that free radicals generation was increased in cancer cells compared with normal cells (Kumar et al., 2008). A nother study suggested that development of PCa may be affected by environmental factors such as smoking, (Gsur et al., 2004) . In this study, the serum PSA levels was found to be significantly higher (p=0.0001) in both PCa and BPH patients when compared to control group. The measurement of PSA is a helpful tool in the diagnosis and follow up of patients, also, PSA is specific for prostate tissue but not for PCa. It is also found in abnormal concentrations in normal and benign changes of the prostate such as BPH and other non–neoplastic prostatic lesions (Lakhey et al., 2010). Besides the role of GSTs in activation and inactivation of oxidative metabolites of carcinogenic compounds associated with cancer, they also detoxify a broad range of substances including carcinogens, environmental toxins, and drugs (Konwar et al., 2010) .

Trace elements are accepted as major constituents of biological structures which have a complex role in development and inhibition of cancer (Kaba et al., 2014). Zn is more abundant in the human prostate than in other tissues (Mehran Mohseni et al., 2015), and it plays an anti–carcinogenic role through structural stabilization of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. Zinc has a protective effect against free– radical injury (Wu et al., 2004), and Zn deficiency could be a risk factor for PCa (Kaba et al., 2014).

In the present study, significantly lower plasma levels of The present study was similar to a study found the loss of Zn from biological membranes could increase the susceptibility of such cells to oxidative damage and impaired cell functions (Xia et al., 1999).

Several studies reported significantly lower levels of both Se and Zn in all categories of PCa patients (Ozmen et al., 2006, Arinola and Marbel, 2008). In different studiess showed that Zn is involved in the pathogenesis of PCa; and that Zn could be efficacious in the prevention and treatment of PCa (Shobeiri et al., 2014, Rodrigo et al., 2015). In vitro, Zn helps to maintain intra – prostatic balance of testosterone (Ganiyu and Mabel 2008). High Cu concentrations can lead cancer by producing DNA damage via toxic free radicals (Theophanides et al., 2002). While the Cu induces angiogenesis, it can potentially cause growth in PCa by improving

blood supply to tumor. This can explain increased Cu levels in involved tissues (Majumder et al., 2009). Similar result was reported by Ozmen et al.(2006), who found a significant increase in serum Cu levels in PCa patients compared with control.

The results of the present study showed that PCa patients had lower concentration of Se. Most of the previous studies determined the association between risk of PCa and trace elements concentration in the plasma (Brooks et al., 2001; Shahar et al., 2009; Adaramoye et al., 2010). Se is a well documented cofactor to antioxidant enzymes (Dunna et al., 2011). The best–known example of Se function is the reduction of hydrogen peroxide and damaging lipid and phosphor lipid hydroperoxides to harmless products by the family of selenium-dependent glutathione peroxidises. This function helps to maintain membrane integrity, protects prostacyclin production and reduces the likelihood of propagation of further oxidative damage to bio–molecules such as DNA, lipoproteins and lipids with the associated increased risk of conditions such as cancer and atherosclerosis (Karimi et al., 2012). The anticancer activity of Se was also attributed to its being a component of glutathione peroxidase, which protects DNA and cell membrane from peroxide damage by catalyzing conversion of peroxides (ROOR) to hydroxyl acids (ROH). Selenium is also vital for immune system function and may help prevent PCa. Low levels of Se in subjects with PSA values >4ng/ml may explain their susceptibility and progression of PCa. (Ganiyu and Mabel 2008).

Prostate carcinogenesis appears to be a highly multifactorial process in which hormones, both androgens and estrogen, play a central role, some studies appear to support the counterintuitive concept that low androgen levels may be related to adverse PCa outcomes (Isbarn et al., 2009). E2 is essential for the initiation of PCa growth by bringing about telomere formation (Friedman 2005).The enzyme GST is repressed in PCa cells due to hypermethylation (Dubey and Apenten 2014), and GST are involved in the intracellular transport of steroid hormones (de Sa et al., 2014). Due to its xenoestrogenic nature, As may also causes reproductive cancer. This indicates that the ingestion of As could be causing more risk of PCa in male inhabitants of the region, possibly suggesting a gene-environment interaction in the area. Reports in literature suggest an association of As with reproductive hazards possibly due to disruption of the steroid hormone signalinug pathway and disruption of the steroid hormone metabolism in human body. Because of prostate epithelial cell sensitivity, As reportedly plays a potential role in prostate carcinogenesis (Nath et al., 2012).

Positive correlation between serum MDA level with PSA, GST, and As in PCa patients was reported in Table 4. It was suggested a cause and effect association, that is if oxidative stress developed, then increase in level of antioxidant and PSA try to nullify the effect. These cascades of events may lead to reduced expression of the detoxifying enzymes or protein, which may result in development of prostate cancer. The important role of GST is in conjugating GSH to the products of endogenous lipid peroxidation. The increase of MDA levels give the evidence of significant alteration of pro–oxidant and antioxidant of BPH and PCa patients .

This observations are in agreement with the study of Merendino et al., 2003, MDA, which found that products of the lipid peroxidation MDA levels are capable of modifying both DNA and proteins, resulting in mutagenic, genotoxic and cytotoxic events. Therefore, high levels of MDA, such as of other reactive aldehydes, may explain DNA base modifications demonstrated not only in PCa, but also in BPH epithelium too. It can speculate that increased circulating MDA levels might be considered a useful marker of lipid peroxidation and inflammation of prostate epithelium (Merendino et al., 2003).

Recent data indicated that malignant transformation is accompanied by a loss of tissue – specific functional features, which leads to a significant reduction in the contents of elements associated with functional characteristics of the human prostate tissue. Therefore, it is plausible that the reason for the emergence and development of adenocarcinoma is associated with abnormally

high concentration of some metals in the prostate tissue of older men (Zaichick, 2016).

In conclusion,trace elements which used in this study (Zn, As, Se, and Cu) can be used a bioindicator for prostate illness. Serum MDA may be considered a marker for predicting prostate cancer as a compliment to PSA. This is the first study was done on patients with PCa and BPH in Missan province in south of Iraq .

References:

1. A Edward Friedman (2005). The Estradiol-Dihydrotestosterone model of prostate cancer. Theoretical Biology and Medical Modelling; 2:10.
2. Ahmedin Jemal, Rebecca Siegel, Elizabeth Ward, Taylor Murray, Jiaquan Xu, Carol Smigal, Michael J. Thun (2006). Cancer Statistics. CA Cancer J Clin; 56:106–130.
3. A Nath, J K Singh, S Ezhil Vendan, Priyanka, Shreya Sinha (2012). Elevated Level of Prostate Specific Antigen Among Prostate Cancer Patients and High Prevalence in the Gangetic Zone of Bihar, India. Asian Pacific J Cancer Prev; 13: 221-223.
4. Andrew W. Roddam, Naomi E. Allen, Paul Appleby, and Timothy J. Key (2008). Endogenous Hormones and Prostate Cancer Collaborative Group. J Natl Cancer Inst;100: 170 – 183.
5. Arinola OG, Marbel CDA (2008). The serum levels of Trace metals in Nigerians males with different PSA values. Malaysian J. Med. Sci.; 15(2): 39-42.
6. Arinola Olatunbosun Ganiyu and Charles Davies Ayebatonyo Mabel (2008). The Serum Levels of Trace Metals in Nigerian Males with Different PSA Values. Malaysian Journal of Medical Sciences;15(2): 39-42.
7. Banas A, Kwiatek WM, Banas K (2010). Correlation of concentrations of selected trace elements with Gleason grade of prostate tissues. J Biol Inorg Chem; 15: 1147-55.
8. Costello LC, Franklin RB. (2006). The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots. Mol Cancer; 5(17).
9. Barry P. Rosen1 and Zijuan Liu (2009). Transport pathways for arsenic and selenium: A miniriew. Environ Int.; 35(3): 512–515.
10. D.S.L. Srivastava and R.D. Mittal (2005). Free Radical Injury and Antioxidant Status in Patients with Benign Prostate Hyperplasia and Prostate cancer. Indian Journal of Clinical Biochemistry; 20(2): 162-165.
11. Guven K, Ozbay C, Unlu E, Satar A (1999). Acute Lethal Toxicity and Accumulation of Copper in Gamma rus pulex. Turk J Biol; 23:513 – 21.
12. Hasan Yari, Mehran Mohseni, Raziyeh Vardi, Adel Mirza Alizadeh and Saeideh Mazloomzadeh (2015). Copper, Lead, Zinc and Cadmium levels in serum of prostate cancer patients by polarography in Iran. J. Chem. Pharm. Res.; 7(2):403-408.
13. Hendrik Isbarn, Jehonathan H. Pinthus , Leonard S. Marks, Francesco Montorsi, Alvaro Morales, Abraham Morgentaler, Claude Schulman (2009). Testosterone and Prostate Cancer: Revisiting Old Paradigms, EUROP EAN UROLOGY; 56: 48–56.
14. Hershey JW, Oostdyk TS, Keliher PN. (1988). Determination of arsenic and selenium in environmental and agricultural samples by hydride generation atomic absorption spectrometry. J Assoc Off Anal Chem.;71(6):1090-3.
15. John A. Buege, and Steven D. Aust. (1978). Microsomal lipid peroxidation. Methods in Enzymology; 52: 302–310.
16. Khalid M. Malik, Ariana Nelson, and Honorio Benzon. (2015). Disease – modifying Antirheumatic Drugs for the Treatment of Low Back Pain: A Systematic Review of the Literature. Pain practice; 12(4).
17. Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK (2008). Oxidative Stress Is Inherent in Prostate Cancer Cells and Is Required for Aggressive Phenotype. Cancer Res.; 68: 1777.
18. Majumder S, Chatterjee S, Pal S (2009). The role of copper in drug-resistant murine and human tumors. Biometals; 22: 377-84.
19. Mehmet Kaba, Necip Pirincci, Mehmet Bilgehan Yuksel, Ilhan Gecit, Mustafa Gunes, Huseyin Ozveren, Huseyin Eren, Halit Demir (2014). Serum Levels of Trace Elements in Patients with Prostate Cancer. Asian Pac J Cancer Prev; 15(6): 2625-2629.
20. Meltem Ozlen Dillioglugil, Haluk Mekık, Bahar Muezzinoglu, T. Alp Ozkan, Cennet Gural Demir, Ozdal Dillioglugil (2012). Blood and tissue nitric oxide and malondialdehyde are prognostic indicators of localized prostate cancer. Int Urol Nephrol; 44(6):1691-6.
21. Nelson WG, De Marzo AM, DeWeese TL, Isaacs WB (2004). The role of inflammation in the pathogenesis of prostate cancer. J. Urol., 172: 6–11.
22. Ozmen H, Eiulas FA, Karatas F, Cukurovali A, Yakin O (2006). Comparison of the concentration of trace metals (Ni, Zn, Co, Cu and Se, Fe, vitamins A,C,E and lipid peroxidation in patients with prostate Cancer. Clin. Chem. Lab. Med.; 44(2): 175-179.
23. Pathak SK, Sharma RA, Steward WP, Mellon JK, Griffiths TR, Gescher AJ. (2005). Oxidative stress and cyclooxygenase activity in prostate carcinogenesis: targets for chemopreventive strategies. Eur. J. Cancer; 41: 61–70.
24. Pirkko L Harkonen and Sari I Makela (2004). Role of estrogens in development of prostate cancer. Journal of Steroid Biochemistry & Molecular Biology; 92: 297–305.
25. PJ Van Bladeren. (2000). Glutathione conjugation as a bioactivation reaction. Chemico-biological interactions; 129 (1): 61-76.
26. Renata Almeida de Sa, Aline dos Santos Moreira, Pedro Hernan Cabello, Antonio Augusto Ornellas, Eduardo Butinhao Costa, Cintia da Silva Matos, Gilda Alves, Ana Hatagima (2014). Human glutathione S-transferase polymorphisms associated with prostate cancer in the Brazilian population. ibju | GSTs and Prostate Cancer; 40(4): 463-473.
27. Rituraj Konwara, Parmeet Kaur Manchandab, Preeti Chaudharya, V. Lakshma Nayaka, Vishwajeet Singhc, and Hemant Kumar Bid. Glutathione S-transferase (GST) Gene Variants and risk of BPH (Benign Prostatic Hyperplasia): A report in north Indian population, Asian Pacific Journal of Cancer Prevention, 11(10):1067-1072.
28. Rosaria Alba Merendino, Francesco Salvo, Antonella Saija, Giuseppe Di Pasquale, Antonio Tomaino, Paola Lucia Minciullo, Giuseppe Fraccica and Sebastiano Gangemi (2003). Malondialdehyde in benign prostate hypertrophy: a useful marker? Mediators of Inflammation; 12(2): 127-128.
29. Savas, M., A. Verit, H. Ciftci, E. Yeni and E. Aktan (2009). Oxidative Stress in BPH. Nma j nepal med assoc.; 48(173): 41-45.
30. Shobeiri MJ, Tabrizi AD, Atashkhoei S, Sayyah-Melli M, Ouladsahebmadarek E, Ghojazadeh M. (2011). Serum levels of Copper,

Zinc and Copper/Zinc Ratio in Patients with Ovarian Cancer. Pak J Med Sci; 27(3): 561-565.
31. Strasak, A., K. Rapp, L. Brant, W. Hilbe, M. Gregory and W. Oberaigner (2008). Association of glutamyl transferase and risk of cancer incidence in men: A prospective study. Cancer Res; 68(10): 3970-3977.
32. Tandon R., U.S. Rath, Deepti Pande, Reena Negi, Kanchan Karki and Hari D. Khanna (2012). Lipid Peroxidation and Thymidine Phosphorylase expression in Prostate Carcinoma. Journal of Stress Physiology & Biochemistry; 8(3): 113-119.
33. Theophanides T, Anastassopoulou J (2002). Copper and carcinogenesis. Crit Rev Oncol Hematol; 42: 57-64.
34. Vaibhav Dubey and Richard Owusu-Apenten. Curcumin Restores (2014). Glutathione-S-Transferase Activity for LNCaP Prostate Cancer Cells. Pure and Applied Chemical Sciences; 2(2): 61 – 72.
35. Vladimir Zaichick and Sofia Zaichick. (2016). Trace Element Contents in Adenocarcinoma of Human Prostate Investigated by Energy Dispersive X-Ray Fluorescent Analysis. Journal of Adenocarcinoma; 1(1):1.
36. Wu T, Sempos CT, Freudenheim JL, Muti P, Smit E (2004). Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. Ann Epidemiol; 14: 195-201.
37. Xia J, Browing JD, ODell BL. (1999). Decreased plasma membrane thiol concentration is associated with increased osmotic fragility of erythrocyte in Zinc-deficient rats. J. Nutr.; 129: 814-819.