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Appraisal of essential metals in the blood of prostate cancer patients in comparison with healthy subjects

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ABSTRACT

Prostate cancer is one of the most common life-threatening malignancies afflicting men and imbalance of the metals has been considered as one of its major risk factor. Selected essential metals (Ca, Mg, Na, K, Sr, Li and Co) were analysed in the blood of prostate cancer patients and counterpart healthy subjects by atomic absorption spectrometry. In addition, prostate specific antigen (PSA) was also measured in the blood serum of the patients using immunoradiometric method. Mean concentrations of Na, Li and Co were found to be significantly higher ($p < 0.05$) and that of K was lower in the blood of the patients compared with the healthy subjects. Most of the metals exhibited higher dispersion and asymmetry in the blood of patients than controls. The correlation study revealed considerably diverse relationships among the metals in blood of both donor groups. Variations in the metal levels were also noted for various stages (I, II, III & IV) as well as types (adenocarcinoma, squamous cell carcinoma, transitional cell carcinoma and small cell carcinoma) of the patients. The study evidenced considerably divergent variations in the metal levels in prostate cancer patients in comparison with healthy subjects.

Keywords: *prostate cancer; metal; blood; PSA; BMI; statistical analysis; Pakistan*

1. INTRODUCTION

Globally prostate cancer ranks 2nd in cancer incidence and 6th in cancer mortality; it is 4th most common cancer of men in Pakistan [1, 2]. There has been an abrupt increase in the last few decades that may be associated with the increased industrialization and urbanization. Generally, it is the consequence of chromosomal aberration and pathological proliferation of cells of the prostate tissue which invades the surrounding tissues and eventually spreads to the lymph nodes, skeletal bones and other parts of the body [1]. The main histological types of prostate cancer are adenocarcinoma, squamous cell carcinoma, transitional cell carcinoma, signet-ring carcinoma and small cell carcinoma [3]. Staging of the cancer is one of the most important factors in order to plan the treatment and it is based on the prostate biopsy results and the prostate specific antigen (PSA) levels [3, 4].

Prostate cancer has a complex aetiology and multiple factors have been identified for its development; commonly the risk of this malignancy increases with age [4]. Both genetic and environmental risk factors including diet, age, race, familial tendency, obesity, smoking, metals imbalances, alcohol consumption, sexual/physical activity, hormones and life style are associated with the risk of this malignancy [5, 6]. Some nutritional/clinical studies suggested that numerous dietary minerals/trace elements have been implicated in the aetiology of prostate cancer [7, 8]. Essential metals play a key role in physiological processes; they exist in delicate equilibrium in the fluid/tissues under normal conditions but deficiency or excess of certain metals disturb their dynamic balance which in turn interrupt the cell functions and ultimately result in cell death [7]. Therefore, increased dietary intake and/or levels of the metals in human body have been linked to the risk of prostate cancer [8].

Monitoring of the metals in prostate cancer patients is very important for maintaining their health and has become a prosperous field in medicine and nutrition [9, 10]. In humans, various biological samples can be used to measure and monitor the metal levels as indices for assessing nutritional status and clinical maladies [9, 11]. Blood is the most widely used and accepted matrix for biomonitoring the trace metal burden. It is classified as a connective tissue with a complex liquid intercellular matrix which measures the component absorbed and temporarily in circulation before excretion and/or storage, thus it clearly reflects recent exposure. In addition, the metal levels are generally higher in the blood compared with the plasma/serum and therefore easy to detect with conventional techniques [12]. However, it is an invasive matrix and can have unpleasant effects on the participant response [13]. It should be stored below freezing point before metal analyses [14].

Prostate specific antigen (PSA) has been demonstrated as the premier tumour marker for prostate cancer in untreated patients. US food and drug administration (FDA) has approved serum PSA for use as a prostate cancer screening laboratory test [15]. It is a glycoprotein produced by the prostate gland having a structural relationship to the glandular kallikreins. PSA is highly specific for prostatic tissue and is important in determining prognosis, detection of recurrent disease and for screening/early diagnosis [16].

Nowadays many researchers are investigating the relationships between the metals and prostate cancer in order to understand the pathogenesis of the disease [7, 8, 17]. In view of the important roles of essential metals in various physiological processes, the present study was designed to evaluate the disproportions in the distribution and covariations of selected essential metals (Ca, Mg, Na, K, Li, Sr and Co) in the blood of prostate cancer patients in comparison with healthy donors with matching age group, socioeconomic status, abode and food habits.

Viable variations in the metal levels with respect to histopathological cancer types/stages were also assessed. It is anticipated that the present study will provide basic data related to the imbalances of the metals which can be linked with the progression of carcinoma. The study will also help for planning preventive and educational interventions regarding prostate cancer in Pakistan.

2. EXPERIMENTAL

2.1. Study population. The blood samples were collected from the newly diagnosed prostate cancer patients admitted in Nuclear Oncology & Radiotherapy Institute (NORI), Islamabad, Pakistan. All the participants (patients and controls) were selected on volunteer basis and their age ranged from 24 and 73 years. Prior to sample collection, the protocol of study was approved by the institutional ethic review committees. Informed written consent was obtained from each participant and the information on age, gender, place of residence, ailment duration, food habits, health status, smoking habits, type of ailment, family history of cancer, medication, hobbies, occupation and tumour stage/type etc., were recorded in a questionnaire at the time of sample collection. Physical and clinical examinations were performed in the hospital. Height and weight of all the participants were measured in order to calculate their body mass index (BMI) that was then used as an indicator of obesity. The inclusion criteria for the prostate cancer patients were; (a) no history of signs and symptoms of recent past chronic illness except malignant prostate tumour; (b) no patients had undergone with blood transfusion/surgery; (c) no treatment with chemotherapy or radiotherapy; (d) no use of multivitamins/mineral supplements and herbal medications or drug during past six months; (e) no history of alcohol consumption. The inclusion criteria for healthy men were; (a) no history of any type of cancer disease; (b) stay in same geographic area; (c) similar socioeconomic status and food habits; (d) matched age group.

2.2. Sample collection and preparation. Prior to the sample collection, the skin of each participant was thoroughly cleaned with 70% saturated isopropyl alcohol. For all the subjects, 3–5 mL of venous blood was collected from antecubital vein using a sterile needle with syringe (10 mL, BD Ref. 305720) and immediately stored into the vacutainer polyethylene tubes. One part of the blood sample was kept at –15°C in a refrigerator for metal analysis [18], while other part was used for separating the sera. The blood was allowed to clot at room temperature for 15–30 min; after complete clotting it was centrifuged for 5–10 min at 3000 rpm. The supernatant fluid was separated by an Eppendorf pipette, labelled and stored at –15°C until further analysis [19].

For digestion, an exactly known amount of the blood sample was transferred from the storage tube to the digestion flask and 10 mL of HNO_3 (65%) was added to each flask and kept for 10 min at room temperature. Then 10 mL of $HClO_4$ (70%) was added and after 10–15 min the flask contents were heated at 80°C (for 3–4 hours) until dense white fumes appeared marking the completion of digestion procedure. After cooling, the digest was quantitatively transferred to 25 mL volumetric flask and the final volume was adjusted by 0.1 M HMO_3 [18]. Blank digest was also carried out in the same way but without blood sample.

2.3. Quantification of the metals. Concentrations of selected essential metals (Ca, Mg, Na, K, Sr, Li and Co) in the digested samples were measured using a flame atomic absorption spectrophotometer (Shimadzu AA-670, Japan) under optimum analytical conditions (Table 1). All the chemicals and reagents used during the analysis were of high purity (>99.9%) purchased from E-Merck (Darmstadt, Germany). Doubly distilled water was used throughout the study for the preparation of the samples and standards. Stock solution (1000 mg/L) of each metal was used to prepare the fresh working standards just before the analysis. To establish the precision of the results, three sub-samples of each sample were treated and run separately onto the spectrophotometer to pool the mean concentration. The samples were also analysed at an independent laboratory for comparison of the results and a maximum of 5% difference was observed in the results of two laboratories. Standard reference material (Bovine Muscle Powder, NIST-SRM 8414) was analysed to check the reliability of the quantified results and the percentage recovery ranges from 98-103%. Generally, the contribution of the blank was <5% of the measured metal levels in the samples.

Table 1. Optimum analytical conditions for the metal analyses along with their detection/quantification limits and certified versus measured concentrations (µg/g, ±SD) of the metals in standard reference material.

Metal	Wavelength (nm)	Slit width (nm)	Limit of Detection (mg/L)	Limit of Quantification (mg/L)	Bovine Muscle Powder, NIST-SRM 8414		
					Certified Level	Measured Level	Recovery (%)
Ca	422.7	0.5	0.004	0.013	145	143.8 ± 3.01	99.2
Mg	285.2	0.5	0.001	0.004	960	955.2 ± 10.4	99.5
Na	589.0	0.5	0.002	0.007	2100	2085 ± 21.6	99.3
K	766.5	0.5	0.003	0.009	15170	15210 ± 33.7	100.3
Sr	460.7	0.5	0.005	0.016	0.052	0.051 ± 0.006	98.1
Li	670.7	0.5	0.003	0.009	-	-	-
Co	240.7	0.2	0.005	0.016	0.007	0.007 ± 0.002	99.2

2.4. Measurement of PSA. Prostate specific antigen (PSA) was measured by total immunoradiometric assay kit procured from Immunotech (Beckman Coulter Company, Czech Republic), in which two monoclonal antibodies against two different epitopes of PSA molecules were used [19]. The samples or calibrators were incubated in the tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labelled with ^{125}I. The bound radioactivity was then determined in a gamma counter

[20]. In this method we used labelled duplicate tubes for total counts (TC), nonspecific binding (NSB), zero standard, standards, control and samples. 100 µL of zero standard was added in each of NSB tubes. Pipetted 100 µL each standards, control and serum samples into the labelled polypropylene tubes. 100 µL of ^{125}I-labelled antibody tracer was dispensed in all the tubes which were incubated for 2 hours with gentle shaking (>280 rpm) on a shaker at room temperature except TC tubes. After shaking the supernatant was decanted from all the tubes (except TC tubes) and washed twice with 2 mL of wash solution (provided in the kit) and aspirated again. Afterwards inverted tubes were struck on thick absorbent paper to shake off all residual droplets. Radioactivity of all the tubes was then determined: counted for one minutes in a gamma counter with window adjusted for ^{125}I. The concentration of unknown was determined by log-logit curve. The counts were directly proportional to the concentration of the analyte [19, 21].

2.5. Statistical analysis. The analytical results were subjected to the detailed statistical analyses using STATISTICA software [22]. The statistical analysis of the data comprised of the basic statistical parameters including range, mean, median, standard deviation (SD), standard error (SE), skewness and kurtosis. The Student-*t* test was used to determine the difference between the cancer patients and healthy subjects ($p < 0.05$ was considered statistically significant). Besides, the Wilcoxon rank-sum test was used for the comparison of the median levels. Pearson's correlation coefficient was used to examine the relationships between the metal concentrations for mutual variations.

3. RESULTS AND DISCUSSION

3.1. Demographic characteristics of the subjects. The demographic data related to the prostate cancer patients and healthy donors are presented in Table 2, which revealed that age of the patients was about 57 years on the average while those of healthy men was about 48 years on the average. Majority of donors in both groups (>50%) were vegetarians in their food habits. About 51% of the patients resided in rural localities while 55% of healthy donors lived in urban area. More than half of the cases (58%) in the patient group and 56% in the control group were not addicted of smoking.

Table 2. Characteristics of the subjects.

Characteristics	Cancer Patients (n = 74)	Healthy Subjects (n = 66)
Age (years)		
Range	32–75	27–68
Mean	56.8	47.8
BMI (kg/m^2)		
Mean	28.11	20.08
PSA (ng/mL)		
Range	317–1640	–
Mean	642.8	–
Diet		
Vegetarian	43 (58%)	38 (58%)
Non-vegetarian	31 (42%)	28 (42%)
Habitat		
Urban	36 (49%)	36 (55%)
Rural	38 (51%)	30 (45%)
Tobacco Use (Smoking)		
No use	43 (58%)	37 (56%)
Use	31 (42%)	29 (44%)
Types of Prostate Cancer		
Adenocarcinoma	42 (57%)	–
Squamous cell carcinoma	14 (19%)	–
Transitional cell carcinoma	10 (14%)	–
Small cell carcinoma	08 (10%)	–
Stages of Prostate Cancer		
Stage-I	19 (26%)	–
Stage-II	20 (27%)	–
Stage-III	12 (16%)	–
Stage-IV	23 (31%)	–

Patients included in the present study were commonly suffering from adenocarcinoma (57%), followed by squamous cell carcinoma (19%), transitional cell carcinoma (14%) and small cell carcinoma (10%). Twenty-six percent (26%) of the patients were diagnosed at stage-I, 27% at stage-II, 16% at stage-III and 31% at stage-IV of prostate cancer (Table 2). The PSA total levels in prostate cancer patients ranged from 317 to 1640 ng/mL with the mean value of 642.8 ng/mL against the normal range of < 6 ng/mL. Thus, the patients exhibited about 100 times higher PSA levels than the normal range in the present study.

3.2. Body mass index and prostate cancer. Higher body mass index (BMI) has been linked to the cancer incidence and mortality worldwide [23]. Several studies have examined the association between BMI and the risk of prostate cancer, but the relationships described in the literature are conflicting and inconclusive [24, 25]. Some cohort studies suggested a positive association between BMI and increasing risk of prostate cancer [26], whereas other studies have found no association [24]. Rodriguez et al., [27] examined BMI and prostate cancer mortality in two large cohorts of men and found prostate cancer mortality rates to be significantly higher among men with higher BMI. In contrast, Nomura [28] completed a follow-up study and found no relationship between BMI and prostate cancer. Giovannucci et al., [29] found an inverse relationship between BMI and prostate cancer risk in younger males. In the present study, BMI of prostate cancer patients was found to be significantly higher than healthy men ($p < 0.05$) as shown in Table 2. A growing body of evidence indicated that a higher BMI provides a favourable biological microenvironment for tumour onset and growth [25]. Men with higher BMI values have also been reported to produce less testosterone, resulting in prostate cancer that is less androgen dependent and consequently more aggressive [30]. In addition, obesity increases the prostate cancer recurrence as well [31].

3.3. Distribution of the metals. Basic statistical distribution parameters for the concentrations of selected essential metals (Ca, Mg, Na, K, Sr, Li and Co) of in the blood of prostate cancer patients and healthy subjects are summarised in Table 3. Statistical evaluation of the data indicated that most of the metals exhibited large variations as shown by the minimum and maximum levels. The tabulated data showed that Na revealed highest concentration at 1,529 µg/g, followed by K (165.2 µg/g), Ca (54.33 µg/g) and Mg (29.43 µg/g). Relatively lower mean levels were noted for Co (5.475 µg/g), Sr (1.301 µg/g) and Li (0.720 µg/g). On the whole, mean metal levels in the blood of the patients showed following

descending order: Na > K > Ca > Mg > Co > Sr > Li. Among the metals, Na, K, Ca and Mg exhibited random distribution pattern and large dispersion as revealed by higher SD and SE on one hand and mismatched mean and median levels on the other hand. Lower magnitude of skewness and kurtosis were observed in favour of Ca and Co thus showing almost symmetrical distribution pattern. Relatively large asymmetry in the distribution of K and Sr was evidenced by higher kurtosis and skewness values.

In the case of healthy subjects (Table 3) predominantly higher mean concentration was found for Na (959.8 µg/g), followed by K (351.6 µg/g), Ca (56.65 µg/g), Mg (35.27 µg/g), Co (2.941 µg/g) and Sr (1.479 µg/g). Lowest average concentration was recorded for Li (0.441 µg/g). On the average basis, the metals revealed following decreasing order in the blood of healthy donors: Na > K > Ca > Mg > Co > Sr > Li. Almost same trend was observed for the median levels. Highest dispersion in terms of elevated SD and SE values were noted for Ca, Mg, K and Na. Relatively large asymmetry was noted in the distribution of Mg, K and Sr as evidenced by highest kurtosis and skewness values.

Table 3. Statistical distribution parameters for the concentrations (µg/g, wet weight) of selected metals in the blood of prostate cancer patients and healthy subjects.

		Ca	Mg	Na	K	Sr	Li	Co
Cancer Patients	Min	5.245	12.94	928.6	85.72	0.102	0.01	0.144
	Max	108.3	57.84	1936	325.1	4.754	2.247	10.85
	Mean	54.33	29.43	1529	165.2	1.301	0.720	5.475
	Median	53.80	28.25	1521	162.7	1.201	0.596	6.220
	SD	25.48	8.662	191.7	40.44	0.844	0.501	3.008
	SE	3.161	1.007	22.28	4.702	0.103	0.059	0.398
	Skew	0.138	0.888	-0.535	2.037	1.240	1.017	-0.144
	Kurtosis	-0.620	1.281	1.164	6.262	3.204	0.836	-1.059
Healthy Donors	Min	4.852	13.55	786.0	81.93	0.032	0.01	0.160
	Max	140.4	92.48	1218	466.4	4.566	1.43	8.170
	Mean	56.65	35.27	959.8	351.6	1.479	0.441	2.941
	Median	46.96	32.06	936.2	351.3	1.133	0.393	2.622
	SD	31.70	13.59	105.3	53.03	1.159	0.320	2.224
	SE	4.355	1.673	12.96	6.528	0.152	0.041	0.308
	Skew	0.650	1.442	0.616	-1.840	1.069	0.886	0.894
	Kurtosis	-0.123	3.964	-0.191	9.487	0.463	0.465	-0.042
	p-value	*NS	*NS	<0.05	<0.05	*NS	<0.05	<0.05

*NS-non significant

3.4. Comparison of the metal levels in prostate cancer patients and healthy subjects. A systematic and detailed comparison (two tailed *t*-test) of the concentrations of the metals in the blood of the patients and healthy subjects explained that mean concentrations of Na, Li and Co were significantly higher ($p < 0.05$) in the patients, whereas average level of K was significantly lower compared with the healthy men ($p < 0.05$) as shown in Figure 1. Nevertheless, average contents of Ca and Mg were also different; however the difference did not reach statistical significance. Likewise, median concentrations of the metals were also compared by Wilcoxon rank sum test which manifested statistically significant differences ($p < 0.05$) for Na, Li and Co in the blood of the patients and healthy donors. The literature reported data also showed some statistically significant differences for Ca, Mg and Co in the case of prostate cancer patients [7, 8, 32].

3.5. Correlation study. Spearman correlation coefficients (r) between selected metals in the blood of the patients and healthy subjects are shown in Table 4 ($p < 0.01$). In the case of patients, significant positive correlations were observed between Li-Mg (r = 0.598), Sr-Mg (r = 0.431), Li-Na (r = 0.371), Li-Sr (r = 0.369), K-Mg (r = 0.337), Li-Ca (r = 0.336) and Sr-K (r = 0.335) thus pointing out some common origin of these metals. The counterpart data for the healthy subjects (Table 4) showed significant correlation coefficients between Sr-Na (r = 0.418), Li-Mg (r = 0.408), Na-Mg (r = 0.338) and Li-Sr (r = 0.319) indicating close associations among these metals and they may share common origin. A significant negative correlation was noted for K-Na (r = –0.465), indicating their opposing variations. Some other metals pairs also revealed inverse relationships but these were not significant. Age of the donors exhibited insignificant positive/negative correlations with the metal contents. Among the metals Co was not significantly correlated with any other metal and therefore exhibited independent variations. The correlation study therefore brings out marked divergences in the variations of the metal contents in prostate cancer patients in comparison with the healthy donors.

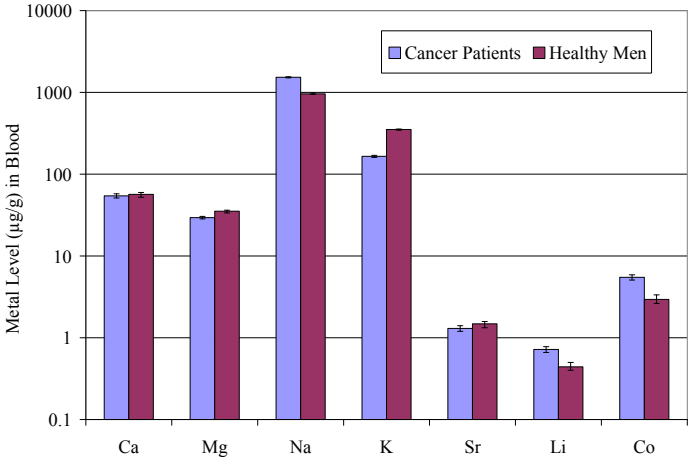

Figure 1. Comparison of average metal levels (±SE) in the blood of prostate cancer patients and healthy subjects.

Table 4. Correlation coefficient (*r*)* matrix for selected metal levels in the blood of prostate cancer patients (below the diagonal) and healthy donors (above the diagonal).

	Age	Ca	Mg	Na	K	Sr	Li	Co
Age	1	0.085	0.205	-0.170	-0.001	0.126	0.051	-0.057
Ca	0.005	1	0.165	0.100	-0.135	-0.042	-0.201	0.010
Mg	-0.009	0.159	1	**0.338**	-0.010	0.149	**0.408**	0.090
Na	0.095	0.207	0.331	1	**-0.452**	**0.418**	0.127	0.097
K	-0.304	0.081	**0.337**	-0.189	1	0.048	0.167	0.019
Sr	0.032	0.031	**0.431**	0.110	**0.335**	1	**0.319**	0.098
Li	0.021	**0.336**	**0.598**	**0.371**	0.255	**0.369**	1	0.278
Co	-0.028	0.033	-0.078	-0.009	0.028	0.008	0.140	1

**r-values > 0.307 or < – 0.307 are significant at $p < 0.001$

3.6. Comparison of the metal levels based on habitat. Average metal levels (± SE) in the blood of prostate cancer patients and healthy subjects inhabiting in urban and rural localities are displayed in Figure 2, for comparative evaluation. Mean levels of Li, Co and Na were significantly higher in the urban patients compared with urban healthy donors, while mean levels of Sr, K and Mg exhibited higher levels in the blood of urban controls. Almost comparable average levels of Na, Mg and Ca were observed in the blood of urban/rural patients, while mean contents of K and Na revealed comparable contribution in the blood of urban/rural healthy subjects. Likewise, in the case of healthy donors, mean contents of Ca and Co were determined at appreciably higher levels in the blood of rural subjects, whereas mean levels of Mg, Sr and Li were noticeably higher in the blood of urban subjects. Average concentrations of Na and Co depicted marginally elevated levels in the urban patients compared to rural controls while mean levels of Mg, K and Sr were comparatively higher in the blood of rural controls compared to the rural patients. Accordingly, noticeable variations in the metal levels among urban and rural subjects evidenced the imbalance of these metals in the blood of prostate cancer patients.

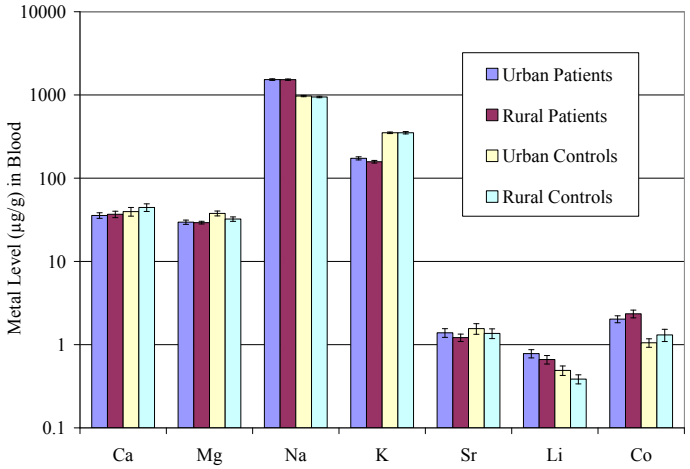

Figure 2. Comparative average concentrations of selected metals (±SE) in the blood of prostate cancer patients and healthy subjects based on habitat

3.7. Comparison of the metal levels based on dietary habit. Comparison of average concentrations of selected metals (± SE) in the blood of prostate cancer patients and healthy donors with vegetarian and non-vegetarian food habits are portrayed in Figure 3. Average levels of Na, Li and Co were appreciably higher in the blood of non-vegetarian patients compared to non-vegetarian controls while, mean contents of Ca, Mg, K and Sr were comparatively higher in non-vegetarian controls. Average concentrations of Co, Na and Li were significantly elevated in the blood of vegetarian patients compared to the vegetarian controls. Besides, mean contents of Ca, Mg and K were appreciably elevated in the blood of vegetarian controls compared to the vegetarian patients. Average levels of Na, Li and Co were appreciably higher in the blood of the patients (vegetarian & non-vegetarian) compared to the controls (vegetarian & non-vegetarian), while mean contents of Ca, Mg and K were relatively higher in healthy donors irrespective of vegetarian & non-vegetarian food habits. In the case of healthy donors, average concentrations of Li and Sr were considerably higher in non-vegetarian healthy subjects, while mean Co level was noticeably high in the blood of vegetarian healthy subjects. From the above discussion, it can be concluded that the dietary attitude had some noticeable effects on the metal levels which was supported by already reported studies [33, 34].

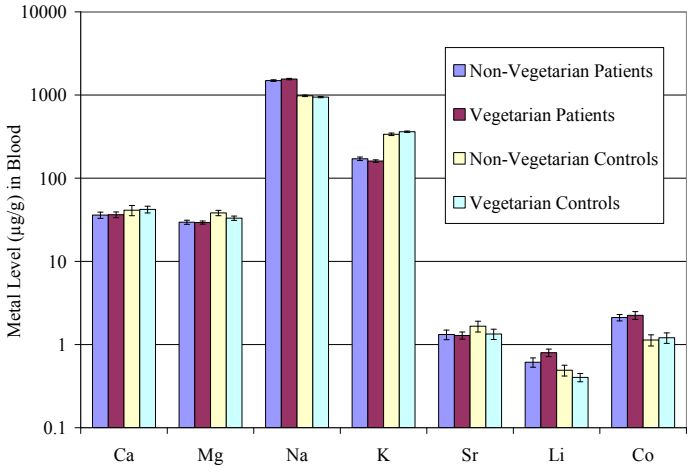

Figure 3. Comparative average concentrations of selected metals (±SE) in the blood of prostate cancer patients and healthy subjects based on dietary habits.

3.8. Comparison of the metal levels based on smoking habit. Mean levels of selected metals (± SE) in the blood of the donors with smoking and non-smoking habits are shown in Figure 4, for comparative appraisal. Average concentrations of Na, Li and Co were found to be significantly higher in the blood of patients compared with healthy subjects (irrespective of smoking & non-smoking), whereas K level was recorded higher in healthy donors. Nonetheless, average levels of Ca, Mg and Sr revealed comparable contributions in the blood of patients and controls. Mean levels of Na, Li and Co were considerably higher in the blood of non-

smoking patients compared with non-smoking controls, nonetheless, Ca, Mg, K and Sr levels were somewhat higher in non-smoking controls. Average levels of Li and Sr were found to be significantly higher in the blood of smoking patients compared with non-smoking patients, however mean level of Co was noticeably higher in the patients with non-smoking habit. Similarly, mean levels of Li, Mg and Co were considerably higher in the blood of non-smoking controls than smoking controls, whereas only Sr was considerably higher in the blood of smoking controls. The comparative proportions of the metals in the blood of prostate cancer patients with smoking/non-smoking habits were significantly different which suggested that the metals distribution was affected by tobacco-use.

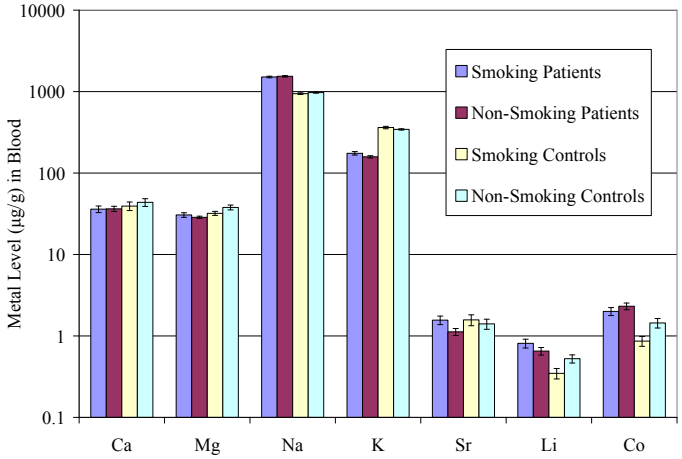

Figure 4. Comparative average concentrations of selected metals (±SE) in the blood of prostate cancer patients and healthy subjects based on smoking habits.

3.9. Comparison of the metal levels based on types and stages of cancer. Comparative evaluation of mean metal concentrations (± SE) in the blood of different types of prostate cancer patients (i.e., adenocarcinoma, squamous cell carcinoma, transitional cell carcinoma and small cell carcinoma) is shown in Figure 5. In the case of small cell carcinoma patients, Sr exhibited highest average concentrations while, Co, Mg and Li showed maximum levels in the blood of squamous cell carcinoma patients. In contrast, lowest level was noticed for Ca in the blood of adenocarcinoma patients while it exhibited almost equivalent contributions in the remaining types of the patients. Nonetheless, mean levels of Na, K and Sr were almost comparable in the blood of adenocarcinoma and squamous cell carcinoma patients. In addition, average contents of Na and K were not appreciably dissimilar in the blood of transitional cell carcinoma and small cell carcinoma patients.

Average concentrations of selected metals (± SE) in the blood of prostate cancer patients at different stages are shown in Figure 6, for comparative assessment. Mean blood concentrations of Sr and Mg were considerably higher at stage-IV, while average levels of Ca and Co in the blood were found elevated at stage-I of the patients. It was noted that mean content of Na in the blood was almost equivalent at all four stages of the patients. Among the metals, Ca, Mg, Li and Co levels were almost similar at stage-II and stage-III of the cancer patients. Mean concentration of K was found lowest at stage-IV while K and Sr were noted minimum at stage-III compared to other stages. Average concentrations of Sr increased in the following order: stage-III > stage-II > stage-I > stage-IV. Mean concentrations of Li and K were comparable at stage-I and stage-II in the blood of the patients.

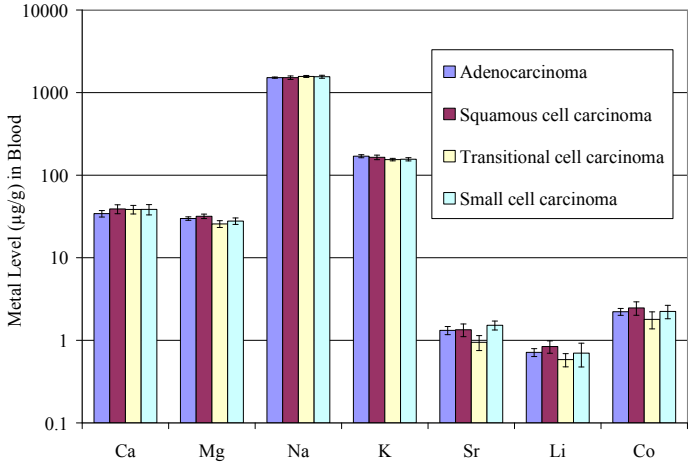

Figure 5. Comparative average concentrations of selected metals (±SE) in the blood of the patients based on prostate cancer types.

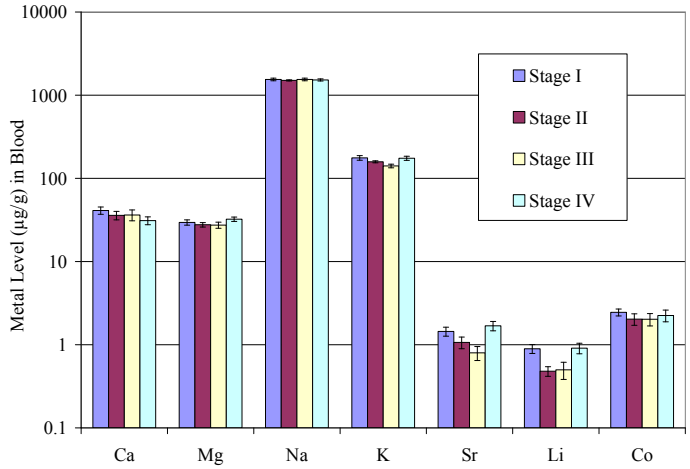

Figure 6. Comparative average concentrations of selected metals (±SE) in the blood of the patients based on prostate cancer stages.

3.10. Metals and oxidative stress in prostate cancer. Numerous studies have reported that some metals undergo redox-cycling reactions generating deleterious free radicals or reactive oxygen species (ROS) which ultimately produce oxidative stress. A cell's state in which ROS level exceeds the ability to detoxify the ROS or to repair the ROS mediated damage is referred as oxidative stress and has been shown to significantly increase the risk of developing prostate cancer [35]. In human body, ROS are produced naturally through variety of mechanism such as oxygen reacting with metal ions and/or mitochondrial electron transport chain reaction that reduces O_2 to H_2O. Mitochondrial mutation could lead to impairment of electron transport chain, which in consequence generates more ROS in prostate cancer cells [36]. Recent studies have indicated that a high oxidative stress can be detected in the epithelium of prostate cancer patients [37]. Indeed, cancer initiation and progression have been linked to oxidative stress by increasing DNA mutations or DNA damage, genome instability, and cell proliferation in prostate [38]. The physiological role of selected metals in relation to human health and prostate cancer is described below.

3.10.1. Calcium. Calcium has long held an interest in cancer with essential messenger roles regulating cell cycle proliferation and apoptosis [39]. Its consumption might also reduce the production of dihydroxyvitamin D; the deficiency of which is associated with

an increased risk of several types of cancer including prostate cancer [40]. Calcium may play a role in prostate carcinogenesis through its effects on the insulin like growth factors system, which possessed mitogenic and anti-apoptotic effects on normal and transformed prostate epithelial cells [41]. A number of prospective studies have investigated the relationship between Ca and prostate cancer risk with mixed results [42]. Some of the studies have advocated that elevated level of Ca is associated with prostate cancer [43, 44]; however a weak negative association was found between Ca and prostate cancer risk [45]. Halthur et al., [46] also found no evidence of an association between prediagnostic serum level of Ca and risk of prostate cancer. Bone is the most common site for metastasis in prostate cancer; it occurs in approximately 80% of the prostate cancer patients and it can lead to disturbances of Ca metabolism [47]. It has been reported that Ni can block Ca channels and hence Ni releases the stored intracellular Ca via a mechanism underlying the interaction between Ni^{2+} and cell surface Ca receptors [48]. The results of the present study manifesting lower Ca level in the blood of prostate cancer patients than healthy men (Table 3) corroborate with the above findings.

3.10.2. Magnesium. Magnesium plays an essential role in ATP production, protein synthesis, muscle contraction, DNA repair, cell differentiation, proliferation, apoptosis, and angiogenesis [49]. It is generally classified as an anticarcinogenic element however the results are controversial [50, 51]. Prior studies in humans found lower Mg level lead to inflammation and insulin resistance which have been linked to progression of prostate cancer [49]. Its deficiency is also linked to the inflammatory response, oxidative stress and in the formation of precancerous cells [52]. In addition, its deficiency may affect multiple pathways toward tumorigenesis across the body [53]. Dai et al., [49] reported that serum Mg level was significantly lower in high grade prostate cancer patients as compared to the controls. Likewise, Mg concentration was somewhat lower in the prostate cancer patients than those in healthy men in the present study (Table 3) thus indicating an essential role in the development of disease. In a previous study, Mg concentration in drinking water was found inversely correlated with prostate cancer mortality [50]. In another study, serum Mg level was found to be significantly higher in the patients with than the controls [7].

3.10.3. Sodium. Sodium is considered essential for the cellular homeostasis and various physiological functions. It is the principal cation of extracellular fluid and a major determinant of intravascular fluid volume [54]. Consumption of too much Na over a long period of time can contribute hypertension, high blood pressure, hypocalcemia, hypokalemia, chronic renal failure, osteoporosis and increasing the risk of stroke [55]. It has been reported that higher intake of Na or salt can be linked to an increase in stomach cancer risk in most of the cases [56]. In the present study, blood Na level was found to be significantly higher in the patient group compared to the healthy subjects (Table 3).

3.10.4. Potassium. Potassium is an essential part of intracellular fluid and its imbalances are potentially life-threatening [57]. Too much K, called hyperkalemia, characterized by irritability, nausea and cardiac arrest. Fatigue is the most common symptom of chronic K deficiency [55]. Nevertheless, high K intake may have beneficial effects including reducing the risk of stroke, protective effects against the risk of kidney stones and reducing the demineralisation of bone or osteoporosis [58]. In a study it was concluded that patients with hyperkaliaemic diseases have reduced cancer rates whereas patients with hypokalaemic disease have increased cancer rates [59]. Generally, the cancer patients are at high risk for electrolyte imbalances which may result in tumour lyses syndrome, chemotherapy side effects and tumours producing ectopic hormone. Failure to recognize this in-vitro phenomenon can lead to unnecessary and potentially harmful interventions [57]. In the present study, the results indicated that there was a significant difference in blood K level between the patients and controls (Table 3).

3.10.5. Strontium. Clinically Sr performs the functions resembling Ca because of in-vivo relevancy and causes Ca to be retained in the body. Strontium is considered helpful in reducing the loss of bone minerals in osteoporosis [60] and it might be useful in the cartilage formation [61]. Animal studies showed that consumption of very large amounts of Sr can be lethal [62]. Under extremely high exposure, the genetic materials in the cells are damaged thus leading to the cancer development [63]. The International Agency for Research on Cancer and the Environmental Protection Agency declared that radioactive Sr is a human carcinogen because it is deposited inside the body and emits beta radiation [62]. Cancers of the bone, nose, lung and blood have been reported in animals due to the high dose of Sr [64]. The present study demonstrated increased Sr concentration in the prostate cancer patients compared to the healthy donors (Table 3); the elevated Sr levels are considered a danger to human health.

3.10.6. Lithium. Lithium is involved in a variety of processes including metabolism, neuronal communication, and cell proliferation but these effects are dose-dependent and cell type [65]. Further, Li stimulates cell proliferation in mammary tumour cells and inhibits proliferation in melanoma and hepatocellular carcinoma [66]. A study reported that increased ROS generation and oxidative stress have the central role in Li cytotoxic mechanism [67]. Previous research work demonstrated that administration with Li for 15 minutes significantly increased intracellular ROS levels in rat hepatocytes [68]. Interestingly, it has been advocated that cancer mortality and incidence are inversely proportional to Li dose in patients taking Li, particularly in cancers of nonepithelial origin [66]. Lithium is an inhibitor of the serine/ threonine kinase GSK-3 (glycogen synthase kinase-3), which is believed to play an important role in the development of some cancers [68]. The present study showed that blood Li concentration was elevated in the patients with prostate cancer compared to the controls (Table 3).

3.10.7. Cobalt. Cobalt is an essential element and an integral part of cobalamin (vitamin B12), but its high concentration is potentially toxic to humans. However, the exact mechanisms of how Co exerts its toxic effects are not fully understood [69]. Literature data indicated that Co is cytotoxic and genotoxic to many cell types, including neural cells and can induce cell death by apoptosis and necrosis [69, 70]. It can cause DNA fragmentation, activation of caspases, increased production of ROS, augmented phosphorylation of mitogen-activated protein kinases, and elevated levels of p53. Cobalt mediates the occurrence of oxidative stress which contributes to toxicity and death [71]. Furthermore, it can inhibit nucleotide excision and DNA repair by the inhibition of incision and polymerization which

is an important mechanism for preventing mutagenesis and carcinogenesis [69]. Thus, Co has been classified as "possibly carcinogenic to humans" (group 2B) by the International Agency for Research on Cancer [72]. Several studies provided clear evidence for the carcinogenic effects of Co in animal models and also enhance the frequency of UV induced mutations and sister-chromatid exchanges in V79 Chinese hamster cells [69]. Recent studies reported higher serum Co concentration in the patients with prostate cancer than the controls [7, 8]. Significantly higher level of Co in the blood of prostate cancer patients compared to the healthy subjects was noticed in the present study, clearly indicating the adverse effect of Co overload in the patients (Table 3).

Prostate cancer is the most destructive type of prostate disease and its projections are useful to plan and prioritize health care services. The numbers of prostate cancer patients by place and type also constitute baseline information and act as indicators of the cancer control. The potential strong points of the present work are that the metal levels were evaluated in relation to various clinical types and stages of the prostate cancer patients. However, the findings of the present study could not be generalized to all types of the cancer patients because of the regional and cultural variation that occurs within and between the population segments. Annual screening for prostate cancer can leads to early detection and treatment. The worldwide prostate cancer burden is expected to grow to 1.7 million new cases and 499000 deaths by 2030 [73]. Further prospective studies are needed to clarify the relationship between various stages/types of the prostate cancer and toxic/trace metal levels in other biological fluids.

4. CONCLUSIONS

In conclusion, the distribution of selected metals in the blood of prostate cancer patients in comparison with healthy subjects was considerably divergent. The average concentrations of Na, Li and Co were significantly higher in the blood of patients compared to the healthy donors but mean levels of K, Ca and Mg were considerably lower in the patient than the healthy subjects. Among types, small cell carcinoma patients showed elevated Sr level, while Mg, Li and Co showed highest concentrations in the blood of squamous cell carcinoma patients. On contrary, lowest Ca level was noticed in the blood of adenocarcinoma patients. Similarly, Sr and Mg levels were considerably higher at stage-IV, while average concentrations of Ca and Co were found elevated at stage-I in the blood of prostate cancer patients. Mean levels of K and Sr was observed lowest at stage-III comparable to all other stages. Correlation study revealed fairly divergent relationships among the metals in the blood of the patients and controls. Significance of these findings either as a cause/effect of carcinogenesis process remains to be further investigated; understanding of such relative and differential metal disparities and their interdependence may be useful in defining the complex metabolic alterations in carcinoma of prostate with potential for development of metal based diagnostic, preventive or therapeutic strategies for the patients.

5. REFERENCES

[1] Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, *International Journal of Cancer*, 136, 5, E359–E386, **2015**.

[2] Siegel R.L., Miller K.D., Jemal A., Cancer Statistics, 2015, *CA-A Cancer Journal for Clinicians*, 65, 5–29, **2015**.

[3] Bracarda S., de Cobelli O., Greco C., Prayer-Galetti T., Valdagni R., Gatta G., de Braud F., Bartsch G., Cancer of the prostate, *Critical Reviews in Oncology/Hematology*, 56, 379–396, **2005**.

[4] Gronberg H., Prostate cancer epidemiology, *Lancet*, 361, 859–864, **2003**.

[5] Shah S.I.A., An update on the risk factors for prostate cancer, *World Cancer Research Journal,* 3, 2, 711e, **2016**.

[6] Cheung E., Wadhera P., Dorff T., Pinski J., Diet and prostate cancer risk reduction, *Expert Review of Anticancer Therapy*, 8, 43–50, **2008**.

[7] Kaba M., Pirincci N., Yuksel M.B., Gecit I., Gunes M., Ozveren H., Eren H., Demir H., Serum levels of trace elements in patients with prostate cancer, *Asian Pacific Journal of Cancer Prevention,* 15, 6, 2625–2629, **2014**.

[8] Ozmen H., Erulas A.F., Karatas F., Cukurovali A., Yalcin O., 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, *Clinical Chemistry and Laboratory Medicine*, 44, 175–179, **2006**.

[9] Vinceti M., Venturelli M., Sighinolfi C., Trerotoli P., Bonvicini F., Ferrari A., Bianchi G., Serio G., Bergomi M., Vivoli G., Case-control study of toenail cadmium and prostate cancer risk in Italy, *Science of the Total Environment*, 373, 77–78, **2007**.

[10] Sapota A., Darago A., Taczalski J., Kilanowicz A., Disturbed homeostasis of zinc and other essential elements in the prostate gland dependent on the character of pathological lesions, *Biometals*, 22, 1041–1049, **2009**.

[11] Esteban M., Castano A., Non-invasive matrices in human biomonitoring: A review, *Environment International*, 35, 2, 438–449, **2009**.

[12] Barany E., Bergdahl I.A., Bratteby L.E., Lundh T., Samuelson G., Schutz A., Skerfving S., Oskarsson A., Trace element levels in whole blood and serum from Swedish adolescents, *Science of the Total Environment*, 286, 129–141, **2002**.

[13] Rockett J.C., Buck G.M., Lynch C.D., Perreault S.D., The value of home-based collection of biospecimens in reproductive epidemiology, *Environmental Health Perspectives*, 112, 94–104, **2004**.

[14] Cocciardi J.A., Operating Safely in Hazardous Environments Review and Refresher. 3rd Edition, *Jones & Bartlett Publishers*, Burlington, USA, **2012**.

[15] Hayes J.H., Barry M.J., Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence, *JAMA*, 311, 11, 1143–1149, **2014**.

[16] Busato W.F.S., Almeida G.L., Prostate cancer screening in Brazil: should it be done or not? *International Brazilian Journal of Urology*, 42, 6, 1069-1080, **2016**.

[17] Guntupalli J.N., Padala S., Gummuluri A.V., Muktineni R.K., Byreddy S.R., Sreerama L, et al., Trace elemental analysis of normal, benign hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique, *European Journal of Cancer Prevention*, 16, 108–115, **2007**.

[18] Qayyum M.A., Shah M.H., Comparative study of trace elements in blood, scalp hair and nails of prostate cancer patients in relation to healthy donors, *Biological Trace Element Research*, 162, 1-3, 46–57, **2014**.

[19] Hanif M., Zaidi P., Kamal S., Idrees S., Rasool S.A., Significance of prostate specific antigen in prostate cancer patients and in non-cancerous prostatic disease patients, *Journal of Pakistan Medical Association*, 57 (5), 248–251, **2007**.

[20] Reiter W., Stieber P., Schmeller N., Nagel D., Hofmann K., Fateh-Moghadam A., Total and free PSA: a methodical and clinical evaluation of five assays. *Anticancer Research*, 17, 6D, 4759–4765, **1997**.

[21] Pfister C., Basuyau J.P., Current usefulness of free/total PSA ratio in the diagnosis of prostate cancer at an early stage, *World Journal of Urology*, 23, 4, 236–242, **2005**.

[22] StatSoft, STATISTICA for Windows, Computer program manual, StatSoft, Tulsa, OK, **1999**.
[23] McGee D.L., Crespo C.J., Body mass index and the risk of prostate cancer, *Open Access Medical Statistics,* 253–260., **2012**.
[24] Skolarus T.A., Wolin K.Y., Grubb R.L., The effect of body mass index on PSA, prostate cancer development, screening and treatment, *Nature Clinical Practice Urology*, 4, 11, 605–614, **2007**.
[25] Hu M.B., Bai P.D., Wu Y.S., Zhang L.M., Xu H., Na R., Jiang W.H., Ding Q., (2015). Higher body mass index increases the risk for biopsy-mediated detection of prostate cancer in Chinese men, *PLoS ONE*, 10, 4, e0124668, **2015**.
[26] Davies N.M., Gaunt T.R., Lewis S.J., Holly J., Donovan J.L., Hamdy F.C., Kemp J.P., Eeles R., Easton D., Jarai Z.K., The effects of height and BMI on prostate cancer incidence and mortality: a Mendelian randomization study in 20,848 cases and 20,214 controls from the PRACTICAL consortium, *Cancer Causes Control*, 26, 11, 1603–1616, **2015**.
[27] Rodriguez C., Freedland S.J., Deka A., Jacobs E.J., McCullough M.L., Patel A.V., Thun M.J., Calle E.E., Body mass index, weight change, and risk of prostate cancer in the Cancer Prevention Study II Nutrition Cohort, *Cancer Epidemiology, Biomarkers & Prevention*, 16, 63–69, **2007**.
[28] Nomura A.M., Body size and prostate cancer, *Epidemiologic Reviews*, 23, 126–131, **2001.**
[29] Giovannucci E., Liu Y., Platz E.A., Stampfer M.J., Willett W.C., Risk factors for prostate cancer incidence and progression in the health professionals follow-up study, *International Journal of Cancer*, 121, 1571–1578, **2007**.
[30] Isom-Batz G., Bianco F.J., Kattan M.W., Mullhall J.P., Lilja H., Eastham J.A., Testosterone as a predictor of pathological stage in clinically localized prostate cancer, *Journal of Urology*, 173, 6, 1935–1937, **2005**.
[31] Wallner L.P., Morgenstern H., McGree M.E., Jacobson D.J., St Sauver J.L., Jacobsen S.J., Sarma A.V., The effects of body mass index on changes in prostate-specific antigen levels and prostate volume over 15 years of follow-up: implications for prostate cancer detection, *Cancer Epidemiology Biomarkers & Prevention,* 20, 501–508, **2011**.
[32] Kukreja S.C., Shanmugam A., Lad T.E., Hypocalcemia in patients with prostate cancer, *Calcified Tissue International*, 43(6), 340–345, **1988**.
[33] Allen N.A., Key T.G., Appleby P.N., Travis R.C., Roddam A.W., et al., Animal foods, protein, calcium and prostate cancer risk: the European prospective investigation into cancer and nutrition, *British Journal of Cancer*, 98, 1574–1581, **2008**.
[34] Wolk A., Diet, lifestyle and risk of prostate cancer, *Acta Oncologica*, 44, 277–281, **2005**.
[35] Nguyen H.L., Zucker S., Zarrabi K., Kadam P., Schmidt C., Cao J., Oxidative stress and prostate cancer progression are elicited by membrane-type 1 matrix metalloproteinase (MT1-MMP), *Molecular Cancer Research*, 9, 10, 1305–1318, **2011.**
[36] Valko M., Morris H., Cronin M.T.D., Metals, toxicity and oxidative stress, *Current Medicinal Chemistry*, 12, 1161–1208, **2005**.
[37] Khandrika L., Kumar B., Koul S., Maroni P., Koul H.K., Role of Oxidative Stress in Prostate Cancer, *Cancer Letters*, 18, 282, 2, 125–136, **2009**.
[38] Oh B., Figtree G., Costa D., Eade T., Hruby G., Lim S., Elfiky A., Martine N., Rosenthal D., Clarke S., Back M., Oxidative stress in prostate cancer patients: A systematic review of case control studies, *Prostate International*, 4, 71–87, **2016**.
[39] Monteith G.R., McAndrew D., Faddy H.M., Roberts-Thomson S.J., Calcium and cancer: targeting Ca^{2+} transport, *Nature Reviews Cancer*, 7, 519–530, **2007.**
[40] Yaturu S., Zdunek S., Youngberg B., Vitamin D levels in subjects with prostate cancer compared to age-matched controls, *Prostate Cancer*, 2012, 524206, **2012**.
[41] Butler L.M., Wong A.S., Koh W.P., Wang R., Yuan J.M., Yu M.C., Calcium intake increases risk of prostate cancer among Singapore Chinese, *Cancer Research*, 15, 70, 12, 4941–4948, **2010**.
[42] Brandstedt J., Almquist M., Manjer J., Malm J., Vitamin D, PTH, and calcium and the risk of prostate cancer: a prospective nested case–control study, *Cancer Causes & Control*, 23, 1377–1385, **2012**.
[43] Aune D., Rosenblatt D.A.N., Chan D.S.M., Vieira A.R., Vieira R., Greenwood D.C., Vatten L.J., Norat T., Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies, *American Journal of Clinical Nutrition*, 101, 87–117, **2015**.
[44] Skinner H.G., Schwartz G.G., Serum calcium and incident and fatal prostate cancer in the National Health and Nutrition Examination Survey, *Cancer Epidemiology, Biomarkers & Prevention*, 17, 9, 2302–2305, **2008**.
[45] Hemelrijck M.V., Hermans R., Michaelsson K., Melvin J., Garmo H., Hammar N., Jungner I., Walldius G., Holmberg L., Serum calcium and incident and fatal prostate cancer in the Swedish AMORIS study, *Cancer Causes & Control*, 23, 8, 1349–1358, **2012**.
[46] Halthur C., Johansson A.L., Almquist M., Malm J., Gronberg H., Manjer J., Dickman P.W., Serum calcium and the risk of prostate cancer, *Cancer Causes & Control*, 20, 1205–1214, **2009**.
[47] Mundy G.R., Metastasis to bone: causes, consequences and therapeutic opportunities, *Nature Reviews Cancer*, 2, 584–593, **2002**.
[48] Kasprzak K.S., Sunderman F.W., Salnikow K., Nickel carcinogenesis, *Mutatation Research*, 533, 67–97, **2003**.
[49] Dai Q., Motley S.S., Smith J.A., Concepcion R., Barocas D., Byerly S., Fowke J.H., Blood magnesium, and the interaction with calcium, on the risk of high-grade prostate cancer, *PLoS ONE*, 6, 4, e18237, **2011**.
[50] Yang C.Y., Chiu H.F., Tsai S.S., Cheng M.F., Lin M.C., Sung F.C., Calcium and magnesium in drinking water and the risk of death from prostate cancer, *Journal of Toxicology and Environmental Health Part A*, 60, 17–26, **2000**.
[51] Castiglioni S., Maier J.A.M., Magnesium and cancer: a dangerous liason, *Magnesium Research,* 24, 3, S92–S100, **2011**.
[52] Nielsen F.H., Magnesium, inflammation, and obesity in chronic disease, *Nutrition Reviews*, 68, 333–340, **2010**.
[53] Nasulewicz A., Wietrzyk J., Wolf F.I., Dzimira S., Madej J., Maier J.A., Rayssiguier Y., Mazur A., Opolski A., Magnesium deficiency inhibits primary tumor growth but favours metastasis in mice, *Biochimica et Biophysica Acta*, 1739, 1, 26–32, **2004**.
[54] Logan A.G., Dietary sodium intake and its relation to human health: a summary of the evidence, *Journal of the American College of Nutrition*, 25, 3, 165–169, **2006**.
[55] Pohl H.R., Wheeler J.S., Murrary H.E., Sodium and potassium in health and disease, *Metal Ions in Life Sciences*, 13, 29–47, **2013**.
[56] D'Elia L., Rossi G., Ippolito R., Cappuccio F.P., Strazzullo P., Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies, *Clinical Nutrition*, 31, 489–498, **2012**.
[57] Handy B.C., Shen Y., Evaluation of potassium values in a cancer patient population, *Lab Medicine,* 36, 2, 95–97, **2005**..
[58] Larsson S.C., Orsini N., Wolk A., Dietary potassium intake and risk of stroke: a dose-response meta-analysis of prospective studies, *Stroke,* 42, 2746–2750, **2011**.
[59] Jansson B., Potassium, sodium, and cancer: a review, *Journal of Environmental Pathology, Toxicology and Oncology,* 15, 2-4, 65–73, **1996**.
[60] Dahl S.G., Allain P., Marie P.J., Mauras Y., Boivin G., Ammann P., Tsouderos Y., Delmas P.D., Christiansen C., Incorporation and distribution of strontium in bone, *Bone*, 28, 4, 466–453, **2001**.
[61] Verberckmoes S.C., De Broe M.E., D'Haese P.C., Dose dependent effects of strontium on osteoblast function and mineralization, *Kidney International*, 64, 534–543, **2003.**
[62] ATSDR, Toxicological profile for strontium. US Department of Health and Human Services Public Health Service, Atlanta, USA, pp. 1–22, **2004**.
[63] Cohen-Solal M., Strontium overload and toxicity: impact on renal osteodystrophy, *Nephrology Dialysis Transplantation,* 17, 30–34, **2002**.
[64] Gould J.M., Strenglass E.J., Sherman J.D., Strontium-90, deciduous teeth as a factor in early childhood cancer, *International Journal of Health Services,* 30, 515–539, **2000**.
[65] Phiel C.J., Klein P.S., Molecular targets of lithium action, *Annual Review of Pharmacology and Toxicology,* 41, 789–813, **2001**.

[66] Nowicki M.O., Dmitrieva N., Stein A.M., Cutter J.L., Godlewski J., Saeki Y., Nita M., Berens M.E., Sander L.M., Newton H.B., Chiocca E.A., Lawler S., Lithium inhibits invasion of glioma cells; possible involvement of glycogen synthase kinase-3, *Neuro-Oncology*, 10, 690–699, **2008**.
[67] Li H., Huang K., Liu X., Liu J., Lu X., Tao K., Wang G., Wang J., Lithium chloride suppresses colorectal cancer cell survival and proliferation through ROS/GSK-3*β*/NF-κB signaling pathway, *Oxidative Medicine and Cellular Longevity*, 2014, 241864, **2014**.
[68] Eskandari M.R., Fard J.K., Hosseini M.J., Pourahmad J., Glutathione mediated reductive activation and mitochondrial dysfunction play key roles in lithium induced oxidative stress and cytotoxicity in liver, *Biometals,* 25, 5, 863–873, **2012**.
[69] De Boeck M., Kirsch-Volders M., Lison D., Cobalt and antimony: genotoxicity and carcinogenicity, *Mutation Research*, 533, 1–2, 135–152, **2003**.
[70] Catelas I., Petit A., Vali H., Fragiskatos C., Meilleur R., Zukor D.J., Antoniou J., Huk O.L., Quantitative analysis of macrophage apoptosis vs. necrosis induced by cobalt and chromium ions in vitro, *Biomaterials*, 26, 2441–2453, **2005**.
[71] Battaglia V., Compagnone A., Bandino A., Bragadin M., Rossi C.A., Zanetti F., Colombatto S., Grillo M.A., Toninello A., Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsic apoptosis in hepatocyte primary cultures, *The International Journal of Biochemistry & Cell Biology*, 41, 586–594, **2009**.
[72] IARC, Cobalt in hard metals and cobalt sulphate, gallium arsenide, indium phosphide and vanadium pentoxide. In: IARC monographs on the evaluation of carcinogenic risks to humans, vol. 86. Lyon, France, **2006**.
[73] Jain S., Saxena S., Kumar A., Epidemiology of prostate cancer in India, *Meta Gene*, 2, 596–605, **2014**.

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