Serum Zn and Cu Levels in Patients with Benign Prostate Hyperplasia (BPH) and Prostate Carcinoma (PCa) and their Association with the Stage of the Disease in Carcinoma of Prostate Patients

M. Ghani[1], M. R. Khan[2*], M. Z. Ahmed[1], S. Mahmood[1], I.A. Shah[1], I. Fayyaz[1] and S. Akram[1]

1. Department of Biochemistry, University of Health Sciences, Lahore, **PAKISTAN**
2. Department of Environmental Science and Policy, Lahore School of Economics, Lahore, **PAKISTAN**

Email: drrafiq@lahoreschool.edu.pk, khanmr1939@yahoo.com

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ABSTRACT

Carcinoma of the prostate (PCa) is one of the most commonly diagnosed malignancy afflicting the men which is a leading cause of carcinoma related mortality in men. Effect of trace elements like zinc and copper has been studied extensively in the recent years to evaluate their role in pathogenesis of various neoplasms including prostate carcinoma. The objective of this study was to seek an association of serum zinc and copper levels with stage of the disease in patients with carcinoma of the prostate. Cross-sectional analytical study design was employed after sampling 60 indoor elderly male patients (30 years age) matched healthy males. 30 patients with organ confined, 30 patients with advanced metastatic carcinoma of the prostate and 30 cases with benign prostatic hypertrophy were included as three groups of patients. The fourth group included was 30 healthy individuals of same age group as controls. Zinc and copper levels in the sera were measured by standard techniques using atomic absorption spectrophotometer. Significant difference was observed in Zn levels of four groups (P<0.001). Zn levels in the sera were significantly decreased in organ confined and metastatic PCa groups (78 ± 14.58, 66 ± 14.24) compared with healthy controls (93 ± 18.21). From the results it may be concluded that a significant percentage of deaths resulting from cancer may be avoided through greater attention to proper and adequate nutrition with due regard to Zn and Cu intake.

Keywords: Serum Zn and Cu levels, Patients with Benign Prostate Hyperplasia (BPH) and Prostate Carcinoma (PCa)**,** Stage of the Disease in Carcinoma of Prostate Patients.

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INTRODUCTION

Cancer is the most dreadful and devastating disease of the human body. It is a health problem that is progressively gaining more and more importance because the people all over the globe are becoming aware of its negative role in affecting the human beings. Cancer is basically of two types: Non-malignant and malignant; former being tolerable to some extent while latter is disastrous. One of the most commonly diagnosed malignancy affecting males is prostate carcinoma (PCa) reported by Van Moorselaar and Voest

(2002) and Li et al. (2005) which is a leading cause of carcinoma related mortality in men second only to lung cancer [1-2].

There is an enormous variation in incidence and mortality of prostate cancer among different geographical regions of the world. In many industrialized nations such as the United States, Western Europe and some countries of Africa it is one of the most commonly encountered cancers and is also among the leading causes of cancer deaths as reported by Parkin et al., [3]. More than 670,000 men were diagnosed worldwide with prostate cancer in 2002, accounting for one in nine of all new cancers in males. In developing countries, it is considered to be less common; however its incidence and mortality has been on the rise as agreed by Ferlay et. al.,[4].

Asians were previously considered to be having the lowest incidence and mortality rate of prostate cancer in the world. However, these rates have been shown to rise rapidly in the past two decades in most Asian countries. According to the results reported by Pu et al.,[5],Sim and Cheng[6] and Jung et al., [7] recent trend in Asian countries reflect increasing incidence of PCa and now the PCa is reported to be one of the leading male cancers in this part of the world. There is a lack of epidemiological data on the exact prevalence of this disease in Pakistan. However, the recent results presented by Bhurgri et al.,[8] has suggested the escalation in incidence and PCa has been reported as one of the most common malignancies among Pakistani males**.**

The etiology of PCa has been extensively studied, yet it remains unclear. The results indicate that this disease has multifactorial origin. Risk factors for cancer include genetic susceptibility as well as age, dietary practices, physical activity, agrochemical exposures, infectious diseases, family history and socioeconomic status [9]. Many of these risk factors are considered unchangeable, but there has been considerable investigation and interest in the effects of modifiable risk factors such as diet and lifestyle on prevention of cancer. It is expected that more than half of cancer deaths could be prevented by a change in diet and lifestyle. Diet alone appears to play an important role in the development and possibly the progression of the disease [10].

Zinc and copper are essential micronutrients whose exclusive role in modifying the etiology of cancer. The significance of zinc and copper has been evaluated by Khanna and Karjodkar[11] and changes in Zn and Cu serum levels have been observed both in human and animal models. Lower level of essential antioxidants in circulation has been found to be a contributory factor for an increased risk of cancer. Zinc and copper are essential for numerous enzymes; therefore, it may be assumed that variations in serum level of these biochemical markers have been implicated in the pathogenesis of prostate cancer. Zinc is an essential mineral in human nutrition and important constituent of more than 300 enzymes in the body [12] that perform different vital biological functions (Ishii et al., 2004) related to zinc deficiency [13]. Among the adult population, the elderly are a potentially vulnerable subpopulation due to multiple reasons such as low dietary Zn intake and an age-associated decrease in intestinal Zn absorption [13].

The recent research has revealed clinical manifestations of Zn deficiency and the significant role Zn plays in physiology and biochemistry of human body [14]. The studies by Ho et al.,[15] conclude that Zn plays a central role in many biological functions, and has diverse functions in relevance to the different cancers. It has been further indicated that oxidative stress is associated with ageing and severe age-related degenerative diseases including cancers, particularly the PCa. The oxidative stress has a definite role to play in pathogenesis and etiology of PCa say Kumar et al.[16]. Zinc is an essential component of Cu/Zn superoxide dismutase (Cu/Zn SOD), a potent antioxidant enzyme and is also involved in stabilization of sulfhydryl groups on proteins to protect them from oxidation as described by Powell [17]. Various in vitro cell culture and in vivo animal studies by Oteiza et al.[18] and Ho et al.[15] have indicated that Zn deficiency may trigger oxidative damage and damage DNA increasing the risk of carcinogenesis.The prostate contains one of the highest levels of Zn and high concentration of Zn is related to physiological

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function in normal and hyperplastic prostate tissues (Ishii et al.[12], Costello et al.[19]). However, the prostate cells which become cancerous have markedly low Zn concentrations, in comparison to the normal prostate tissue in this region[20]. The secretory epithelial cells of the peripheral zone of the prostate are highly specialized cells and are responsible for Zn accumulation report Liang et al.[21]. A number of studies such as by Franklin et al. [20] and Costello et al. [19] have appeared that correlate variation in prostate zinc level and cancer cells proliferation also offer the explanation of these changes. One of these studies conducted by Feng et al. [22] indicates that low Zn as well as low citrate concentration is characteristic of prostate cancer cells and there is substantial evidence which suggests that changes occur early in prostate carcinogenesis. Thus, low Zn concentration provides an environment in prostate cancer cells which supports uncontrolled proliferation and cancer progression. Some studies e.g. Kolonel et al.[23], Leitzmann et al.[24] have explored the correlation between Zn status, dietary Zn intake and prostate cancer risk. and also no correlations between Zn and prostate cancer development Vlajinac et al.[25].The role of Cu in causation and progression of tumors has been extensively studied for the past few decades. The distribution of Cu has been found to be altered in tumor bearing mice, rats and also in humans (Brewer, 2005, Demirhan et al., 2010). Despite being essential element for various biological processes Cu can become toxic for cells at elevated concentrations is reported by Theophanides and Anastassopoulou [26].

Copper stimulates the proliferation and migration of endothelial cells and also activates several proangiogenic factors. The level of Cu required for angiogenesis may be higher than that is required for essential Cu dependent cellular functions, Copper is thought not only to activate endogenous angiogenic factors but it also binds to several proteins like heparin and ceruloplasmins

Several reports in the literature elucidate the Cu levels in serum and tumor tissue of cancer patients and elevated Cu levels have been documented in a wide variety of tumors in comparison with healthy subjects. Studies conducted by Kuo et al.[27] ,Saxena et al.[28], Boz et al.[29], Demirhan et al.[30], Zuo et.al. [31] on patients from different geographical locations of the world also established the similar fact. The most striking finding in most of these studies is the correlation of Cu levels with cancer progression. The studies showed that the concentrations of antioxidant like Zn were significantly lower in cancer patients while the Cu concentrations were almost always found to be either elevated or significantly elevated compared to age matched samples (Kuo et al. [27] and Zuo et.al. [31].Furthermore, it has also been shown that the Cu/Zn ratios are almost always higher in malignant patients compared to normal subjects as reported by Lightman et al.[32] . Although various studies concerning the relative Cu and Zn levels in serum of healthy individuals and patients with carcinoma of the prostate have been carried out in several countries e.g. Aydin et al.[33] and Anetor et.al.[34] ,yet the results have been inconclusive. Similar studies so far have not been conducted in Pakistan. Therefore, there is a need to substantiate the fact that essential trace elements have a definite role to play in PCa and how Cu and Zn are associated with prostate carcinogenesis.

Here, the work reviewed above was extended to study the effect of Zn and Cu levels on physiology/epidemiology of prostate cancer. The specific goal and objectives were as under:

1.   Determination of the serum Zn and Cu levels in patients with BPH, PCa and compare them with levels in normal controls.

2.   Looking into the nature of association of serum Zn and Cu levels with the stage of the disease in patients with carcinoma of the prostate.

MATERIALS AND METHODS

Study design and Samples: Cross-sectional analytical study design was employed. A total of 90 indoor elderly male patients were sampled. All the patients were between 50 and 75 admitted to urology units of two local hospitals of Lahore. The patients were further subdivided into three groups each comprised of 30 patients. Group 1 had been diagnosed of benign prostatic hyperplasia, 2 and 3 were diagnosed of histopathologically confirmed cancer. The control group consisted of 30 age matched healthy males. The selection criteria were as under:

1.   Participants in the study had received no medication known to interfere with bone metabolism and no multivitamin or mineral supplements.

2.   They had no signs of acute or chronic inflammatory conditions of the prostate, diabetes mellitus, hepatic, renal or bone diseases, and other malignancies.

3.   The selected samples were elderly patients diagnosed with benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCa) based on clinical examination including digital rectal examination, ultrasonography, serum Prostate specific antigen (PSA) and histopathological staining.

4.   Prior written and informed consent of all the subjects was obtained.

5.   A detailed history of each patient was taken and examination was conducted and the observations and findings were recordedon a specially designed proforma.

Sample Collection and Processing: Five ml of venous blood was drawn from each patient and control and transferred to metal free test tubes. Blood samples were centrifuged at 2000rpm for 10 min within an hour after venipuncture. Sera were separated and aliquoted in eppendorf tubes which were labeled with the allotted individual sample identification number and stored at –20°C until assayed for Zn and Cu. Clinically cleaned glassware was used for serum separation and subsequent analysis to ensure exclusion of any possibility of contamination with Zn or Cu. 6-Zowczak. The subsequent laboratory investigations were carried out at the Department of Biochemistry, University of Health Sciences Lahore in collaboration with the University of Veterinary and Animal Sciences Lahore.

Determination of Zinc in the Serum: The level of Zn was measured in the sera by atomic absorption spectroscopy. Serum sample was prepared as described by Goel and Sankhwar [35]. To 1 mL of serum, 2 mL of 5% trichloric acid and 7 mL of deionized water were added to bring up to a total volume of 10 mL. The interfering protein was precipitated and the mixture was centrifuged to settle down insoluble precipitates. The clear supernatant was separated and again brought up to a volume of 10 mL. This ten-fold diluted sample was aspirated into acetylene atomic absorption flame. The Zn level was determined by comparing the absorption of diluted serum with absorption of standard solution prepared in nitric acid in Atomic Absorption Spectrophotometer Hitachi model Z8230 under the conditions at 213.9 nm using zinc Hollow Cathode Lamp. Each sample was run in duplicate

Determination of Copper in the Serum: The serum Cu level in the sera was also measured by atomic absorption spectroscopy as done in Zn using the same instrument. The procedure of sample preparation was the same as above. Ten- fold diluted samples were aspirated into the atomic absorption flame. The Cu level was determined by comparing the absorption of diluted sera with absorption of standard solution which were prepared in nitric acid at 324.8 nm using copper Hollow Cathode Lamp..

Statistical Analysis: The data was analyzed by using SPSS software version 16.0 (SPSS Inc, Chicago, IL, USA). Mean $\pm$ S.E.M was calculated for normally distributed quantitative variables while categorical data was presented as percentages and frequencies. Sphario-Wilk test was applied to check normality of quantitative variables. One-way ANOVA test was applied to observe group mean differences and Post HOC Tukey test was applied to observe which group means differ. Pearson Chi-square test was applied to observe associations between qualitative variables. Pearson's coefficient was applied to observe correlation between quantitative variables. A p-value of <0.05 was considered as statistically significant.

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RESULTS AND DISCUSSION

Comparative picture of the age of control and cancer patient groups: The statistical average of age noted during sampling of the persons in all groups including controls is shown in table 1 and their comparative picture displayed in fig. 1. These were control (60±6.3), **BPH** (59±5.8), organ confined prostate cancer (N_0M_0) (63±5.0) and metastatic prostate cancer (M_1) (67±3.8). Significant difference was found between the ages of groups (P<0.0001). No significant difference was found between the age of controls and the age of patients in BPH and N_0M_0 groups. The mean age of the patients in M_1 group (67±3.8) was significantly high (P<0.0001) compared to the control group (60±6.3). The mean age of patients in M_1 (67±3.8) and N_0M_0 (63±5.0) groups were significantly high (P<0.0001) and (P<0.05) when compared with BPH group (59±5.8).
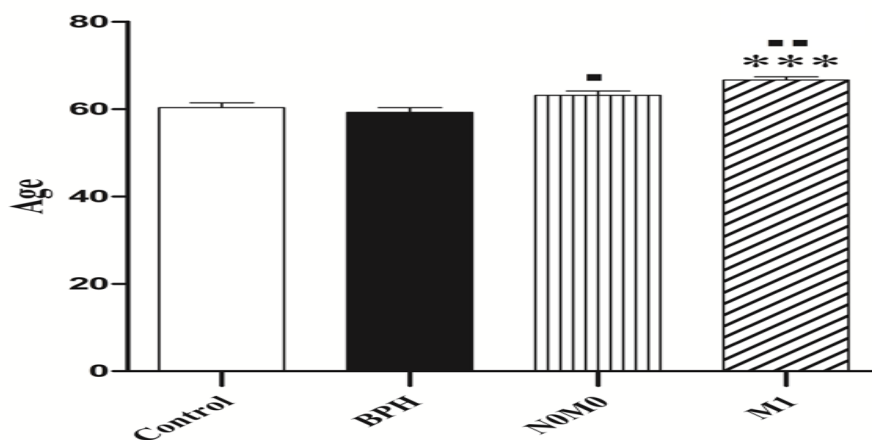

Figure1. Comparison between mean age in study groups

Fig 1 shows significant difference between the groups when compared with Control group*, between the groups when compared with BPH group[■] and between the groups when compared with N_0M_0 group[f].

Table1: Age, level of Cu, Zn µg/dl and the Cu/Zn ratio of the studied subjects.

Parameter	Control Group n=30 Mean ± SD	BPH Group n = 30 Mean ± SD	N_0M_0 Group n =30 Mean ± SD	M_1 Group n = 26 Mean ± SD	p-value
Age in years	60±6.3	59±5.8	63±5.0	67±3.8	0.0001
Copper µg/dl	97 ± 18.52	99 ±18.56	140 ± 27.75	190 ±36.55	0.0001
Zinc µg/dl	93 ± 18.21	89±17.71	78 ± 14.58	66 ± 14.24	0.0001
Cu/Zn ratio	1.1±0.42	1.2±0.54	1.8±0.53	2.9±1.0	0.0001

There was a significant different between the groups (p<0.0001) and no difference was found between control and BPH group.

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Level of Copper and Zinc in Sera: Table 1 shows the level of Cu and Zn in the study groups. It indicates that the level of Zn was high in the sera of control group as compared to the sample groups. The level of Zn in the sera of patients in N_0M_0 (78 ± 14.58 µg dl^{-1}) and M1 (66 ± 14.24µg dl^{-1}) groups was significantly low (P<0.01) and (P<0.001) respectively compared with Control group (93 ± 18.21µg dl^{-1}). N_0M_0 and M1 groups when compared with BPH (89±17.71 µg dl^{-1}) group, the level of Zn was remarkably low (P<0.05) and (P<0.001) respectively (Fig.2). Zinc level of N_0M_0 group and M1 group showed no significant difference (78 ± 14.58µg dl^{-1} Vs 66 ± 14.24µg dl^{-1}, p=0.51)**.**
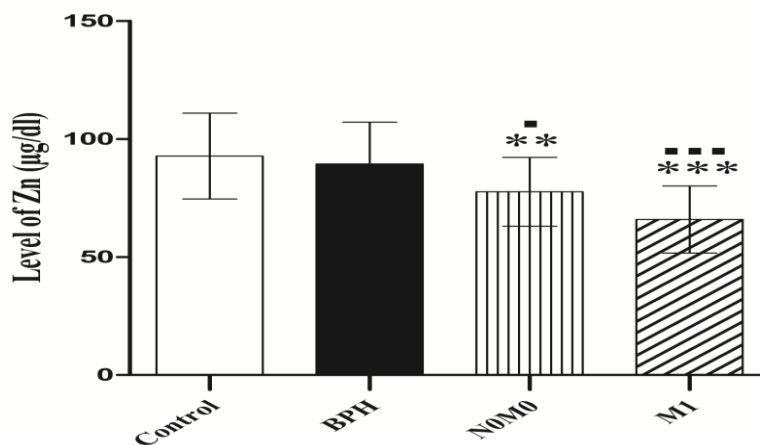

Figure2. Comparison between levels of Zn in study groups

Fig. 2 shows the difference between the groups when compared with Control group*, significant difference between the groups when compared with BPH group■ and significant difference between the groups when compared with N_0M_0 group ^{f}.

Similarly, Table 1 shows that the level of Cu in the sera of patients in N_0M_0 (140 ± 27.75 µg dl^{-1}) and M_1 (190 ±36.55 µg dl^{-1}) groups was significantly high (P<0.001) and (p<0.001) respectively compared with Control group (97 ± 18.52 µg dl^{-1}). Significant difference (P<0.001) was observed when level of Cu concentration in the sera of patients in group N_0M_0 and M_1 was compared with BPH group (99 ±18.56). Similarly, Cu level of N_0M_0 group and M_1 group also showed significant difference (140 ± 27.75 µg dl^{-1} Vs 190 ±36.55 µg/dl), p<0.01. The M_1 group showed significantly higher levels than N_0M_0 group.
A comparison between levels of Cu in study groups is made in Fig.3.
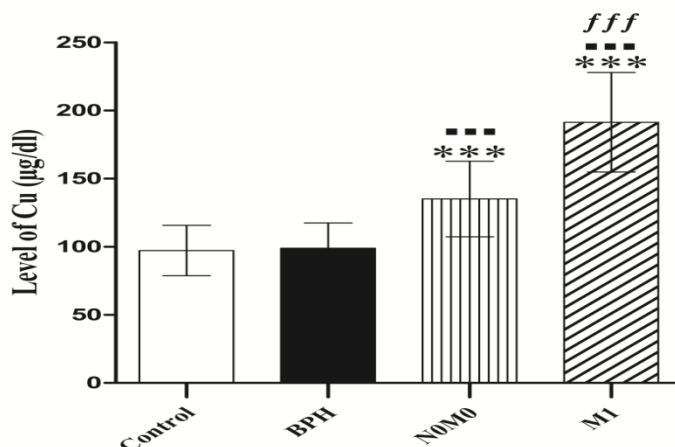

Figure3. Comparison between levels of Cu in study groups

Fig. 3 shows significant difference between the groups when compared with Control group, significant difference between the groups when compared with BPH group[■] and significant difference between the groups when compared with N0M0 group[f].

Furthermore, the Cu/Zn ratio in control, BPH, N_0M_0 and, M_1 group were 1.1±0.42, 1.2±0.54, 1.8±0.53 and 2.9±1.0. Significant difference was observed in the Cu/Zn ratio between four groups, p<0.001. Cu/Zn ratio of control group and metastatic PCa (M_1) group (1.1±0.42 Vs 2.9±1.0), p<0.01, showing that Cu/Zn ratio of M_1 group is higher than the control group. Significant difference was also observed between Cu/Zn ratio of control group and organ confined PCa (N_0M_0) group (1.1±0.42 Vs. 1.8±0.53), p<0.01, again showing that Cu/Zn ratio of N_0M_0 group is also higher than the control group. Significant difference was observed between Cu/Zn ratio of BPH and M_1 group (1.2±0.54 Vs 2.9±1.0), p<0.01) showing that Cu/Zn ratio of M_1 group is also higher than the BPH group. Statistically significant difference was also observed in Cu/Zn ratio of BPH and organ confined carcinoma group (1.2±0.54 Vs 1.8 ± 0.53), p<0.01 showing that Cu/Zn ratio of N_0M_0 group is higher than BPH group. Cu/Zn ratios were significantly higher in M_1 group as compared to N_0M_0 group, BPH and control groups (Fig.3). Cu/Zn ratio of organ confined PCa group was also significantly higher as compared to both BPH and control group. Cu/Zn ratio of N_0M_0 group and M_1 group showed significant difference (1.8 ± 0.53 Vs 2.9 ± 1.0), p<0.01. There was no significant difference between Cu/Zn ratio of control group and BPH group (1.2 ± 0.54 Vs 1.1±0.42), p= 0.963
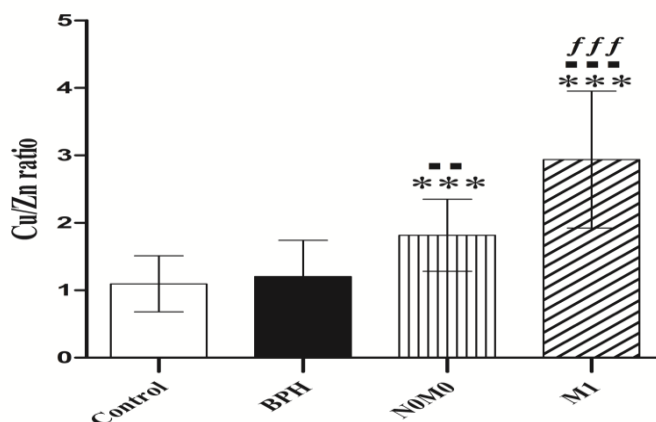Comparison between Cu/Zn ratio in study groups is made in Fig. 4

Figure4. **Comparison between Cu/Zn ratio in study groups**

Fig. 4 shows significant difference between the groups when compared with Control group[*], significant difference between the groups when compared with BPH group[■] and significant difference between the groups when compared with N0M0 group[f].

Correlation of age with Cu and Zn, and correlation between Cu and Zn in study groups: The correlation calculated from the age to levels of Zn and Cu variables are displayed in Fig.5A and 5B (patients) and 6A and 6B (control) There was significant (p< 0.0001) and positive correlation (r= 0.4437) between age and Cu levels in the sera of the all persons including control and patients (Fig.5A). On the other hand, a significant (p<0.0056) and negative correlation (-0.2593) was observed between age and the level of Zn in sera (Fig.5B). The correlation data shows with increasing age the level of Cu increased while the level of Zn decreased
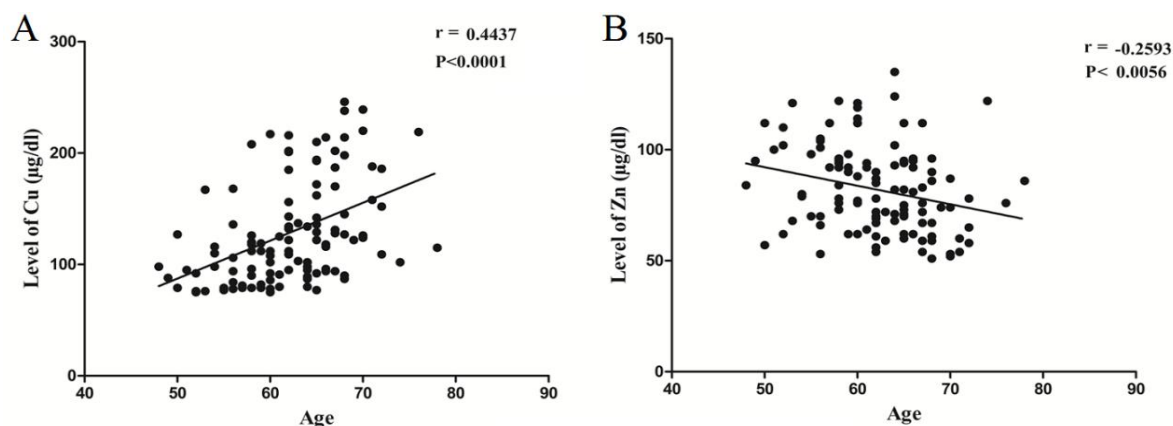
Fig. 5, Correlation of age of the persons included in the study with levels of Cu and Zn.
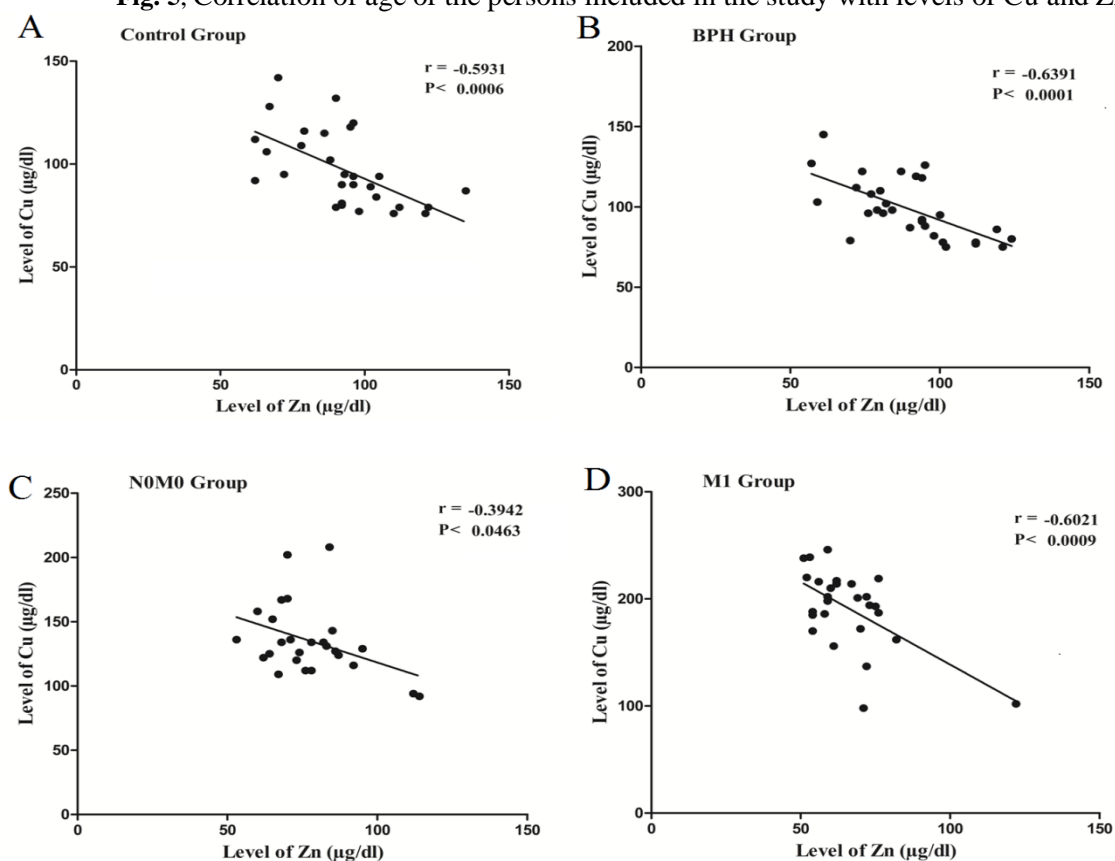

Fig.6: Correlation between the Zinc (Zn) and Copper (Cu).

There was significant (p< 0.0006) and (P<0.0001) inverse (r= -.5931) and (r= -0.6391) correlation between Cu and Zn in the Control and BPH group respectively (Fig.6 A, B). Similarly, in organ confined PCa (N_0M_0) and metastatic PCa (M_1) groups, significant (p<0.0463) and (p<0.0009) inverse (r= -0.3942) and (r= -0.6021), correlation was observed between Cu and Zn in the sera of N_0M_0 and M_1 group (Fig. 6 C, D).
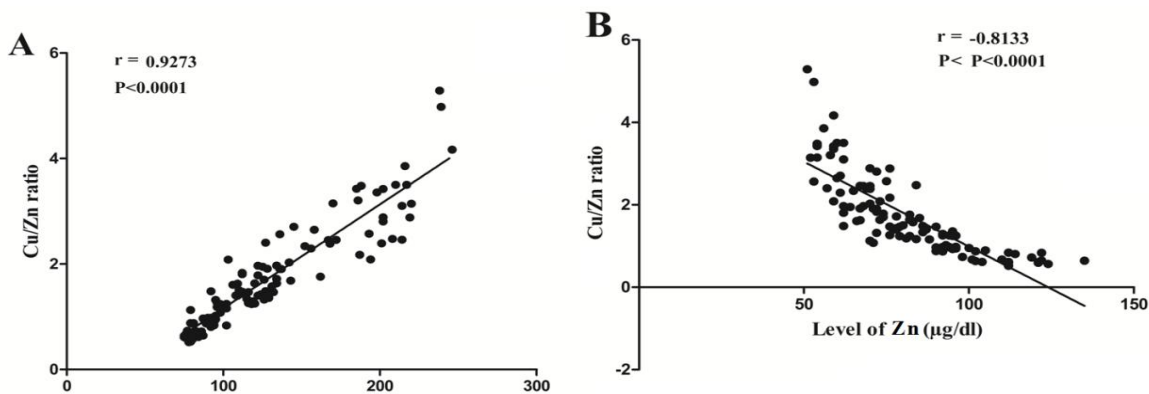
Figure 7: Correlation between the Cu/Zn ratio and level of Cu and Zn.

Significant (P<0.0001) positive (r=0.9273) correlation was observed between Cu levels and Cu/Zn ratio which indicated that with increasing Cu levels there was increase in the Cu/Zn ratio. Significant (P<0.0001) inverse (r=0.8133)) correlation was observed between Zn levels and Cu/Zn ratio. This showed that with decreasing Zn level, the Cu/Zn ratio was increasing.

Prostate cancer predominantly is considered to be a disease of elderly men and age is a well-established risk factor for PCa (Schaid, [36]). In this study the mean age of control group was 60 ± 6.3 years. The mean age of BPH group, organ confined prostate cancer group (N_0M_0) and metastatic prostate cancer group (M_1) was 59 ± 5.8, 63 ± 5.0 and 66 ± 3.8 years respectively. The choice of the age groups well qualifies because most of the studies have reported average age in cancer patients to be above 60 years (Sadjadi et al., [37]). Our choice of age is also is in agreement with a study conducted in Pakistan based on the mean age of patients as 68 ± 10 years (Iqbal and Chaughtai, [38]) and the one by Bhurgri et.al,[39] which also demonstrated the similar age group of the patients with PCa.

For determination of age of PCa patients the studies done in Europe and America suggested that the men who are above 70 have more likelihood of developing aggressive tumor (Delongchamps et al., Sun et al.,[40,41]. The lower mean age of patients in metastatic group in our study may be due to overall lower life expectancy in Pakistan (Aziz et al., [42]).

Various in vitro and in vivo studies have established the fact that Zn deficiency leads to increased oxidative stress and DNA damage (Ho et al., [15]). Limited availability of cellular Zn due to Zn deficiency can result in loss of activity of the Zn-dependent proteins involved in the maintenance of DNA integrity and may contribute to the increased potential for cancer risk. Zinc deficiency can cause impairment of the DNA binding abilities of the tumor suppressor protein, p53, nuclear factor κB (NFκB), and AP-1 transcription factors.

Here, significant reduction was observed in serum levels of Zn in two groups of patients with PCa localized and advanced, as compared to controls, while no significant difference was observed in the levels between BPH group and controls. The decreased level of Zn in carcinoma patients signifies its role in carcinogenesis. The average Zn level of metastatic PCa group was 66 ± 14.24 µg/dl and significant difference was observed in Zn levels between study groups. So results are in agreement with a large study conducted in China that found reduced levels of Zn in patients of PCa as compared to the normal subjects. These results are also consistent with investigations of Tiwari et. al.[43].They demonstrated that the plasma Zn levels were markedly lowered in patients with carcinoma prostate and more interestingly they also could not find any significant difference in Zn levels of patients with and without metastases. The

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results are also in conformity with a study on association of Zn with PCa conducted in Turkey who observed a significant difference in the serum Zn level in PCa and BPH.

The only difference between the study here and that referred above is that it did not present detailed data of patients with different stages of PCa such as how Zn behaved with advancement of the disease but here it was presented.

The results of this study are in also in agreement with those of Aydin et.al. [33] found that the concentration of Zn was significantly lower in malignancy compared to healthy subjects. They also showed that Zn level was significantly lower in patients with BPH as compared to the controls. Similar to the results hinted above a study done by Feustel et al.[44] found significant differences in concentrations of serum Zn in carcinoma patients as compared to normal population. One possible explanation for these findings is that absorption of Zn may be impaired in these patients and reflect low level of Zn. Other alternative explanation would be that sequestration by liver or other tissues leading to lowering of circulating Zn level. These results were similarly interpreted by Zhang et al.[45] while studying association of multivitamin and mineral use with risk of prostate carcinoma. They observed that long term use of Zn doubled the risk of prostate cancer**.** While investigating the role of Zn supplementation Ko et al. found that intraprostatic Zn concentration can be improved by administering high dietary Zn but they were of the opinion that high Zn concentration instead of preventing the prostate intraepithelial neoplasm, promoted the malignancy. An interesting point in this study is that Zn supplementation did not cause the development of tumor; rather was associated with progression of tumor. However, in other reports, Zn supplementation in elderly males has been proved effective against PCa (Gonzalez et al.[47]).

The results being reported here did not agree with the findings of Goel and Sankhwar [35], in some respects. These researchers reported significant reduction in Zn levels in patients with PCa but no significant effect of metastasis on serum Zn levels whereas, here an inverse correlation between levels of Zn and stage of the disease is reported. The Zn level decreased with the severity of disease as its level was much lower in patients with metastatic carcinoma as compared to controls and patients with BPH and organ confined PCa (Fig.2). However, significant difference in serum Zn levels of controls and patients with BPH was observed.

Banas et. al. [47] were of the same opinion as corroborated by Zhang et al.[45] and Ko et al.[48] that Zn is involved in tumor growth and malignant transformation. They found high Zn concentration in PCa patients as compared to healthy subjects.

Unlike these conflicting findings the results of our study and studies done by Liang et al.[21]and Costello et. al. [19] are in favor of hypothesis that there is inverse relationship between Zn and PCa. These studies showed inhibitory effect of Zn on human PCa cell growth possibly due to induction of cell cycle arrest and apoptosis. They also suggested that Zn inhibits mitochondrial aconitase and exerts its effect on the citrate metabolism of prostatic epithelial cells.

So, the studies conducted on serum Zn level in prostate cancer, do not come to a single conclusion, there is controversy in the level of serum Zn in either control and cancer patients or different stages of PCa. These findings suggest that there are certain potential factors beside the prostate cancer that affect the serum Zn level. However, at this stage, it is difficult to ascertain whether the low Zn level due to the effect of the cancer, to lower dietary intake of Zn, or to some other unknown factor. This is also one of the limitation of this study. The increased metabolic requirements of Zn by cancer cells might result in an increased uptake from serum (Issell et al,[49]). Inflammation of the prostate and tissue damage, which are often associated with PCa, also play a significant role in hypozincemia. Both of these factors lead to liberation of leukocyte endogenous mediators from neutrophils which, in turn, results in decrease in serum Zn and concomitant uptake of Zn by the liver (DiSilvestro and Cousins, [50]. Deficiency of Zn in patients with neoplastic

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diseases has been attributed to loss of Zn from catabolic tissue and enhanced urinary excretion of Zn subsequent to its mobilization from interleukins (Prasad,[51]).

Copper plays an important role in biological systems and is an essential co-factor in many biochemical reactions. However, this micronutrient may become toxic to cells when its concentration exceeds certain optimal level.

Like Zn the abnormal levels of Cu have also been reported in patients with different cancers including carcinoma of prostate (Goyal et.al., Saxena et al., [35,28] but results have been inconclusive.

In this study, it was observed variation in Cu level that was statistically significant in prostate cancer groups. The results are strongly favored by Nayak et al.[52] as Cu levels were elevated significantly in patients with PCa as compared to controls and it has been suggested that determination of this trace element may be of value in early diagnosis of cancer. The results are also in concordance with findings of Yaman et al .who reported existence of a relationship between malignancy of prostate and increased Cu level; higher Cu level in malignant patients as compared to benign prostate cancer patients. Another study found that there is significantly higher serum Cu level in patients of PCa as compared to control group (Ozmen et al.[53]). The marked increase of serum Cu level in patients with localized and advanced PCa clearly indicates its association with development and progression of tumor. Copper being one of the promoters of the Fenton reaction, may participate in the prostate carcinogenesis by changing its oxidation state. This ultimately leads to increased oxidative stress and attendant oxidant injury (Gaetke and Chow [54]).

A research conducted in Turkey by Aydin et. al. [33] has shown no significant difference in control and patients groups. The results of this study are in partial concordance with our study, as in Control and BPH group there is no significant difference in the Cu level in both studies. Consistent with the findings of Turkish study,

The reason for increase in Cu levels among cancer patients is not well known. It may result from increased liver production of copper–containing ceruloplasmin as an inflammatory response to the cancer or from a tumor –induced decrease in catabolism of the serum ceruloplasmin (Lowndes and Harris,[55]).

Earlier workers considered that rise in serum Cu levels in malignant lesion could be due to destruction and necrosis of the tissue involved, leading to release of Cu present in the affected tissue into circulation. Another possible mechanism underlying the elevation of Cu level in malignancies could be lowering of intestinal barrier causing rapid absorption of Cu and uptake by the liver where more ceruloplasmin is formed and released into circulation. Its metabolism is altered including enhanced intestinal absorption and diminished turnover of whole body Cu (Tapiero et al., [56]). Whatever the reasons maybe of elevated Cu in patients with PCa but still these findings show a strong association of Cu with PCa.

In our study the levels of Zn and Cu were compared in terms of the Cu/Zn ratio. A significant correlation (r=-0.8133, r=0.9273)in the patient group and control group was encountered. Therefore, instead of analyzing the effect of Cu and Zn individually, it may be proposed to evaluate them in relation to Cu/Zn ratio, which in cancer patients was higher than in the control group. A study conducted in Nigeria has shown that Cu /Zn ratio is significantly higher in PCa patients as compared to control found an increase in serum Cu/Zn ratios in patients with cancers of the lung, breast, gastrointestinal tract and gyneocological malignancy

In this study it was observed that this ratio also increased with the advancement of the disease and correlated with the strength of the disease. The highest ratio was observed in patients with localized as well

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as advanced carcinoma. The fact that ratio gradually increased with advanced disease makes it clinically significant.

Taking into consideration that a change of Cu and Zn serum levels is also attributable to ageing (Wood et al., [57]), the correlation of age with the serum levels of Zn (p<0.0056) and (r=-0.2593) and Cu (p< 0.0001) and (r= 0.4437) were also determined. It was observed that with increasing age, there was a significant decrease in serum level of Zn, whereas, Cu level showed direct correlation with age and its level increased with the increase in age. Some researchers have suggested that lower serum zinc is more related with disease process rather than with age *(*Mezzetti et al.,[58]), while elevated Cu has been reported to be correlated with ageing (Baumgartner,[59]).

APPLICATIONS

The results of this research article are applicable for treatment of patients with benign prostate hyperplasia and prostate carcinoma by their nutrition with copper and zinc to adjust zinc to copper ratio levels in blood. Although highly advanced methods can be applied to estimate metals like Cu and Zn [60,61] , still the method applied here using Atomic Absorption Spectrometer which is relatively cheaper is expected to find extensive application in estimation of these minerals in different medical fluids such as blood and others to draw important conclusions.

CONCLUSIONS

The study suggests that a significant percentage of deaths resulting from cancer may be avoided through greater attention to proper and adequate nutrition. Although many dietary compounds have been suggested to contribute in the prevention of cancer, yet there is a strong evidence to support that Zn has essential role to play in this regard. The findings of this study show an inverse relationship between Zn deficiency and prostate carcinogenesis. The deficiency may be due to under-utilization and mal-utilization of Zn in the diet and lack of supplementation for Zn. These could be tied to ignorance and poverty. The present study also shows the altered level of Cu in serum of patients with PCa and exhibits positive correlation with extent of the disease.

It is also concluded that determination of serum Zn, Cu and Cu/Zn ratio can be of importance in estimating the extent of the disease and prognosis of a patient with prostate cancer. Our results do not allow to conclude whether Zn deficiency and Cu level, preceded or occurred as a result of cancer. However, the low mean serum Zn and high Cu levels in cancer patients as compared to controls indicates their strong association with prostate cancer in Pakistani population.

The project can be extended by undertaking future studies with large sample size to confirm or disprove the role of trace elements in predicting PCa occurrence and progression. Finally, the relationship between serum Zn level and prostate cancer demands additional exploration in order to establish the optimal intake of this essential micronutrient for prostate tissue health and cancer prevention.

REFERENCES

[1]   R. J. A. Van Moorselaar, E. E.Voest, Angiogenesis in prostate cancer: its role in disease progression and possible therapeutic approaches, *Molecular and cellular endocrinology*, **2002,** 197(1-2), 239-250.
[2]   Li, X. M., Zhang, L., Li, J., Li, Y., Wang, H. L., Ji, G. Y., Kuwahara, M., Zhao, X. J. Measurement of serum zinc improves prostate cancer detection efficiency in patients with PSA levels between 4 ng/mL and 10 ng/mL, *Asian J Androl*, **2005, 7**(3), 323-8.

1259

[3]   D. M. Parkin, F. Bray, J. Ferlay, P.Pisani, Global cancer statistics, 2002, CA *Cancer J Clin*, **2005,** 55(2), 74-108.

[4]   J.Ferlay, F. Bray, P. Pisani, D.M. Parkin, Cancer Incidence, Mortality and Prevalence Worldwide, IARC Cancer Base No. 5 Version 2.0. Lyon, France: IARC Press; **2004.**

[5]   Pu, Y. S., Chiang, H. S., Lin, C. C., Huang, C. Y., Huang, K. H. and Chen, J. et al., Changing trends of prostate cancer in Asia, *Aging Male*, **2004, 7**(2), 120-32.

[6]   H. G. Sim, C.W. Cheng, Changing demography of prostate cancer in Asia, *Eur J Cancer*, **2005,** 41(6), 834-45.

[7]   K. W. Jung, Y. J. Won, S. Park, H. J. Kong, J.Sung, H.R. Shin et al., Cancer statistics in Korea: incidence, mortality and survival in 2005, *J Korean Med Sci*, **2009,** 24(6), 995-1003.

[8]   Y. Bhurgri, A.Bhurgri, S. Nishter, A. Ahmed, A. Usman, S.Pervez, et al., Pakistan--country profile of cancer and cancer control 1995-2004, *J Pak Med Assoc*, **2006,** 56(3), 124-30.

[9]   D. G.Bostwick, L.Liu, M. K. Brawer, J. Qian, High-grade prostatic intraepithelial neoplasia. *Rev Urol*, **2004,** 6(4), 171-9.

[10]   G. Chodak, Prostate cancer: epidemiology, screening, and biomarkers, *Rev Urol***, 2006,** 8**:** Suppl 2(S3-8).

[11]   S. S. Khanna, F. R. Karjodkar, Circulating immune complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer: a randomised, controlled clinical trial, *Head Face Med,* **2006,** 2**,** 33.

[12]   K. Ishii, T. Otsuka, K. Iguchi, S. Usui, H. Yamamoto, Y.Sugimura, K. Yoshikawa, S. W. Hayward, K. Hirano, Evidence that the prostate-specific antigen (PSA)/Zn2+ axis may play a role in human prostate cancer cell invasion, *Cancer Lett*, **2004,** 20**7**(1), 79-87.

[13]   M. Mirsamadi, I. Nourmohammadi, Correlation of human age-related cataract with some blood biochemistry constituents, *Ophthalmic Res*, **2003,** 35(6), 329-34.

[14]   W. Maret, H. H. Sandstead, Zinc requirements and the risks and benefits of zinc supplementation, *J Trace Elem Med Biol*, **2006,** 20(1), 3-18.

[15]   E. Ho, C. Courtemanche, B. N. Ames, Zinc deficiency induces oxidative DNA damage and increases p53 expression in human lung fibroblasts, *J Nutr*, **2003,** 133(8), 2543-8.

[16]   B. Kumar, S. Koul, L. Khandrika, R. B. Meacham, H. K. Koul, Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype, *Cancer Res*, **2008,** 68(6), 1777-85.

[17]   S.R. Powell, The antioxidant properties of zinc, *J Nutr*, **2000**, 130(5S Suppl): 1447S-54S.

[18]   P. I. Oteiza, M. S. Clegg, M. P. Zago, C. L. Keen, Zinc deficiency induces oxidative stress and AP-1 activation in 3T3 cells, *Free Radic Biol Med*, **2000,** 28(7), 1091-9

[19]   L. C. Costello, R. B. Franklin, P. Feng, Mitochondrial function, zinc, and intermediary metabolism relationships in normal prostate and prostate cancer, *Mitochondrion*, **2005,** 5(3), 143-53.

[20]   R. B. Franklin, P. Feng, B. Milon, M. M. Desouki, K. K. Singh, A. Kajdacsy-Balla, O. Bagasra, L. C. Costello, hZIP1 zinc uptake transporter down regulation and zinc depletion in prostate cancer, *Mol Cancer,* **2005**, 4**,** 32.

[21]   J. Y. Liang, Y. Y. Liu, J. Zou, R. B. Franklin, L. C. Costello, P. Feng, Inhibitory effect of zinc on human prostatic carcinoma cell growth, *Prostate*, **1999,** 40(3), 200-7.

[22]   V. Zaichick, T. V. Sviridova, S. V. Zaichick, Zinc in the human prostate gland: normal, hyperplastic and cancerous, *Int Urol Nephrol*, **1997,** 29(5), 565-74.

[23]   L. N. Kolonel, C. N. Yoshizawa, J. H. Hankin, Diet and prostatic cancer: a case-control study in Hawaii, *Am J Epidemiol*, **1988,** 127(5), 999-1012.

[24]   M. F. Leitzmann, M. J. Stampfer, K. Wu, G. A. Colditz, W. C. Willett, E. L. Giovannucci, Zinc supplement use and risk of prostate cancer, *J Natl Cancer Inst*, **2003,** 95(13), 1004-7.

[25]   H. D. Vlajinac, J. M. Marinkovic, M. D. Ilic, N. I. Kocev, Diet and prostate cancer: a case-control study, *Eur J Cancer*, **1997,** 33(1), 101-7.

[26]   T. Theophanides, J. Anastassopoulou, Copper and carcinogenesis, *Crit Rev Oncol Hematol*, **2002,** 42(1), 57-64.

1260

[27]   H. W. Kuo, S. F. Chen, C. C. Wu, D. R. Chen, J. H. Lee, Serum and tissue trace elements in patients with breast cancer in Taiwan, *Biol Trace Elem Res*, **2002,** 89(1), 1-11.

[28]   P. Saxena, S. Yadav, S. Salhan, K. T. Bhowmick, Serum copper levels in carcinoma of ovary and cervix, *Indian J Physiol Pharmacol*, **2002,** 46(2), 159-66.

[29]   A. Boz, O.Evliyaoglu, M. Yildirim, N. Erkan, B. Karaca, The value of serum zinc, copper, ceruloplasmin levels in patients with gastrointestinal tract cancers, *Turk J Gastroenterol*, **2005,** 16(2), 81-4.

[30]   O. Demirhan, A. Demirkaya, E. Ersen, M. Ercan, K. Kaynak, Analysis of the alterations of trace elements in plasma and tissue, in lung cancer, *Tuberk Toraks*, **2010,** 58(1), 53-8.

[31]   Zuo, X. L., Chen, J. M., Zhou, X., Li, X. Z. and Mei, G. Y., Levels of selenium, zinc, copper, and antioxidant enzyme activity in patients with leukemia, *Biol Trace Elem Res*, **2006,** 114(1-3), 41-53.

[32]   A. Lightman, J. M. Brandes, N. Binur, A. Drugan, O. Zinder, Use of the serum copper/zinc ratio in the differential diagnosis of ovarian malignancy, *Clin Chem,* **1986,** 32**,** 101-3.

[33]   A. Aydin, Z. Arsova-Sarafinovska, A. Sayal, A. Eken, O. Erdem, K. Erten, et al., Oxidative stress and antioxidant status in non-metastatic prostate cancer and benign prostatic hyperplasia, *Clin Biochem*, **2006,** 39(2), 176-9

[34]   J. I. Anetor, F. Ajose, G. O. Anetor, A. A. Iyanda, O. O. Babalola, F. A. Adeniyi, High cadmium / zinc ratio in cigarette smokers: potential implications as a biomarker of risk of prostate cancer, *Niger J Physiol Sci,* **2008,** 23, 41-9.

[35]   T. Goel, S. N. Sankhwar, Comparative study of zinc levels in benign and malignant lesions of the prostate, *Scand J Urol Nephrol*, **2006,** 40(2), 108-12.

[36]   D. J. Schaid, The complex genetic epidemiology of prostate cancer, *Hum Mol Genet*, **2004,** 13, 103-21.

[37]   A. Sadjadi, M. Nooraie, A. Ghorbani, M. Alimohammadian, M. J. Zahedi, S. Darvish-Moghadam, The incidence of prostate cancer in Iran: results of a population-based cancer registry, *Arch Iran Med,* **2007,** 10(4), 481-5.

[38]   N. Iqbal, N. Chaughtai, Evaluation of the diagnostic use of free prostate specific antigen/total prostate specific antigen ratio in detecting prostate cancer, *J Pak Med Assoc*, **2005,** 55(8), 318-20.

[39]   Y. Bhurgri, N. Kayani, S. Pervez, R. Ahmed, I. Tahir, M. Afif, et al., Incidence and trends of prostate cancer in Karachi South, 1995-2002, *Asian Pac J Cancer Prev,* **2009,** 10, 45-8.

[40]   N. B. Delongchamps, C. Y. Wang, V. Chandan, R. F. Jones, G. Threatte, M. Jumbelic, et al., Pathological characteristics of prostate cancer in elderly men, *J Urol,* **2009,**182**,** 927-30.

[41]   L. Sun, A. A. Caire, C. N. Robertson, D. J. George, T. J. Polascik, K. E. Maloney, et al., Men older than 70 years have higher risk prostate cancer and poorer survival in the early and late prostate specific antigen eras, *J Urol,* **2009,** 182**,** 2242-8.

[42]   Z. Aziz, S. Sana, S. Saeed, M. Akram, Institution based tumor registry from Punjab: five year data based analysis, *J Pak Med Assoc,* **2003,** 53(8), 350-3.

[43]   Tiwari, V. S., Agrawal, A. Mohd. S.and Agrawal, V. Comparative study of zinc levels in benign and malignant lesions of prostate, *Ind J Sur*, **2004**, 66(6), 352-355.

[44]   A. Feustel, R. Wennrich, B. Schmidt, Serum-Zn-levels in prostatic cancer, *Urol Res*, **1989,** 17(1): 41-2.

[45]   Y. Zhang, P. Coogan, J. R. Palmer, B. L. Strom, L. Rosenberg, Vitamin and mineral use and risk of prostate cancer: the case-control surveillance study,*Cancer Causes Control*, **2009,** 20(5), 691-8.

[46]   A. Gonzalez, U. Peters, J. W. Lampe, E. White, Zinc intake from supplements and diet and prostate cancer, *Nutr Cancer,* **2009,** 61**,** 206-15.

[47]   A. Banas, W. M. Kwiatek, K. Banas, M. Gajda, B. Pawlicki, T. Cichocki, Correlation of concentrations of selected trace elements with Gleason grade of prostate tissues, *J Biol Inorg Chem,* **2010** ,15, 1147-55.

[48] Y. H. Ko, Y. J. Woo, J. W. Kim, H. Choi, S. H. Kang, J. Lee, et al., High-dose dietary zinc promotes prostate intraepithelial neoplasia in a murine tumor induction model, *Asian J Androl,* **2010,** 12(2), 164-70.

[49] B. F. Issell, B. V. Macfadyen, E. T. Gum, M. Valdivieso, S.J. Dudrick, G. P. Bodey, Serum zinc levels in lung cancer patients, Cancer, **1981,** 47(7), 1845-8.

[50] R. A. Disilvestro, R. J. Cousins, Mediation of endotoxin-induced changes in zinc metabolism in rats, *Am J Physiol,* **1984,** 247**,** E436-41.

[51] A. S. Prasad, Zinc: an overview, *Nutrition,* **1995,** 11(1 Suppl), 93-9.

[52] S. B. Nayak, V. R. Bhat, D. Upadhyay, S. L. Udupa, Copper and ceruloplasmin status in serum of prostate and colon cancer patients, *Indian J Physiol Pharmacol,* **2003,** 47, 108-10.

[53] H. Ozmen, F. A. Erulas, F. Karatas, A. Cukurovali, O. Yalcin, Comparison of the concentration of trace metals (Ni, Zn, Co, Cu and Se), Fe, vitamins A, C and E and lipid peroxidation in patients with prostate cancer, *Clin Chem Lab Med*, **2006,** 44(2), 175-9.

[54] L. M. Gaetke, C. K. Chow, Copper toxicity, oxidative stress and antioxidant nutrients, *Toxicology*, **2003,** 189(1-2), 147-63.

[55] S. A. Lowndes, A. L. Harris, The role of copper in tumour angiogenesis, J *Mammary Gland Biol Neoplasia*, **2005,** 10(4), 299-310.

[56] Tapiero, H., Townsend, D. M. and Tew, K. D., Trace elements in human physiology and pathology. Copper, *Biomed Pharmacother*, **2003,** 57(9), 386-98.

[57] R.J. Wood, P.M. Suter, R.M. Russell, Mineral requirements of elderly people, *Am J Clin Nutr. Am J Clin Nutr,* **1995,** 62(3), 493-505.

[58] Mezzetti, A., Pierdominico, S.D., Costantini, F., Romano, F., De Cesare, D. and Cuccurullo, F. et al., Copper/zinc ratio systemic oxidant load: effect of aging and aging-related degenerative diseases, *Free Radical Biol Med,* **1998,** 25**,** 676– 81.

[59] T. G. Baumgartner, Trace éléments in clinicat nutrition, *Nutr. Clin Prac.t,* **1993,** 8,251– 63

[60] S .Zafar, M.R. Khan, Analysis of Kasur Reclaimed Soil for the Toxicity of Heavy Metals, *Journal of Applicable Chemistry*, **2014,** 3 (6), 2435-2440.

[61] M.R .Khan and S. Ahad, Analysis of Kasur Tannery Pre Treated Wastewater For Its Metal Toxicity, *Journal of Applicable Chemistry,* **2015,** 4 (2), 572-577

AUTHORS' ADDRESSES

1. **Mansoor Ghani**
   Department of Biochemistry, University of Health sciences, Lahore
   mansoorgw@gmail.com

2. **M. R. Khan**
   Department of Biochemistry, University of Health Sciences, Lahore,
   drrafiq@lahoreschool.edu.pk

3. **M. Zamir Ahmed**
   Department of Biochemistry, Allama Iqbal Medical College/Jinnah Hospital, Lahore
   drzamirahmad2hotmail.com

3. **M. Zamir Ahmed**
   Department of Biochemistry, Allama Iqbal Medical College/Jinnah Hospital, Lahore
   drzamirahmad@hotmail.com

4. **Sadia Mehmood**
   Department of Biochemistry, Central Park Medical College, Lahore
   saadiah786@yahoo.com

5. **Imran Ali Shah**
   University Of Health Sciences, Lahore
   immishah@yahoo.com

6. **Irum Fayyaz**
    Department of Biochemistry, Lahore CMH Medical College, Lahore
    iram_nadeem@yahoo.com
6. **Shama Akram**
    Department of Biochemistry, Rashid Latif Medical College, Lahore
    shamaakram@yahoo.com