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ORIGINAL RESEARCH ARTICLE

Multivariate Statistical Evaluation of 20 Metals/Metalloid Levels in the Serum of Patients with Prostate Gland Diseases

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Abstract The prostate gland diseases are associated with benign prostatic hyperplasia (BPH) and prostate cancer (PC) and exposure to toxic trace elements may promote the prostatic disorders in men. The present study is intended to analyze the concentrations of twenty elements (Al, Sb, Ca, Se, Cd, Fe, Hg, As, Zn, Mn, Na, Li, Cu, Co, Mg, Sr, Ni, K, Cr and Pb) in the serum of BPH ($n=188$) and PC ($n=217$) patients and in comparison with controls ($n=233$). Nitric acid-perchloric acid mixture was used for serum digestion followed by determination of the metals/metalloid by atomic absorption spectrophotometry. This study elucidates the imbalances of the elements with BPH/PC patients and healthy subjects. For multiple comparisons, Bonferroni test was applied and principal component analysis was performed for measuring the multiple metals/metalloid exposure. Mean concentrations of Al, Cr, Pb, Cd, Na, Ni and K were found higher significantly ($p<0.05$) in the serum of BPH patients compared with healthy controls, while average levels of Sb, Al, Cd, As, Mn, Sr, K and Pb were significantly ($p<0.05$) elevated in PC patients than controls. The correlation patterns revealed significantly different mutual associations among the metals/metalloid in patients as compared to controls. Multivariate statistical methods showed substantially divergent grouping of the metals/metalloid for both groups of patients and healthy controls. Significant variations in the elements levels were also detected in various PC types (small cell prostate, transitional cell, squamous cell carcinomas and adenocarcinoma) and PC stages. Significant differences in the metals/metalloid levels were also noted with abode, dietary and smoking habits of donor groups.

Keywords Benign prostatic hyperplasia · Prostate cancer · Serum · PSA · Metals · Multivariate analysis

Introduction

Progressive urbanization, industrialization, vehicular emissions and poor food quality contribute to the rising burden of several diseases including cancer in humans. Cancer of prostate is a heterogeneous disease develops in the prostate gland organ among men. It is one of the most common non-cutaneous malignancies in elderly males with an estimated more than 1.4 million new cases and 375,000 deaths in 2020 [1]. Globally, prostate cancer (PC) is the 2nd most commonly diagnosed tumor and is the 5th leading cause of cancer mortality in males and represents a substantial public health burden [2]. After about two decades of declining incidence, PC cases increased by 3% per year in USA [3]. Benign prostatic hyperplasia (BPH) is a non-cancerous cellular proliferative process resulting prostatic enlargement leading to obstruction of the urethra thereby can significantly impair quality of life [4]. Among men, BPH is the most common urologic disorder after the age of 50 years representing serious health issue especially in industrial countries [5].

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However, PC and BPH coexist in the same prostatic zone is only 20% of cases [5]. Generally, prostate malignancy progresses very slowly and can often be treated successfully, especially if diagnosed in the early stage. Accordingly, cancer stages represent the progression of cancer pertaining the spread of tumors from unspread to growing tumors penetrating in the adjacent tissues/other organs [6]. The stage of cancer helps to predict the course of treatments and to identify clinical trials [7]. Despite intense efforts on cancer research, the exact etiology of BPH and PC are unknown, various factors such as family history, hormones, ethnicity, diet, age-related changes, infection-related inflammation, alcohol and smoking as well as exposure to metals/metalloid are linked to prostate gland disease development [8–11]. Moreover, as mentioned, various epidemiological studies have explored the associations among non essential heavy metals exposures and risk of prostatic disorders in patients [11, 12]. Hence, various toxic elements such as Cd, As, Pb, Cr, Ni and Hg are persistent in the environment and are considered to contribute to the augmented risk of this disease [4, 10]. Further, long-term exposure to various toxic metals may disturb the dynamic balance in the body causing enhanced generation of free radicals via oxidative stress, modification of tumor suppressor gene expression, damage of nucleic acid and the activation of redox-sensitive transcription factors resulting in many ailments including prostate disorders [12]. It has been recognized that both PC and BPH develop due to DNA damage and mutations, which could arise for various reasons, including environmental contaminants such as Cd, Pb and Ni exposure [8–10]. Recent scientific data revealed the associations of toxic metals like Pb and Cd to BPH development and progression through the altered pro-oxidant–antioxidant balance in the patients [4, 5]. Consequently, these studies also concluded that Cd and Pb play an important role in the pathogenesis of prostatic disease [5]. For instance, Cd is a potential risk factor for PC based on substantial supportive evidence from experimental/clinical research for such an association [13]. Various biological fluids/tissues (plasma, blood, hair, nails and tissues) exposed alterations of elements in patients with throat, colorectal, breast, ovarian and prostate cancers etc., [7, 14, 15]. Prostate specific antigen (PSA) is a tumor marker and is utilized for screening of prostate gland diseases [16]. The epithelium of prostate tissue excreted PSA which is a single-chain glycoprotein [12]. PSA level increases slightly with age as the prostate gland grow bigger. Nevertheless, along with prostate disease, PSA level can also be influenced by a variety of other factors, such as lifestyle choices and exposure to metals [7]. Hence, high concentration of PSA in serum is a sign of prostate disease in the prostate gland including BPH and PC in patients [17].

Quantification of metals/metalloid levels in the human blood serum can be used as a clinical diagnostic tool, which explains the effects of environment and/ or nutrition on the elemental levels in the body [14]. Further, many epidemiologic and biochemical research data on the relationship among many of metals/metalloid and incidence of prostate disease is incomplete. Little is known about the relationships (beneficial or harmful) between serum metals levels and BPH patients and PC in patients. The relationship of these metals exposures with serum PSA, a marker used for PC screening, is unknown as well. To our knowledge, relationships between metals/metalloid and serum PSA have not been examined in Pakistani Population. Keeping in view, it is necessary to explore strategies that might reduce the incidence of prostate diseases. To our knowledge, this is the first study to investigate the relationship between the levels of metals/metalloid and elevated serum PSA in BPH and PC in Pakistani patients. Based on the above facts, the present study was designed to measure the concentrations of toxic and essential metals/metalloid (Al, Sb, Ca, Se, Cd, Fe, Hg, As, Zn, Mn, Na, Li, Cu, Co, Mg, Sr, Ni, K, Cr and Pb) levels in the serum of BPH patients and PC patients in comparison with counterpart healthy donors. Mutual associations among the metals/metalloid and PSA levels were assessed by spearman correlation study. Further multivariate methods such as cluster analysis and principal component analysis were implicated for the apportionment of metals in the serum of BPH patients and PC patients as well as healthy donors. Variations in the metals/metalloid concentrations with respect to abode, food habits, smoking and non-smoking habits, types of PC (small cell prostate, transitional cell, squamous cell and adenocarcinoma) and stages (I, II, III and IV) of the donors were also assessed. Different analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), X-ray fluorescence spectroscopy and atomic absorption spectroscopy (AAS) can be applied to identify and measure metals and metalloid. AAS is an accurate, reliable, reproducible and frequently used analytical tool to quantify most metals and metalloids in fluids and tissues, whether for toxicological investigation or for therapeutic indications. This method offers sufficient sensitivity for many applications and is relatively interference free. The outcomes of studies may be impacted by the sensitivity and specificity differences between various trace element assays [14].

Materials and Methods

Study Setting

The study subjects were recruited from the Punjab Institute of Nuclear Medicine (PINUM) Faisalabad Pakistan, one of the large public cancer's Institute in Faisalabad-Pakistan,

where a total of 638 study participants were recruited in 2020–2021. The inclusion criteria for patients were: age between 40 and 85 years old, diagnosed with benign prostatic hyperplasia and prostate carcinoma based on pathological/biopsy examination, no surgery, no blood transfusion, treatment with chemotherapy or radiotherapy has not yet started; not used multivitamins from last six months, no endocrine disorder or any other chronic diseases, lived in Punjab province for more than 5 years. The exclusion criteria were: treated with radiotherapy or chemotherapy, post-operative patients, distant metastasis, history of other tumors and serious diseases of the liver or kidney, not willing to participate in the present study etc., took nutritional/vitamin supplements or antioxidant within the past three months, drinking alcohol abuse one month before enrollment. The BPH patients did not start any treatment for cure. The healthy donors enrolled from the same Institute PINUM belong to the same area of respective patients, no prior history of tumor disease at the time of recruitment, matched to the cases by age, sex and concurrent infection, or chronic infection, similar socioeconomic status and nutrition habits and no history of drug abuse. Prior to sample collection, the study was approved by the Ethics Committee of Punjab Institute of Nuclear Medicine, Faisalabad (Ref. No. UEFC/2022/R437), and all participants signed the informed consent performa and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. At the time of sample collection, information regarding age, residence, occupation, nutritional habits, health status, smoking habits, type of ailment, medication, and tumor stage and type of tumor etc., were filled by trained research staff using a standardized questionnaire via respective participant. Routine medical examinations were performed in the Institute-PINUM of each participant.

Sample Collection

After 10 h fasting, trained staff nurse was collected the blood of each subject from an antecubital vein at morning hours. Appropriate precautions were managed to inhibit exogenous contamination. Around ten milliliter blood (venous) of PC patients, BPH patients and healthy controls were drawn at fasting conditions. Immediately, the blood samples were transferred in two metal free sterile polyethylene tubes almost equally. One tube was sent to special chemistry laboratory for prostate specific antigen (PSA) test and other blood sample tube was kept in water bath until all the blood in the tube was completely clotted. Then the samples (blood) were centrifuged by centrifuge machine for 15 min around 4000 rpm. The buffy coat was removed with the help of pipette and recentrifuged the blood. Then the serum was separated by Appendorf pipette and transferred to a new screw capped tube followed by stored at − 10 °C until elemental analysis [15].

Sample Processing

Accurately known amount of serum sample of each participant was relocated from storage tube to the digestion flask. 5 mL of HNO_3 was added in each tube and remained at the room temperature for 30 min and stirred occasionally. Then, placed on hot plate and heated for 30 min at 50 °C followed by cooling at room temperature. Two mL of $HClO_4$ was added in each of the digestion flask and the mixture was transferred to the hot plate followed by heating at 70–80 °C. This heating process to a soft boil followed by evolving of white dense fumes was signal of completion process of mineralization. The serum samples were taken off the hot plates and then cooled at ambient temperature [14]. The digested solutions after mineralization were transferred to 25 mL volumetric flasks and then diluted with doubly distilled water. A sample blank was processed with each batch of the 6 samples.

Measurement of Essential and Toxic Metals and Metalloid Levels

Serum samples concentrations of Al, Sb, Ca, Se, Cd, Fe, Hg, As, Zn, Mn, Na, Li, Cu, Co, Mg, Sr, Ni, K, Cr and Pb in BPH patients, PC patients and healthy controls were measured by using flame atomic absorption spectrophotometer (AAS) (Shamidzu AA670, Japan). Serum samples levels of Se, Hg, and As were determined in all participants were made using an GBC 932b plus atomic absorption spectrophotometer (GBC Scientific Equipments Pvt. Ltd. Australia). All measurements were performed in duplicate and runs separately onto the AAS in order to get pool average levels of specific element. The accuracy of the analytical process and measurements were controlled using certified reference materials (CRM), NIST–SRM 8414 (bovine Muscle Powder). Overall percentage recoveries were 95–105% of the assigned analytical values. The selected metals/metalloid were also determined by an independent laboratory in the serum samples for comparison of the results and maximum of ± 3% difference was noted in the results of two laboratories. In addition, working standards were prepared by serial dilution of the stock standard solutions (1000 mg/L) prior to the analysis [15].

Measurement of Prostate Specific Antigen (PSA) Levels

Prostate specific antigen is a single chain glycoprotein produced by prostatic epithelial cells, lining the acini and ducts of the prostate gland [17]. It is widely acknowledged PSA levels elevate in the serum of males with normal, BPH

patients and PC patients tissue as well as prostatic inflammation/infection [12]. Accordingly, raised level of PSA in blood serum is a sign of prostate disease in the prostate gland including PC [16]. The Atellica IM PSA assay is a 2 2-site sandwich immunoassay utilizing direct chemiluminometric technology. This uses constant amounts of 2 antibodies. A direct connection finds among the amount of PSA present in the patient serum sample and the amount of relative light units detected by the system. The PSA was quantified on the Atellica IM Analyzer (Siemens Healthineers, Germany) as the assay protocol provided in the kit. The PSA level of less than 5 ng/mL is used as the cut off value.

Statistical Analysis

Metals and metalloid data were analyzed by STATISTICA (6.0) software for statistical analysis [18]. All results were expressed as mean range, median, standard deviation, standard error and skewness as the basic statistical parameters. Chi-square test was used to examine the frequency distributions of variables including smoking habits, nutrition habits and habitat between cases and controls. The donors groups comparisons were also performed by using *t*-test. Further *p* values less than 0.05 were noted as statistically significant. For the comparison of the median levels, Wilcoxon rank-sum test was applied. Pearson's correlation coefficient was applied to establish the relationships between the essential and toxic metals/metalloid levels for mutual variations. The one-way analysis of variance (ANOVA) and Bonferroni's test was employed to measure the statistical significance ($p < 0.05$) of concentrations of the selected metals/metalloid among the BPH patients, PC patients and healthy controls.

Chemometric Analysis

Chemometric analysis is a discipline that manipulates information from multivariate chemical data utilizing tools of mathematics and statistics. Multivariate analysis concerned more than one statistical outcome variable at a time and interrelationships among several variables. Principal component analysis (PCA) is a multivariate technique that is used to reduce the data size by calculating the most important variables in the formation of phenomenon/system [19]. The primary use of PCA is to extract the important information from the statistical data preserving as much 'variability' (i.e. statistical information) as possible, enhancing interpretability and minimizing information loss at the same time. PCA creates a set of new orthogonal variables known as principal components (PC), and to exhibit the pattern of similarity between the observations and of the variables which reduces to solving an eigenvalue/eigenvector problem, and hence making PCA an adaptive data analysis technique [20]. Cluster analysis (CA) is a multivariate tool/exploratory analysis via unsupervised classification that tries to partition/identify a set of data objects (or observations) into subsets called clusters [21]. The observations which are similar to one another remained within the same cluster and the dissimilar observations to the objects persists other clusters. It is a quantitative form of classification that combines meaningful aggregations of objects with little or no a prior information and identify useful patterns within a large data set [22].

Results and Discussion

Demographic Characteristics

The demographic characteristics for the BPH patients, PC patients and healthy donors/controls are noted in Table 1, which demonstrated that the subjects were closely matched for their ages. The average age of BPH patients was 53 (range 43–73) years and PC patients was 54 (41–70) years. The mean age of healthy controls was 51 (range 46–77) years. About 40% of the BPH patients and 59% of the PC patients as well as 44% of healthy controls were used cigarette smoking on continuous basis. Among use of smoking/no use of smoking habits, there was not existed significant difference between patients and healthy controls as shown in Table 1. 52–54% patients fitted to vegetarian class, while 48% of the healthy controls were also belonged to vegetarian class. Less than 50 percent of patients were non-vegetarian in their dietary habits as shown in Table 1. Based on the habitat, > 50% of the BPH patients & PC patients resided in urban and rural areas, respectively. However, 44% of the BPH patients and 47% of the PC patients were belonged to rural and urban localities respectively. There were not found significant difference between patients and healthy subjects regarding vegetarian and non vegetarian habits.

Based on types of PC, adenocarcinoma patients represented the major type of PC (29%) patients in the present study. 27% of patients were suffered from the squamous cell carcinoma. Transitional cell carcinoma patients were 24% followed by 20% from small cell prostate cancer patients. In the present study, patients were diagnosed 24% at stage I, while stage II revealed 26% of the PC patients. Stage III demonstrated 28% followed by 22% of the PC patients were diagnosed at stage IV of the PC as publicized in Table 1.

Distribution of Metals/Metalloid Levels and Prostate Specific Antigen (PSA) Levels

Average metals levels along with the basic statistical parameters concerning to the distribution in the serum of BPH patients, PC patients and healthy controls are publicized in Table 2. A wide range of levels as noted by the minimum and maximum concentrations were shown by most of

Table 1 Characteristics of the patients and healthy controls

Parameters	Serum			*p* value
	Benign prostatic hyperplasia patients	Prostate cancer patients	Healthy controls	
	n = 188	n = 217	n = 233	
Age (years)				
Range (mean)	43–73 (53)	41–70 (54)	46–77 (51)	
Smoking habits				
Use	75 (40%)	96 (59%)	103 (44%)	0.602
No use	113 (60%)	121 (41%)	130 (56%)	
Nutrition habits				
Vegetarian	97 (52%)	113 (54%)	112 (48%)	0.652
Non-vegetarian	91 (48%)	104 (46%)	121 (52%)	
Habitat				
Urban	106 (56%)	109 (47%)	117 (50%)	0.365
Rural	82 (44%)	108 (53%)	116 (50%)	
Types of prostate cancer				
Small cell prostate cancer	–	44 (20%)	–	
Transitional cell carcinoma	–	51 (24%)	–	
Squamous cell carcinoma	–	59 (27%)	–	
Adenocarcinoma	–	63 (29%)	–	
Pathological stages of prostate cancer				
Stage I	–	51 (24%)	–	
Stage II	–	57 (26%)	–	
Stage III	–	61 (28%)	–	
Stage IV	–	48 (22%)	–	

the metals and metalloid. In BPH patients, comparatively elevated mean concentrations were noted for Ca (3983 µg/dL), Zn (3930 µg/dL), Na (3881 µg/dL), Mg (2671 µg/dL), Sr (2160 µg/dL), Fe (2171 µg/dL), K (2129 µg/dL) and Se (1286 µg/dL) in the serum. Relatively lower mean levels of Cu (341.7 µg/dL), Ni (294.1 µg/dL), Sb (88.97 µg/dL), Al (48.69 µg/dL), Mn (41.30 µg/dL), Cr (34.76 µg/dL), Li (31.41 µg/dL) and Co (27.94 µg/dL) were calculated in the serum of BPH patients. Further, lowest average Cd (4.293 µg/dL), Pb (3.535 µg/dL), Hg (2.965 µg/dL) and As (1.400 µg/dL) concentrations were found in the serum of BPH patients. On the average level, overall the decreasing trend of the metals and metalloids contents in the serum of BPH patients involved the following order: Ca > Zn > Na > Mg > Sr > Fe > K > Se > Cu > Ni > Sb > Al > Mn > Cr > Li > Co > Cd > Pb > Hg > As. Some of the metal(loid)s (Pb, As, Hg and Cd) revealed moderately normal distribution pattern supported by lowest SE values. Large skewness values for As, Ni, K, Pb and Cr revealed their significant asymmetric distribution while for rest of the metal(loid)s showed moderately symmetrical distribution representing modest skewness values of these metals in the serum of BPH patients. The metals/metalloid levels of Zn, Na, Mg, Sr and K exhibited non-Gaussian distribution pattern as supported by higher SD values. Nevertheless, the rest of the metals demonstrated relatively Gaussian distribution in their levels as evidenced by smaller SD values in BPH patients. The PSA levels (mean) in the serum of BPH patients was 41.91 ng/mL with ranged from 9.790 to 81.56 ng/mL against the cut off value of < 5 ng/mL.

In the serum of PC patients (Table 2), highest average levels were observed in Na (4359 µg/dL), Zn (3962 µg/dL), Ca (3784 µg/dL), Sr (2764 µg/dL), Mg (2318 µg/dL), Fe (2054 µg/dL), K (2243 µg/dL) and Se (1249 µg/dL). Average Cu (378.6 µg/dL), Ni (277.1 µg/dL), Sb (92.23 µg/dL), Mn (54.95 µg/dL), Al (48.75 µg/dL), Cr (31.99 µg/dL), Li (27.63 µg/dL) and Co (22.20 µg/dL) contents revealed relatively lower values in the serum of PC patients. Average levels of Cd (4.299 µg/dL), Pb (3.689 µg/dL), As (2.757 µg/dL) and Hg (1.731 µg/dL) were exhibited lowest in the serum of PC patients as shown in Table 3. The selected metal(loid)s in the serum of PC patients presented following descending order in their mean concentrations: Na > Zn > Ca > Sr > Mg > Fe > K > Se > Cu > Ni > Sb > Mn > Al > Cr > Li > Co > Cd > Pb > As > Hg. Among the metals, Ca, Zn, Na, Mg, Sr, and K pointed out elevated dispersion as disclosed by their large SD values in the serum of PC patients. Large skewness values for Cr, As and Mn exhibited their asymmetric distribution in the serum of PC patients while modest skewness values for Al, Sb, Ca, Se, Cd and Fe indicated moderately

Table 2 Statistical summary for the levels of metal(loid)s (µg/ dL), PSA (ng/mL) in the serum of benign prostatic hyperplasia patients, prostate cancer patients and healthy controls

	Benign prostatic hyperplasia (BPH) patients							Prostate cancer (PC) patients							Healthy controls (HC)							BPH versus HC	PC versus HC	BPH versus PC
	Min	Max	Mean	Med	SD	SE	Skew	Min	Max	Mean	Med	SD	SE	Skew	Min	Max	Mean	Med	SD	SE	Skew	P1	P2	P3
Al	1.400	88.20	48.69	41.6	0.029	0.004	−0.069	1.400	92.00	48.75	49.10	0.028	0.004	−0.230	5.000	50.00	22.58	20.50	0.010	0.002	0.801	0.000**	0.000**	1.000^{ns}
Sb	7.000	193.0	88.97	92.0	0.053	0.008	0.299	7.000	211.0	92.23	97.00	0.057	0.008	0.290	4.100	106.0	52.23	54.00	0.029	0.005	−0.030	0.003*	0.001**	1.000^{ns}
Ca	2088	5700	3983	4000	0.883	0.130	−0.055	1124	6000	3784	3821	1.139	0.163	−0.370	2088	6100	4311	4347	0.973	0.156	−0.178	0.412^{ns}	0.048*	1.000^{ns}
Se	76.00	1840	1286	1580	0.595	0.087	−0.948	30.00	1870	1249	1580	0.626	0.089	−0.870	77.00	1820	1443	1605	0.454	0.072	−1.822	0.606^{ns}	0.338*	1.000^{ns}
Cd	1.000	7.000	4.293	4.000	0.002	0.001	−0.311	1.000	7.000	4.299	4.200	0.002	0.001	−0.320	1.000	6.500	3.918	5.200	0.001	0.002	−1.446	0.205^{ns}	0.021*	1.000^{ns}
Fe	60.00	3930	2171	2100	0.715	0.107	−0.092	60.00	3930	2054	2033	0.745	0.108	0.210	1020	3040	1881	1820	0.569	0.091	0.354	0.169^{ns}	0.733^{ns}	1.000^{ns}
Hg	0.001	6.400	2.965	2.729	0.002	0.001	0.731	0.001	9.400	1.731	1.885	0.002	0.002	1.380	0.002	7.040	3.945	3.925	0.002	0.003	−0.223	0.041*	0.010*	0.000**
As	0.001	5.000	1.400	1.000	0.001	0.002	2.379	0.001	5.000	2.757	1.000	0.001	0.003	3.470	0.001	5.000	1.252	1.646	0.001	0.004	1.990	0.138^{ns}	0.011*	0.000**
Zn	1703	5739	3930	4280	1.124	0.168	−0.287	1121	5910	3962	4365	1.298	0.187	−0.560	2074	5830	4325	4461	1.053	0.167	−0.609	0.367^{ns}	0.410^{ns}	1.000^{ns}
Mn	0.001	93.00	41.30	46.77	0.032	0.005	0.072	1.000	661.0	54.95	43.00	0.094	0.013	5.780	1.200	86.70	46.3	53.20	0.030	0.005	−0.389	1.000^{ns}	0.044*	0.035*
Na	1071	8617	3881	2943	2.172	0.320	0.876	1071	9053	4359	4040	2.306	0.329	0.510	1210	8570	3328	2240	2.063	0.326	0.806	0.036*	0.087*	0.029*
Li	0.001	91.02	31.41	19.90	0.029	0.004	0.834	0.001	93.75	27.63	16.89	0.028	0.004	1.120	2.000	91.96	41.64	41.56	0.030	0.005	0.265	0.320^{ns}	0.080^{ns}	1.000^{ns}
Cu	23.80	808.0	341.7	289.2	0.195	0.028	0.774	23.80	984.0	378.6	355.6	0.221	0.032	0.670	14.80	952.4	353.3	345.2	0.228	0.036	0.712	1.000^{ns}	1.000^{ns}	1.000^{ns}
Co	0.001	92.00	27.94	23.00	0.024	0.004	0.935	1.060	82.90	22.20	15.91	0.019	0.003	1.150	0.001	92.00	25.06	10.00	0.028	0.004	0.970	1.000^{ns}	1.000^{ns}	0.014*
Mg	397.0	5530	2671	2500	1.135	0.166	0.812	397.0	5420	2318	2240	1.036	0.148	1.220	1080	6500	2917	2212	1.554	0.246	0.937	1.000^{ns}	0.076^{ns}	0.500^{ns}
Sr	46.00	5529	2160	1908	1.580	0.236	0.682	46.00	7778	2764	2568	1.836	0.268	0.580	46.00	9091	1724	1604	1.732	0.293	2.267	0.937^{ns}	0.032*	0.028*
Ni	17.00	992.0	294.1	232.9	0.247	0.037	1.287	11.00	992.0	277.1	227.0	0.243	0.036	1.410	14.40	878.7	248.5	222.7	0.180	0.030	1.401	0.004**	1.000^{ns}	1.000^{ns}
K	1021	5490	2129	1605	1.286	0.190	1.449	474.0	5490	2243	1679	1.419	0.205	1.070	781.0	4167	1767	1569	0.716	0.115	1.526	0.034*	0.043*	1.000^{ns}
Cr	2.100	162.0	34.76	26.80	0.030	0.004	2.348	2.100	162.0	31.99	26.96	0.027	0.004	2.970	11.14	90.42	30.53	27.70	0.019	0.003	1.513	0.044*	1.000^{ns}	1.000^{ns}
Pb	1.000	9.000	3.535	2.200	0.003	0.001	1.016	1.000	9.000	3.689	3.000	0.002	0.003	0.820	1.000	9.000	2.605	4.000	0.002	0.001	0.566	0.043*	0.001**	1.000
PSA	9.790	81.56	41.91	40.00	23.56	4.102	0.264	3.390	460.0	161.5	142.0	139.7	21.560	0.636	0.006	3.523	3.466	3.776	0.270	0.271	0.124	0.064^{ns}	0.000**	0.000**

ns Non-significant ($p > 0.05$)

*Significant ($p < 0.05$); **Highly significant ($p < 0.01$)

Table 3 Correlation coefficient (r) matrix of selected metal(loid)s in the serum of benign prostatic hyperplasia patients

	Al	Sb	Ca	Se	Cd	Fe	Hg	As	Zn	Mn	Na	Li	Cu	Co	Mg	Sr	Ni	K	Cr	Pb	PSA
Al	1.000																				
Sb	0.013	1.000																			
Ca	−0.116	−0.205	1.000																		
Se	−0.107	**0.601**	0.306	1.000																	
Cd	0.118	−0.004	0.055	**0.766**	1.000																
Fe	−0.077	−0.156	0.323	−0.073	0.124	1.000															
Hg	−0.109	−0.002	**0.615**	0.191	−0.054	0.252	1.000														
As	0.154	−0.022	0.201	−0.173	0.009	0.084	0.007	1.000													
Zn	−0.171	**0.527**	0.131	0.448	**0.535**	−0.012	0.139	**−0.549**	1.000												
Mn	−0.193	0.213	0.051	**0.633**	0.380	−0.107	0.030	−0.093	**0.523**	1.000											
Na	−0.069	0.086	0.244	0.213	0.122	0.348	**0.514**	−0.017	0.310	0.179	1.000										
Li	−0.044	**0.665**	−0.014	**0.563**	**0.579**	−0.074	−0.002	−0.098	0.250	0.310	0.200	1.000									
Cu	−0.018	0.154	0.287	**0.529**	0.398	0.030	0.403	−0.076	0.218	0.305	−0.146	0.272	1.000								
Co	**0.673**	0.266	0.031	0.316	0.043	0.015	0.154	0.096	**0.648**	**0.648**	**0.576**	0.338	0.098	1.000							
Mg	**−0.565**	−0.022	0.133	0.224	0.065	−0.152	−0.072	−0.056	0.282	0.280	−0.075	−0.111	0.173	−0.172	1.000						
Sr	−0.192	0.208	0.124	0.361	0.116	**−0.528**	0.153	−0.036	0.256	0.167	0.014	0.355	0.196	−0.044	**0.504**	1.000					
Ni	0.140	0.077	0.054	0.477	0.277	−0.101	0.073	**0.524**	0.207	0.323	0.190	**0.664**	0.349	**0.556**	0.343	0.286	1.000				
K	**0.554**	0.185	−0.013	0.293	0.191	−0.186	0.005	0.023	0.160	0.115	**−0.531**	0.181	**0.674**	**−0.562**	0.082	0.012	0.132	1.000			
Cr	**0.619**	**−0.588**	0.274	0.258	0.178	−0.075	0.150	−0.097	−0.107	0.057	0.030	−0.090	0.176	0.113	0.057	−0.038	**0.597**	0.143	1.000		
Pb	−0.083	−0.259	0.066	−0.054	0.045	0.178	−0.004	−0.064	**−0.527**	−0.042	0.190	0.074	0.044	0.010	−0.149	**−0.539**	−0.053	−0.032	−0.085	1.000	
PSA	0.135	−0.363	−0.070	−0.330	**0.568**	0.046	−0.008	0.117	**−0.558**	−0.174	−0.042	−0.125	−0.205	0.053	−0.169	**−0.508**	−0.203	−0.219	−0.030	**0.678**	1.000

Bold r-values are significant at $p < 0.05$

symmetrical distribution of these metals and metalloid in the serum of PC patients. Among the enzyme, PSA levels varied from 3.390 to 460 ng/mL with a mean value of 161.5 ng/mL in the serum of prostate cancer patients. Thus, the PSA levels were increased in the serum of patients as presented in Table 2.

In case of healthy controls, maximum concentrations were determined in the serum were Zn (4325 µg/dL), Ca (4311 µg/dL), Na (3328 µg/dL), Mg (2917 µg/dL), Fe (1881 µg/dL), K (1767 µg/dL), Sr (1724 µg/dL) and Se (1443 µg/dL) followed by Cu (353.3 µg/dL), Ni (248.5 µg/dL), Sb (52.23 µg/dL), Mn (46.25 µg/dL), Li (41.64 µg/dL), Cr (30.53 µg/dL), Co (25.06 µg/dL) and Al (22.58 µg/dL) on the other side noted relatively lower average concentrations. Among the rest of the metals, relatively lowest contributions were displayed for Hg (3.945 µg/dL), Cd (3.918 µg/dL), Pb (2.605 µg/dL) and As (1.252 µg/dL) in the serum of healthy controls (Table 2). Mean elemental contents in the serum of healthy controls showed the following trend: Zn > Ca > Na > Mg > Fe > K > Sr > Se > Cu > Ni > Sb > Mn > Li > Cr > Co > Al > Hg > Cd > Pb > As. Relatively lower SD values and skewness values exhibited by Cd, Hg, As, Al, Cr and Pb compared with the other metals showing rather Gaussian and symmetrical distribution in the serum of healthy controls. However, Na, Sr, Mg and Zn indicated more randomness and asymmetry in the healthy controls as expressed by their relatively higher SD and higher skewness values as pointed out in Table 2. Overall, prostate gland diseases patients exhibited reasonably higher asymmetry and randomness in the metals and metalloid distribution than the healthy controls, which revealed considerably lower randomness in the serum of their concentrations than the patients. The mean PSA level was 3.466 ng/mL against the cut off value < 5 ng/mL in the serum of healthy controls as shown in Table 2.

Comparison of Average Metals/Metalloid Levels

Mean metals levels in serum samples of the BPH patients, PC patients and healthy controls are compared to find out the significant differences by applying one way ANOVA test (Table S1-supplementary material) and Bonferroni test (Table 2). Average concentrations of Al, Cd, Na, Ni, K, Cr and Pb were found to be significantly elevated in serum samples of BPH patients when compared to healthy controls. However, in case of PC patients, the mean serum levels of Sb, Al, Cd, As, Mn, Sr, K and Pb revealed to be significantly increased than the healthy controls demonstrating considerable associations of these metals with the prostate disease. Similarly there were significantly differences among Hg, Co, As, Mn, Na, Se and Sr concentrations in the serum of BPH patients and PC subjects. On contrary, average Se, Ca, Cu, Li, Zn and Hg contents were found raised significantly ($p < 0.05$) in the serum of healthy controls than patient's subjects. Wilcoxon rank-sum test was applied to compare the median levels of metalloids in the serum of patients and health subjects. Concentrations of Sb, Ni, Cd, Co, Pb and Al were significantly ($p < 0.05$) elevated in patients groups (BPH & PC) than healthy controls.

A comparison between prostate gland diseases (BPH & PC) patients and healthy controls using one-way analysis of variance (ANOVA) was shown in Table S1 (supplementary material). The results revealed that there were significant differences in Al, Cd, Sb, K, Hg, Co, As, Pb, and Sr concentrations in samples collected from patients subjects and healthy controls, whereas no significant differences were found for the rest of metals and metalloids. These were also found the significant differences in PSA values in the patients and healthy subjects. From the above discussion, it appears important in the present study that levels of toxic metals (Cd, Ni, Co and Pb) are related to prostate gland diseases such as BPH and PC in patients. Accordingly many epidemiological and experimental studies advocated that toxic and essential metals in biological substrates may be linked with the risk of prostate gland diseases especially PC [4, 7, 23, 24]. For instance Cd has been suggested to a carcinogen by acting as a catalyst in the production of reactive oxygen species (ROS), escalating lipid peroxidation, disrupts cell signalling and depleting glutathione and protein-bound sulfhydryl groups which in turn promote oxidative stress causes DNA damage [25]. Recent data advocated that exposure of Cd even at low level could lead to DNA fragmentation, microsatellite instability in cells. In addition, exposure to Cd may trigger the release of tumor necrosis factor-alpha, a cytokine linked to a number of cancer risks [26]. Various epidemiological studies concluded a link between exposure to Cd and PC risk/mortality [27]. However the results are often inconsistent. Experimental studies recognized that Cd can induce PC in laboratory animals as well [28]. Concentration of Cd was found significantly elevated in the plasma of PC patients when compared to controls as demonstrated in the recent study [29]. In a meta-analysis study displayed that enhanced exposure to Cd is a risk factor for PC development in occupational settings [30]. In a study conducted 2022 belonged to Serbia, significantly elevated Cd contents were noted in the blood of PC patients when compared to controls [7]. An association between Cd exposure markers in serum and prostate gland disease (BPH & PC) was also observed in patients.

Arsenic is naturally occurring metalloid and epidemiological evidences supports its carcinogenic potential as demonstrated by IARC [31]. Arsenic induced malignancies through oxidative stress by production of ROS leading to genomic aberrations. Epigenetic changes of gene expression via the disruption of DNA methylation patterns, histone modification, and expression of microRNAs [32]. Chronic exposure to As has been related to cancers such as skin, lung, bladder,

kidney, liver, and prostate [31]. Serum concentration of As revealed significantly high in PC patients than the controls [11] and similar findings have were examined in the current study which corroborates the above arguments (Table 2). On contrary, Pizent et al. [7] reported lower concentrations of As in PC patients when compared to controls.

Zinc has a crucial role in signaling pathways such as proliferation, differentiation, apoptosis, cell cycle regulation, and immune functions. As a cofactor of metalloenzymes, Zn involved in cellular activities against oxidative stress, DNA repair, integrity and immunity [33]. Recent scientific literature exhibited that Zn suppresses tumor growth in prostate progression. Nonetheless, it was submitted that Zn deficiency could be a risk factor for PC [34]. Considerable decrease Zn levels were observed in the tissues of PC than the BPH patients and healthy controls [35]. In serum Zn levels were also decreased in PC patients than normal prostate men and BPH as examined in a meta-analysis [36–38]. Alteration occurs in Zn pools from normal to malignant prostate have also been reviewed [39]. Multiple studies have recorded lower Zn levels in the serum of PC cases than the healthy controls [36]. Taken together, these results revealed that the progression of PC was strongly linked to reduction of Zn in malignant cells and the circulating level in plasma [40]. It is evident from the present study that BPH patients or PC may be associated with a reduction in the levels of Zn in the serum of patients. It is documented that long exposure to Cr can cause DNA damage, sister chromatid exchange, chromosomal aberrations, single-stranded and double-stranded DNA breaks [41, 42]. Some epidemiological data confirmed that Cr is capable of epigenetic changes such as suppression of DNA repair and tumor suppressing genes [43]. It was reported that chronic exposure to Cr can induce cell apoptosis in non tumorigenic human prostate cells resulting PC development [44]. However, the exact relationship among Cr level in biological substrates and PC remains relatively unknown. Zhang et al. [45] found that exposure to Cr promoted PC cell growth both in vivo and in vitro, proving the pathogenic effect of Cr as a carcinogenic risk factor which is in agreement with the findings of the present work (Table 2).

Aluminum is a metallic toxicant, causes oxidative stress via ROS produces genotoxic profile, immunologic alterations, pro-inflammatory effect, peptide transformation, apoptosis and lipid peroxidation [46, 47]. Recent reports suggested that chronic exposures to Al involves in cancer, cyst, pancreatitis, anemia and diabetes mellitus [47, 48]. Other scientific date revealed that exposure to Al promoted elevated incidence of BPH and, to a lesser extent, the occurrence of squamous metaplasia and glandular inflammation [49]. In the present study, serum Al was elevated in the patients when compared to healthy subjects. Lead is classified as probable (Group 2A) carcinogen by IARC and scientific literature recognized that exposure to Pb causes DNA damages, chromosomal damages, and tumorigenesis through impairment of DNA repairs system [50]. Many scientific efforts have shown a link between Pb and lung, gastrointestinal and bladder cancers [7, 51, 52]. Epidemiologic studies suggested weak or no associations among exposure to Pb and lung, kidney and brain cancers. Nevertheless, a positive relationship between Pb exposure and PC risk may occur [53]. However the role of Pb in prostate carcinogenesis remains unclear. Elevated concentrations were noted in the blood of PC cancer patients and BPH patients as observed by Guzel et al. [54]. In another study, concentration of Pb in blood revealed lower in PC patients when compared to the controls [7] but Lim et al. [50] did not find a significant link among serum Pb and PC risk. In the serum, significantly elevated Pb levels were found in PC as compared to the controls [55], require further studies for its role in the prostate disease development.

Correlation Study of Metals/Metalloid Levels and PSA

Table 3 showed the correlation coefficient matrix among the metals and metalloid levels in the serum of the BPH patients, PC patients and healthy controls as well as PSA. wherein significant *r*-values are exposed in bold at $p < 0.05$.

The following pairs between the metals revealed positive correlations in the serum of the BPH patients pairs according to the coefficient (*r*) magnitude:

(1) Strong correlations ($0.800 < r > 0.600$): Cd–Se, K–Cu, Co–Al, Li–Sb, Co–Zn, Co–Mn, Cr–Al, Hg–Ca and Se–Sb.
(2) Significant correlations ($0.600 < r > 0.500$): Cr–Ni, Li–Cd, Co–Na, Li–Se, Ni–Co, K–Al, Zn–Cd, Cu–Se, Zn–Sb, Ni–As, Mn–Zn, Na–Hg and Sr–Mg in the serum of BHP patients.
(3) Strong significant positive correlations were pointed out among Pb-PSA and Cd-PSA in the serum of BPH patients. However negative relationship was existed between Sr-PSA as well. The correlation study exposed that toxic metals demonstrated significant positive correlations with elevated serum PSA levels in the patients. A significant inverse correlation was pointed out between Zn and PSA in the serum as shown in Table 4. Significant correlation among Se, Zn, K, Ca with Cd, Co and Hg in the present study pointed out their critical roles in the onset and progression of prostate gland disease, which were advocated by several epidemiological studies [4, 5]. Besides these strong and significant positive correlations among the metals, some metals/metalloid also revealed significant negative correlations with each other; such as Al–Mg, Pb–Sr, Cr–Sb, Pb–Zn, K–Na, Co–K, Sr–Fe and As–Zn. These inverse correlations demonstrated the depletion

Table 4 Correlation coefficient (r) matrix of selected metal(loid)s in the serum of prostate cancer patients

	Al	Sb	Ca	Se	Cd	Fe	Hg	As	Zn	Mn	Na	Li	Cu	Co	Mg	Sr	Ni	K	Cr	Pb	PSA
Al	1.000																				
Sb	**0.523**	1.000																			
Ca	0.188	0.111	1.000																		
Se	0.471	0.370	0.177	1.000																	
Cd	−0.134	−0.193	**−0.514**	0.094	1.000																
Fe	0.070	0.471	0.036	0.304	−0.188	1.000															
Hg	−0.336	**−0.502**	0.063	−0.087	0.168	**−0.513**	1.000														
As	0.032	0.024	0.135	0.017	−0.060	0.087	0.006	1.000													
Zn	0.130	0.143	0.037	0.418	0.223	0.166	−0.043	0.199	1.000												
Mn	0.369	0.353	0.200	**0.621**	0.215	0.043	−0.304	−0.035	0.254	1.000											
Na	0.332	0.336	0.228	0.309	−0.183	0.206	−0.189	−0.027	−0.093	0.304	1.000										
Li	0.288	0.266	0.126	**0.619**	0.085	0.165	0.084	0.053	0.198	0.279	0.244	1.000									
Cu	0.064	0.090	0.086	**0.545**	0.298	−0.135	0.133	−0.031	0.166	0.487	0.044	0.406	1.000								
Co	0.059	0.126	−0.001	0.418	−0.068	0.037	0.116	0.067	0.240	**0.526**	0.310	**0.659**	0.332	1.000							
Mg	0.046	0.126	−0.106	0.095	−0.163	0.185	−0.030	0.018	0.074	0.107	−0.108	−0.186	0.203	0.150	1.000						
Sr	0.453	0.287	0.092	0.432	−0.026	**0.648**	−0.213	**−0.517**	0.265	0.205	**0.506**	0.333	0.087	0.067	**−0.537**	1.000					
Ni	0.278	0.326	0.173	0.539	0.061	0.192	−0.138	−0.009	0.238	**0.554**	0.319	0.350	0.297	**0.504**	0.044	0.307	1.000				
K	0.264	0.188	0.098	0.351	**−0.515**	0.259	−0.149	−0.008	0.289	0.062	0.199	0.142	0.016	**−0.584**	**−0.573**	0.253	−0.143	1.000			
Cr	0.048	0.182	0.013	0.344	0.083	0.145	−0.070	0.203	0.200	0.112	−0.137	0.012	0.180	0.040	0.004	−0.098	0.274	0.144	1.000		
Pb	0.121	−0.014	**0.596**	0.112	−0.267	−0.218	0.006	−0.012	−0.154	0.234	**0.557**	**0.506**	0.013	0.225	−0.338	**−0.507**	**0.586**	0.203	**−0.513**	1.000	
PSA	−0.429	−0.391	−0.215	**−0.525**	**0.522**	−0.104	0.153	−0.089	−0.136	−0.369	−0.079	−0.416	−0.281	−0.212	−0.030	−0.289	−0.201	−0.138	−0.063	**−0.539**	1.000

Bold r-values are significant at $p < 0.05$

of essential elements (K, Zn, Mg) on the expense of toxic metals (Pb, Co, As) which may increase risk of the prostate disease. The remaining elements revealed positive and negative correlation but they were non significant.

For serum samples of PC patients (Table 4), strong significant correlations were observed for Co–Li, Sr–Fe, Mn–Se and Li–Se. Further Sb–Al, Cu–Se, Co–Mn, Ni–Mn, Ni–Co, Pb–Ni, Pb Li, Sr–Na, Cd–Se, Cr–Al, Cu–Ca, Cr–Ca, Cu–Se, Fe–Sb, Mg–Ca, Ni–Co, Pb–Ca, Sr–Ca, Cr–Li, Li–Mn, Mg–Sr, Pb–Mn, Pb–Sr, Mn–Ca, and Mg–Na are noted as significant correlations in the serum of PC patients as noted in Table 4. Strong and significant positive correlations were recorded among Cd and PSA in the serum of PC patients. Significant negative correlations were existed between Se-PSA, Pb-PSA and Al-PSA in the serum of PC patients. Inverse significant relationships were also noted between Hg–Sb, Cd–Ca, K–Cd, Hg–Fe, Cr–Pb, Co–K, Mg–K, Sr–Mg and As–Sr in the serum of PC patients as noted in Table 5. Scientific studies reported the crucial roles of Cd, As, Pb, Ni and Hg in the development of prostate malignancy.

In case of healthy controls (Table 5), significantly strong correlations were noted among Fe–Mg, Li–Fe, Pb–As, Na–K, K–Se, Co–Se, Zn–Se, Na–Se, Li–Se, Cu–Se, Co–Cd, Sr–Se, Cd–Co. Cr–Hg, K–Na, Mn–K, Zn–Ca, Co–Mn, Zn–Sr, Li–Na, Mn–Na, Mn–K and Co–Mn. Strong significant positive correlations were noted among Se-PSA. Some metals pairs (Hg and Al, Ni and Mg, As and Sb, Cu and Mg, and As and Cd, Mg and Ca, Cu and Fe, Mg and Zn) presented significant inverse correlations as manifested by their correlation coefficient values. The correlation study revealed that Cd, Co, Ni and Hg (which are the toxic metals/metalloid) established strong positive correlations with Se, Ca and Na (which are essential metals/metalloid) in the patients but there were no such correlations in the controls. Some non significant positive and negative correlations were also observed among the metals/metalloids which probably revealed the uptake/deplete of metalloids in the serum of subjects. Consequently, unlike the patients, no significant interferences of toxic metals/metalloid were found in the healthy controls. Overall, the correlation score/outcome of the metalloids for the healthy controls remained significantly dissimilar when compared to that for the patients groups, which may be recognized to the imbalances of the trace and essential elements in the prostate diseases patients.

Comparison of the Metals/Metalloid Levels Based on Demographic Characteristics

Comparison of the metals levels based on smoking habits, food habits and habitat in the serum of BPH patients, PC patients and healthy controls are shown in Fig. 1a. In smoking habits based comparison, mean levels of Cd, Se, Hg, Mn, Na and As exposed peculiar behavior by demonstrating elevated concentrations in the serum of smoker BPH patients than smoker healthy controls. However, mean levels of Al, Sb, Fe, Cu, Zn, Cr and Co demonstrated higher contribution in the serum of smoker healthy controls when compared to smoker BPH patients. Ca, K, Sr, Pb and Li displayed comparable concentrations in the serum of BPH patients and healthy controls. Average Cd, Pb, Mg, Co, As and Hg contents were exhibited maximum in the serum of nonsmokers BPH patients as compared to nonsmoker healthy controls while mean levels of Al, Sb, Se, Fe, Zn, Mn, Na, Li, Cu, Sr, Ni, K, Cr and Ca revealed higher in nonsmoker healthy controls. In smoker PC patients, average Mn, Se, Ca, Sb, Al, Co and Mg concentrations revealed higher in the serum of PC patients than smoker healthy controls. Nonetheless, mean levels of Zn, Na, Li, Sr, Ni, Cr and Pb found maximum in the serum of smoker healthy controls. In case of nonsmoker PC patients, average concentrations of As, Hg, Li and Co were higher than nonsmoker healthy controls in the serum. Similarly, Al, K, Sr, Na, Cu, Mn and Sb were increased in concentrations than nonsmoker PC patients. Comparable levels were observed in Pb, Ca, Cr, Ni, Se, Cd, Fe, Mg and Ni of nonsmoker PC patients and healthy controls. The values of PSA were found higher in the serum of patients than healthy controls subjects. However, PSA concentration was raised in smoker PC patients than non smoker PC patients.

Figure 1b depicts the comparison of the average concentrations of the metals in relation to the food habits (vegetarian and non-vegetarian) of the BPH patients and PC patients and controls. The average contents of Al, Ca, Sb, Fe, Cr, K, Mg and Zn in the serum of non-vegetarian PC patients were elevated than non vegetarian healthy control while mean Ni, Sr, Co, Cu, Na, Mn and Hg were found to be highest in non vegetarian healthy controls. Se, Cd, Hg, Li and As were revealed almost comparable in the serum of non-vegetarian PC patients and healthy controls. Mean Al, Sb, Se, Pb, Ni, Li and Co levels were higher in the serum of vegetarian PC patients when compared to vegetarian healthy donors. However, Cd, Fe, Na, Sr, K, Hg, Zn, As, Mn and Cr were increased in vegetarian healthy donors than vegetarian PC patients as shown in Fig. 1b. Mean Cd, Pb, Ni, Hg, Mg, As, Li and Co contents were observed to be higher in the serum of vegetarian BPH patients than vegetarian healthy controls, whereas, average concentrations of Al, Sb, Fe, Mn and Na were noted to be higher in vegetarian healthy controls. In non vegetarian BPH patients, Li, As, Ca, Cd, Mg, Cd and Hg were shown maximum concentrations in the serum than non vegetarian healthy controls. Mean contents of Sb, Ni, Sr, Al, Fe, Cu, Na and Co were noted higher in non vegetarian healthy donors than non vegetarian BPH patients. The contents of

Table 5 Correlation coefficient (r) matrix of selected metal(loid)s in the serum of healthy controls

	Al	Sb	Ca	Se	Cd	Fe	Hg	As	Zn	Mn	Na	Li	Cu	Co	Mg	Sr	Ni	K	Cr	Pb	PSA
Al	1.000																				
Sb	0.404	1.000																			
Ca	0.169	0.414	1.000																		
Se	0.460	0.554	0.404	1.000																	
Cd	−0.020	0.098	0.302	0.201	1.000																
Fe	0.150	0.471	0.084	0.316	−0.085	1.000															
Hg	**−0.576**	−0.067	0.084	−0.011	0.038	−0.408	1.000														
As	0.017	**−0.577**	−0.303	−0.138	**−0.535**	0.120	−0.170	1.000													
Zn	0.104	0.138	**0.564**	**0.599**	−0.031	−0.036	0.171	0.274	1.000												
Mn	0.090	0.181	0.413	0.372	0.239	−0.027	0.070	−0.057	0.354	1.000											
Na	0.174	0.328	0.275	**0.530**	0.189	0.191	−0.027	−0.019	0.052	0.149	1.000										
Li	0.387	0.348	−0.009	**0.550**	0.016	**0.676**	−0.169	−0.121	0.237	0.063	**0.663**	1.000									
Cu	0.159	0.067	**0.521**	**0.526**	0.074	**−0.522**	0.324	−0.207	0.367	0.358	0.204	0.387	1.000								
Co	0.219	0.439	0.217	**0.610**	**0.558**	0.178	−0.055	−0.037	0.201	**0.503**	0.386	0.356	0.088	1.000							
Mg	0.205	0.213	**−0.571**	−0.096	−0.408	**0.677**	−0.139	0.016	**−0.597**	−0.159	−0.231	−0.119	**−0.548**	0.018	1.000						
Sr	0.213	**0.527**	0.202	**0.575**	0.342	0.193	−0.041	−0.123	**0.578**	0.245	0.354	0.349	0.356	0.217	−0.301	1.000					
Ni	0.272	0.396	0.227	0.468	0.030	0.232	−0.178	0.052	0.093	0.100	0.274	0.335	0.219	0.373	**−0.563**	0.304	1.000				
K	0.125	0.134	0.248	**0.630**	−0.145	0.230	−0.068	−0.053	0.286	**0.533**	**0.572**	0.230	0.267	0.047	0.150	0.060	−0.058	1.000			
Cr	0.188	0.140	−0.112	0.278	−0.047	0.151	**0.549**	0.200	0.178	0.052	−0.070	0.047	0.197	0.029	−0.031	0.039	0.300	0.216	1.000		
Pb	0.006	−0.076	0.092	0.153	−0.014	0.074	−0.210	**0.663**	**−0.648**	0.028	0.487	0.107	**−0.748**	0.199	−0.294	0.220	**0.697**	**0.629**	0.146	1.000	
PSA	−0.160	0.187	0.163	**0.529**	0.117	0.054	0.126	−0.070	0.193	0.211	0.093	0.048	−0.045	0.113	−0.078	0.195	0.057	0.158	0.120	0.143	1.000

Bold r-values are significant at $p < 0.05$

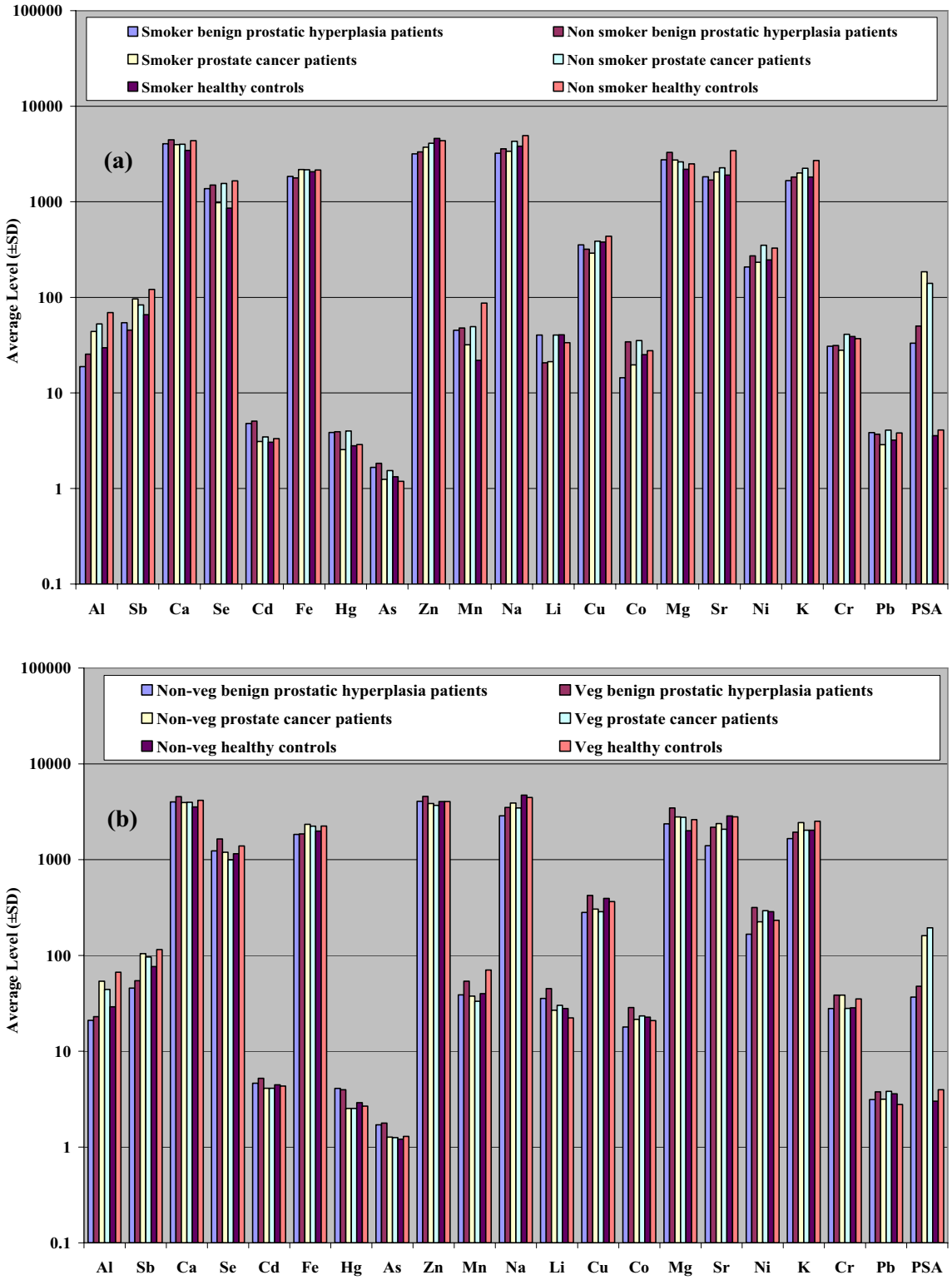

Fig. 1 Comparative mean metals/metalloid levels (μg/dL, ± SE) and PSA (ng/mL) levels in serum of benign prostatic hyperplasia patients, prostate cancer patients and healthy controls based on **a** smoking habits, **b** food habits and **c** habitat

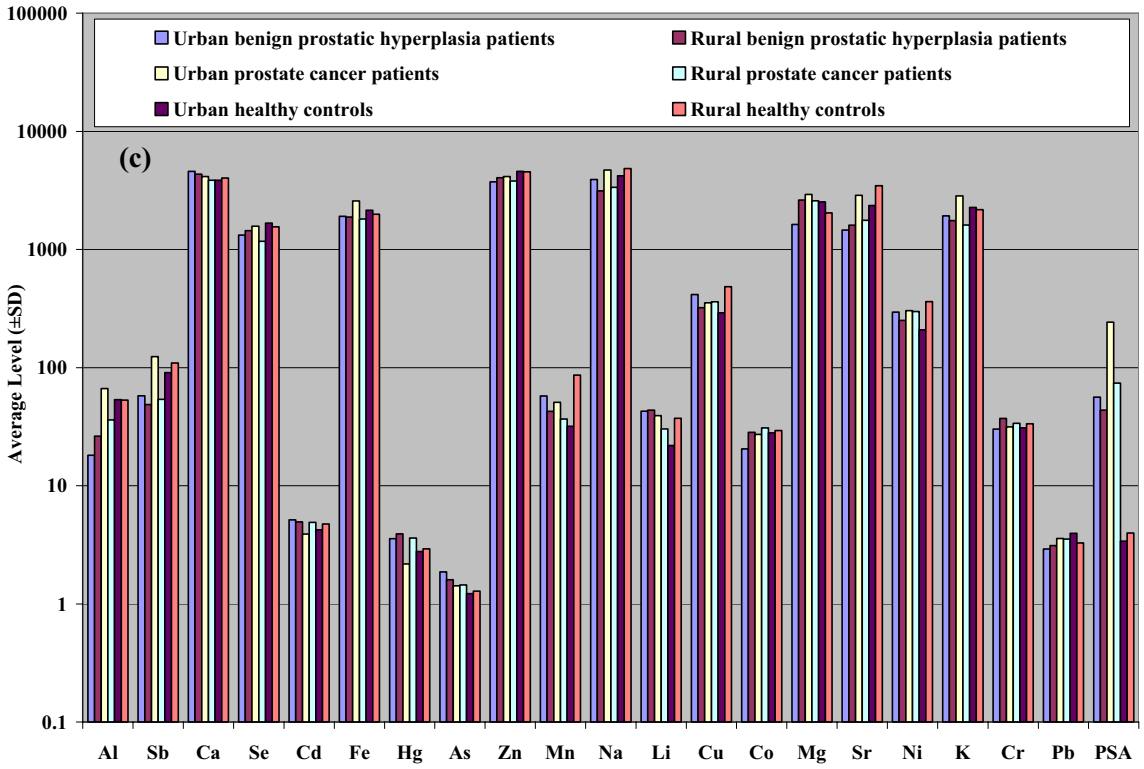

Fig. 1 (continued)

PSA revealed maximum in patients with vegetarian habits when compared with non vegetarian patients. In serum, cancerous patients possessed higher levels of PSA than BPH patients followed by healthy controls (vegetarian & non vegetarian).

Habitat-based comparison of metals levels as showed in Fig. 1c exhibited comparatively higher average concentrations of Pb, Ni, Mg, Co, Cu, Li, Mn, Hg, As, Cd and Sb in the urban BPH patients than counterparts' urban healthy controls which revealed elevated levels of K, Al, Mg, Se, Fe, Zn, Sr, Co and Pb in their serum. Nevertheless, mean contents of Sb, Fe, K, Ni, Sr, Ma, Li, Cu and Mg were found to be high in urban cancer patients when compared to urban healthy controls. In the case of rural healthy controls, mean K, Al, Sb, Se, Zn, Mn, Na, Li, Cu, Sr and Ni levels were found to be higher in the serum than rural PC patients whereas Mg, As and Hg contents were revealed higher. Average As, Mn, Na, Li, Cu, Mg, Sr, K and Ni contents were found to be higher in the serum of urban PC patients compared to urban healthy controls. Similarly mean concentrations of Al, Sb, Se, Fe, Hg, Zn and Pb were displayed higher concentrations in the serum of urban healthy controls as shown in Fig. Ic. Urban (BPH and PC) patients exhibited higher PSA levels in the serum than the rural (BPH and PC) patients. Overall, the concentrations of PSA showed higher in the serum of patients than healthy controls.

Comparison of the Metals/metalloid Levels Based on Types/Stages of Prostate Cancer Patients

Comparative evaluation of the mean metals levels and PSA values in the serum of various types (small cell prostate, transitional cell, squamous cell and adenocarcinoma) of the PC showed in Fig. 2a. Small cell prostate cancer patients displayed relatively higher concentrations of Sb, Se, Sr and K while the value of PSA found highest. In transitional cell carcinoma, toxic metals like Pb, Cr, Ni, Al and Mn exhibited significant contributions in the serum and essential metals showed somewhat less contributions. Mean elevated levels of As, Mg, Cu, Ca rather lower levels were exhibited in the serum of squamous cell carcinoma patients. The PSA value found lowest in squamous cell carcinoma patients as compared to rest of the types of PC in patients. In case of adenocarcinoma, mean Cd, Zn Hg, Li and Co revealed highest contribution in the serum of PC patients as shown in Fig. 2a.

Based on stages of prostate cancer (Fig. 2b), relatively higher levels of Al, Sb, Se, Cd, Fe and Na were exhibited in the serum of patients at stage-I. Elevated levels of Cr, Co and Pb revealed maximum at stage-II in the serum of PC patients. At stage-III of PC, mean As, Ni, Sr and K contents were found relatively higher in the patients serum. Hg, Li, Mn, Zn concentrations exhibited higher in the serum of PC patients as compared to other stages of PC. Average Hg, Li,

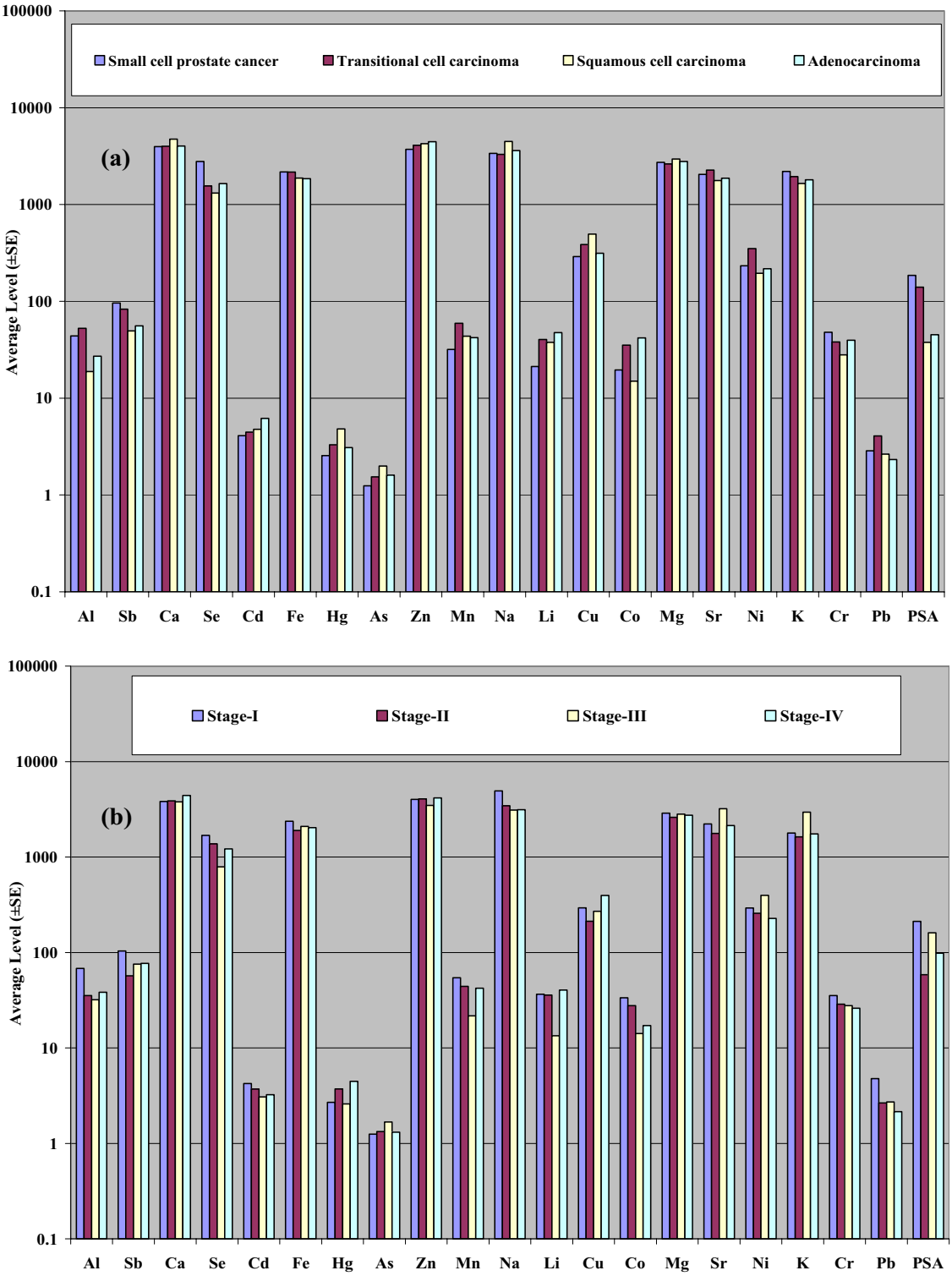

Fig. 2 Comparative mean metas/metalloid (μg/dL, ± SE) levels and PSA (ng/mL) levels in serum of prostate cancer patients at various **a** types, **b** stages

Ca and Cu contents revealed higher in the serum at stage-IV of PC patients (Fig. 4). Similarly average concentration of PSA was found maximum at stage-I in the serum of PC patients. The decreasing trend of the PSA in the serum of PC patients at stage levels: stage-I > stage-III > stage-IV > stage-II as shown in Fig. 2b.

Multivariate Analysis

Interrelationships between the metals and PSA in the serum of the BPH patients, PC patients and healthy controls were determined by multivariate statistical methods. Principal component analysis (PCA) of the metals/metalloid and PSA levels in the serum of BPH patients extracted by using varimax normalised rotation on the data-set (which are given in Table S2-supplementary material). Seven PCs comprised of more than 75% of the cumulative variance of the data were achieved, whereas eigenvalues greater than 1. Figure 3 portrayed the corresponding CA in the form of a dendrogram. PC 1 is loaded by Cu, Sr and Li with a cluster of these metal(loid)s is existed in CA. PC2 has Ca and Cd loadings. These metals/metalloid were strongly supported by a combined cluster in CA suggesting the nutritional intake/dietary sources and environmental pollution of the subjects. PC3 contained greater amount of Se, Mn, Ni and PSA loadings, while Na and Pb unveiled maximum loadings in PC4. These results were verified by CA. This PC pointed out the interference of toxic element (Ni) with the PSA in the BPH patients. The fifth and sixth PCs comprised of Hg, Mg and As, & Cr, K and Zn loadings respectively, indicating anthropogenic activities and contamination of biological segments in the environment. Last PC revealed highest loading for Fe, Co, Sb and Al with similar cluster also presented in the CA as displayed in Fig. 3.

In the case of PC patients, PCA of the metals data yielded seven PCs with eigenvalue greater than 1, commutatively elucidation approximately 72% of the total variance of data as shown in S2 (supplementary materials). The CA of metal(loid)s data pertaining to the PC patients is exposed in Fig. 4. PC 1 disclosed increased loading for Li, Se, Cu and Cd. A similar cluster of these metals presented in CA. PC 2 consisted of maximum loadings for Al, Sb and Ca with a parallel cluster of the elements in CA. PC 3 indicated raised loading for Ni, Co and Mn along with a similar cluster in CA. These three PCs of metals were contributed by the nutritional sources and environmental contamination by anthropogenic sources. Mg, Cr and As exhibited elevated loadings with PC 4 while PC 5 indicated higher loadings of Hg and PSA which also unveiled common cluster in CA. This PC revealed the interference of toxic metalloid (Hg) with the PSA in the PC patients and they were believed to be mainly contributed by anthropogenic sources. Na and Pb displayed elevated loadings in PC 6 while last PC consisted of K, Zn, Sr and Fe with significant clusters showed also in CA. The former PC also directed the interference of toxic metal (Pb) with essential metal (Na) which were mostly associated with anthropogenic sources and food sources as shown in Fig. 4.

In the case of healthy controls, seven PCs were yielded with eigenvalue greater than one which explained more than 71% of the total variance of data as publicized in S2 (supplementary materials). The CA based on ward's

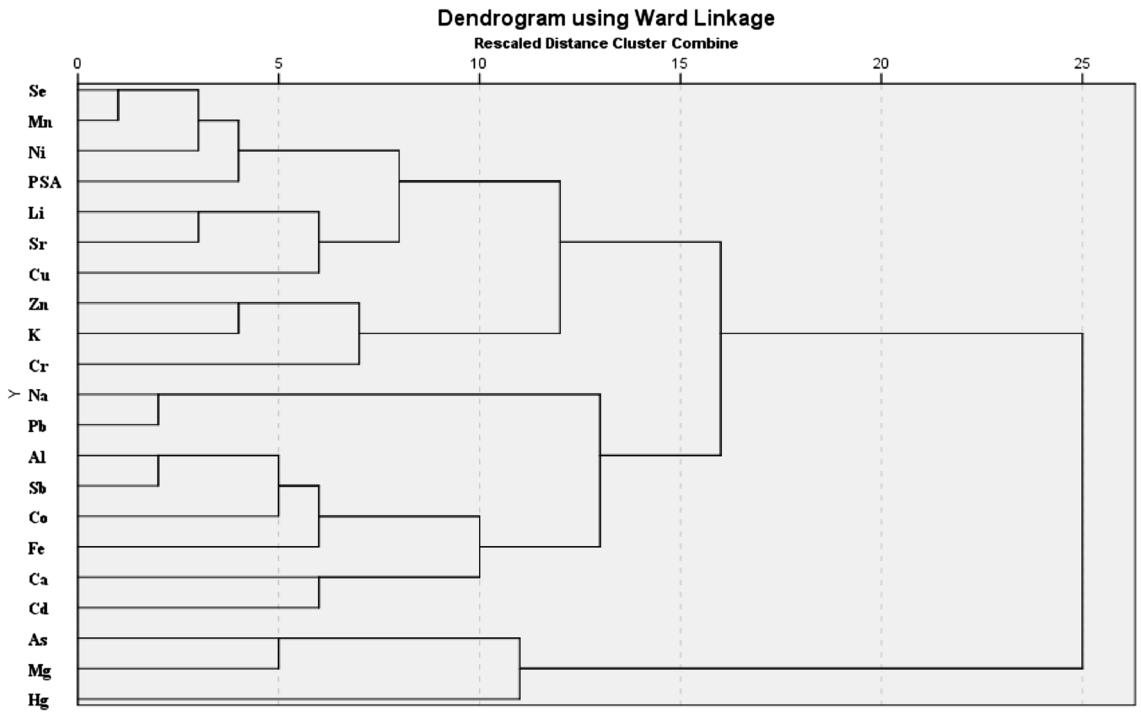

Fig. 3 Cluster analysis of metals/metalloid levels in the serum of benign prostatic hyperplasia patients

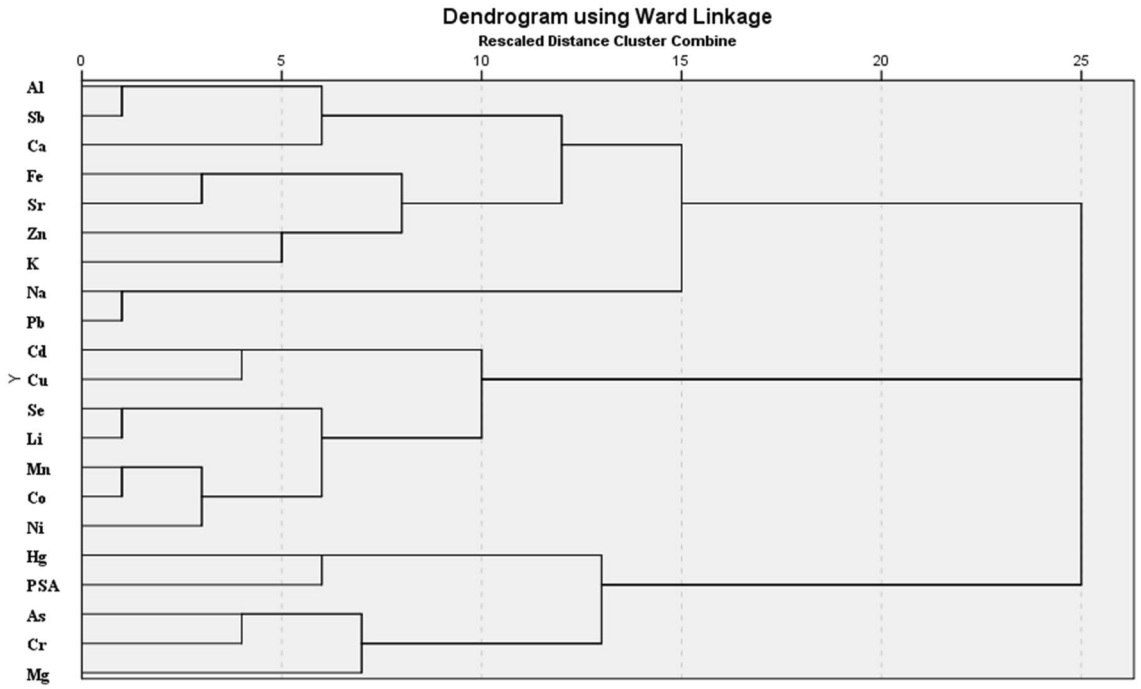

Fig. 4 Cluster analysis of metals/metalloid levels in the serum of prostate cancer patients

method of metals data concerning to the healthy controls is exposed in Fig. 5. The CA of the metals data in the serum of healthy controls demonstrated strong clusters of Mg–Sr–Cr–Ca–Co, K–Pb, Al–Fe–Zn, Se–Li, Hg–Ni, Sb–Cd–Mn and As–Cu–Na–PSA. PC1 directed elevated loadings for Sr, Mg, Cr, Ca and Co supported by a cluster of

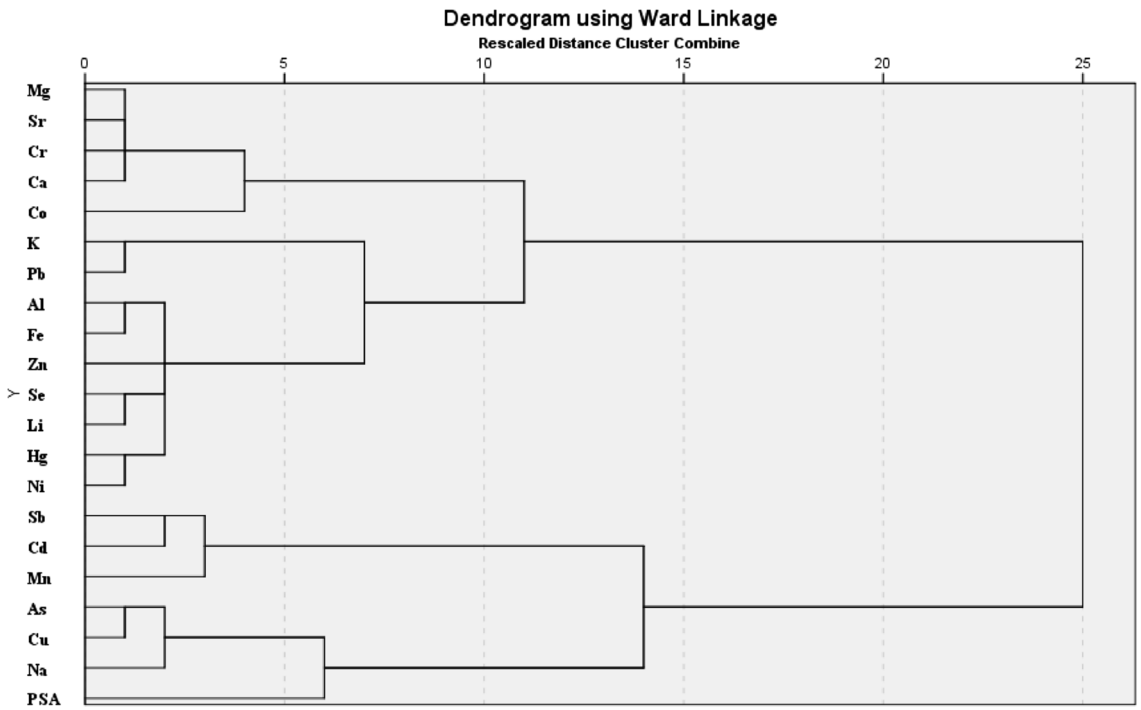

Fig. 5 Cluster analysis of metals/metalloid levels in the serum of healthy controls

metals in CA. These elements were mainly concerned with the dietary habits and also regulated by internal body metabolism. PC 2 presented higher loadings for As, Cu, Na and PSA with a similar cluster is displayed in CA. PC 3 for the serum samples of healthy controls designated higher loading for Mn, Cd and Sb along with a similar cluster in CA. PC 4 and PC 5 showed significant loadings of Ni & Hg and Li & Se, respectively as displayed in S2. The common sources of these metals were anthropogenic contaminations and environmental pollutants. Al, Fe and Zn presented higher loadings in PC 6. These elements showed a common cluster in CA. Last PC presented maximum loadings of Pb and K. These metalloids were traced with mixed sources i.e., traffic emissions and internal body metabolism. Overall, the multivariate apportionment (PCA & CA) of the selected metals in the serum of BPH patients and PC patients revealed considerable interventions of the toxic metals with the essential elements; it was significantly divergent compared with the healthy controls.

The relatively small sample size of the subjects diminished statistical power of analyses. We did not observe the occupational exposure of metals and the metalloid to the subjects. Another limitation was that there was lacked follow-up information (i.e., family history) for some men of prostate disease. Exposures to metal(loid)s may have altered over time which may provide a different study findings than the ones found in the present study. Individuals (small number) with metals levels that were below the limits of detection (LOD) were not included in the present data was also a limitation. A major strength of the present study is that exposure of metal(loid)s were quantified at various stages and types of the PC, which have received little attention before. Comparatively large number of metals and metalloid were quantified in the serum of BPH patients, PC patients and healthy controls and also measured PSA to check whether there were any associations among these variables established or not. Nevertheless, more studies are still needed to explore more essential/toxic metals with large masses (various Nations) and find the risk factors and preventive methods of prostate gland disease development.

Conclusions

In conclusion, the comparison of metals/metalloid levels revealed significant disruption in selected metals balances in BPH patients, PC patients and the healthy controls. Mean Al, Cd, Cr, Na, Pb, Ni and K were found significantly higher in the serum of BPH patients compared with healthy controls, while average levels of Sb, Al, Cd, As, Mn, Sr, K, Pb and PSA were significantly elevated in the serum of PC patients than healthy controls represents the variation in body metals homeostasis. These metals imbalances may be a triggering factor for the development of prostate gland diseases. The correlation study reveals appreciably diverse associations of the metals and metalloid in serum samples of the all donor groups. Moreover, significant correlations were detected between toxic elements (Pb, Cd and Al) and PSA in the serum of patients. The mean concentrations of the majority of the metals demonstrated significant variations based on demographic characteristics of the subjects in all donor groups. Multivariate CA in the serum of metals of BPH patients, PC patients and healthy subjects based on the metals and metalloid levels revealed clearly separate groupings for the patients and healthy controls, while multivariate PCA revealed significantly dissimilar grouping of the metals/metalloid in the patients and controls. Strong significant correlations were pointed out among Cd and PSA levels in the serum of BPH patients and in PC patients. The results of this study provided guidelines to the other researchers investigating the role of metals/metalloid in the prostate gland disease. Among types, small cell prostate cancer patients displayed higher concentrations of Sb, Se, Sr and K while the value of PSA found highest. In the case of transitional cell carcinoma, Pb, Cr, Ni, Al and Mn were found maximum. Mean elevated levels of As, Mg, Cu, Ca was exhibited in the serum of squamous cell carcinoma patients. The PSA value found lowest in squamous cell carcinoma patients. Average Cd, Zn Hg, Li and Co revealed highest contribution in the serum of adenocarcinoma patients. Based on various stages of PC patients, higher levels of Al, Sb, Se, Cd, Fe, Na and PSA were exhibited at stage I while levels of Cr, Co and Pb revealed maximum at stage II. At stage III, As, Ni, Sr and K was found higher & Hg, Li, Ca and Cu concentrations exhibited higher at stage IV. These findings support the hypothesis that toxic elements contribute to the pathogenesis of prostate gland diseases. To come to this point, further studies will help to clarify weather along clinical diagnosis; serum metals/metalloids levels are useful in the differential diagnosis of BPH disease patients and prostate malignancy patients.

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Author Contributions The manuscript was written with the contributions of all authors. All authors have agreed and approved the final version of the manuscript. MHRM, MAQ, and TF studied conception and designed: AI and SI were involved in providing samples with clinical guidance. NH and MAQ were involved in the analysis of the data. Critical revision of manuscript was completed by MHRM and TF.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ethical Review Committee, PINUM Faisalabad & Allied Hospital, Faisalabad Medical University, Faisalabad-Pakistan Ref. No. UEFC/2022/R437 and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all patients and controls for being included in the present research work. All the participants agreed voluntary in the present study.

Consent to Publish All authors have read and approved the final submitted manuscript. We certify that this manuscript is original and not previously published in any form including on preprint servers, nor is it being considered elsewhere.

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