Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2415-2420

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19994

A Proportional Evaluation Based on Gender Dependence of Selected Elements in Hepatitis C Patients and Healthy Donors in Pakistan

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Received: 26 March 2016; *Accepted*: 27 June 2016; *Published online*: 10 August 2016; AJC-18009

The concentrations of selected elements (Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Na and Ni) in the blood samples of chronic hepatitis C patients (men and women) and healthy donors were determined. The biological samples were determined by using flame atomic absorption under optimum spectrophotometric analytic conditions. In the blood samples of men hepatitis C virus patients, the dominant mean levels (mg/L) were found for Na, Ca, Fe and K, followed by Cr, Cd, Co, Cu, Ni and Mn. In women hepatitis C virus patients the higher concentrations were noted for Na, Fe, Ca and K, while Cu, Cd, Ni, Cr, Mn and Co were followed. In healthy subjects the relatively higher concentrations were observed for Ca, Na, Fe and K followed by very low value for Mn, Ni, Cu, Cr, Cd and Co. The data on element to element correlations in the blood samples of hepatitis C virus patients and healthy subjects exhibited significant variation among selected elements. The multivariate cluster analysis of the selected trace metals in the blood samples of hepatitis C virus patients and controls evidenced a marked disproportion, manifesting the imbalance in the essential elements (Ca, K, Fe and Na) by the toxic elements (Cd, Cr, Co and Ni). The distribution and correlation of trace elements in the blood samples of hepatitis C virus patients (men and women) revealed notable variations in comparison with healthy donors.

Keywords: Elements, Hepatitis C, Multivariate cluster analysis, Flame atomic absorption.

INTRODUCTION

A number of evidences from the recent research indicate that many trace elements play vital role in biological processes through nerve conduction and muscle contraction there by affecting the permeability of cell membranes and inhibiting or promoting enzymatic reactions [1]. A balance between concentration of trace elements and other nutrients is necessary for a healthy life [2]. An imbalanced diet of these elements causes disease. Deficiency or elevated level of these metals may have adverse effects on body [3-5]. Recently, trace elements in human body have been used as a tool for diagnosing various diseases including hepatitis [6,7]. Hepatitis B and C lead to chronic liver disease and together cause liver cancer and cirrhosis [8].

Trace elements also have some essential role in metabolic activity of liver like enzyme activation, formation of proteins, antioxidant protection, immune function and repairing of tissues after viral damage [9]. During immune response against hepatitis some oxidants are produced which cause heptocellular carcinoma. So, a change in concentration of trace elements results in oxidative stress which is one of most important factors for hepatitis disease [10-14].

For determination of trace elements in biological research, blood is an important specimen used at larger scale. As transportation and metabolism of trace elements occur through blood, it provides exact concentration of these elements. For determination of trace metals level of a person, whole blood is a proper sample. The concentration of these elements can be used as a marker for identification of changes in environment or in human body [15-18].

The main purpose of this study was to determine the concentration of some trace elements in blood of hepatitis C virus patients and healthy volunteers of same age and group in Bhimber zone to assess the possible impact on the metabolic pathway of the patients. A comparison between level of these metals in women, men hepatitis C virus patients and in healthy volunteers has also been made.

EXPERIMENTAL

A total of 25 men and 25 women suffering from hepatitis C virus were included in this study. The sample collection was carried out from patients admitted in DHQ Bhimber AJK and Al-Rehman Children Hospital Bhimber on volunteer basis. The healthy subjects were randomly selected with comparable, age, sex and environment conditions.

The information related to their age, sex, health status, food habits and environmental conditions were also collected. Generally, the relatives of patients were selected as controls to keep similarity between their food habits and socio-economic status. Venous blood about 3 to 5 mL was ejected with the help of pre-cleaned syringe and then transferred to the evacuated poly-ethylene tubes. The samples were stored at -20 °C for analysis [19,20]. For digestion blood samples were transferred to digestive flask from storage tube. Then, 10 mL of nitric acid was added in it and after 5 min, 10 mL of perchloric acid were added. Then, screw capped flasks were kept for 15 min at room temperature. The blood samples were placed in microwave oven at 500 watt for 5 min. Finally, a room temperature cooling was done. This process was repeated thrice, thus a total 15 min were given to samples in microwave oven. Same procedure was repeated for blank sample. The digested sample contents were transferred to volumetric flask and desired volume was made [21,22]. For metal analysis, Perkin Elmer analyst 800 atomic absorption spectrometer was used (Table-1).

All reagents procured from E-Merck Germany having ultrahigh purity were used such as, nitric acid (65 %) and perchloric acid (70 %). Fresh working solutions were prepared just before the analysis and apparatus was washed with detergent solution (5 % v/v), then soaked in 5 % HNO_3 solution for 24 h and rinsed with distilled water and then dried before use [23]. The multivariate cluster analysis (CA) of the selected essential and toxic metals in the biological samples of the healthy donors based on complete-linkage method was also carried out [24,25].

RESULTS AND DISCUSSION

The basic statistical parameters for the distribution of selected trace elements levels in the blood samples of men hepatitis C virus patients (mg/L) are shown in the Table-2. Dominant mean levels were found for Na, Ca, Fe and K. A relative lower level of Cr, Cd, Co, Cu, Ni and Mn was followed. On the average basis, the decreasing trend of element levels in the blood of men infected with hepatitis C revealed following order: Na > Ca > Fe > K > Cr > Cd > Co > Cu > Mn > Ni. Some of the elements exhibited appreciable randomness in their distribution pattern as manifested by large standard error. Relatively large spread of the concentration was noted in case of Fe, K and Ca. A few elements like Cd, Cr, Co, Cu, Mn and Ni exhibited moderately normal distribution pattern supported by low standard error values. Comparatively low skewness values for Mn, Na, Cr, Cd and Co showed their symmetric distribution.

In case of women hepatitis C virus patients, the most of the trace elements exhibited variation in their concentration (mg/L) as shown by range values in Table-3. On the average, predominantly higher concentrations were noted for Na, Fe, Ca and K, while Cu, Cd, Ni, Cr, Mn and Co was estimated at the low concentration level. The trace metals in the blood of women hepatitis C patients showed following order in their mean concentration: Na > Fe > Ca > K > Cd > Cu > Ni > Cr > Mn > Co. Na, Fe, K and Ca exhibited randomness as revealed by their higher standard error values. While Cd, Co, Cr, Cu, Mn and Ni exhibited rather normal distribution as shown by lower standard deviation and standard error values.

In the blood samples of healthy subjects, relatively higher concentrations (mg/L) for Ca, Na, Fe and K followed by very low value for Mn, Ni, Cu, Cr, Cd and Co were observed (Table-4). The decreasing trend of mean element levels in the blood of healthy subjects revealed following pattern: Ca > Na > Fe > K > Mn > Ni > Cu > Cr > Cd > Co. Gaussian distribution of Na, Mn, Cu, Co, Cr and Cd was shown by the lower standard deviation and standard error values. A balanced distribution of elements like Ca, Cd, Co, Cu, Fe, K, Mn and Ni was shown by lower skewness values.

TABLE-1
INSTRUMENTAL CONDITIONS FOR METAL DETERMINATION BY FLAME ATOMIC SPECTROMETER

Parameters	Ca	Cd	Co	Cr	Cu	Fe	K	Mn	Na	Ni
Wavelength (nm)	422.7	228.8	240.7	357.9	324.8	284.3	766.5	279.5	589.0	232.0
Slit width (nm)	0.5	0.5	0.2	0.5	0.5	0.2	0.5	0.4	0.5	0.2
Support	Air	–	–	–	–	–	–	–	–	–
Fuel	Acetylene	–	–	–	–	–	–	–	–	–
Lamp current (mA)	10	4	7	5	4	5	4	5	10	4
Measurement time (s)	3									
Replicate number	3									

TABLE-2
STATISTICAL DISTRIBUTION PARAMETERS FOR THE SELECTED ELEMENT CONCENTRATIONS IN THE BLOOD SAMPLES OF MEN HEPATITIS C PATIENTS (mg/L)

Parameters	Range	Mean	Median	Standard deviation	Standard error	Skew
Ca	20.40-40.20	32.92	33.56	6.690	1.73	-0.03
Cd	0.122-0.523	0.280	0.230	0.140	0.04	0.59
Co	0.110-0.339	0.210	0.230	0.080	0.02	0.03
Cr	0.073-0.920	0.450	0.430	0.280	0.07	0.23
Cu	0.062-0.272	0.180	0.230	0.090	0.02	-0.078
Fe	11.83-32.10	26.57	28.21	7.470	1.93	-0.50
K	14.12-28.42	22.24	20.56	7.410	1.91	2.41
Mn	0.001-0.029	0.015	0.010	0.010	0.00	0.30
Na	20.00-62.40	41.22	39.78	12.37	3.19	0.10
Ni	0.010-0.029	0.010	0.020	0.010	0.00	-0.52

TABLE-3
STATISTICAL DISTRIBUTION PARAMETERS FOR THE SELECTED ELEMENT CONCENTRATIONS IN THE BLOOD SAMPLES OF WOMEN HEPATITIS C PATIENTS (mg/L)

Parameters	Range	Mean	Median	Standard deviation	Standard error	Skew
Ca	16.42-34.60	25.67	26.08	5.77	1.49	-0.02
Cd	0.363-0.876	0.559	0.53	0.25	0.07	-0.01
Co	0.002-0.046	0.034	0.03	0.01	0.00	-0.76
Cr	0.069-0.127	0.098	0.09	0.02	0.00	1.15
Cu	0.072-0.270	0.151	0.14	0.08	0.02	0.08
Fe	23.78-44.80	34.65	34.26	6.69	1.80	0.05
K	15.66-30.90	24.72	23.89	6.50	1.68	0.18
Mn	0.027-0.140	0.090	0.08	0.03	0.01	-0.15
Na	20.10-60.20	39.19	35.56	13.41	3.46	0.30
Ni	0.047-0.160	0.103	0.11	0.05	0.01	-0.87

TABLE-4
STATISTICAL DISTRIBUTION PARAMETERS FOR THE SELECTED ELEMENT CONCENTRATIONS IN THE BLOOD SAMPLES OF HEALTHY SUBJECTS (mg/L)

Parameters	Range	Mean	Median	Standard deviation	Standard error	Skew
Ca	40.50-152.0	83.58	72.45	32.53	8.40	0.87
Cd	0.042-0.122	0.079	0.07	0.03	0.01	-0.63
Co	0.047-0.092	0.072	0.07	0.02	0.00	-0.14
Cr	0.010-0.242	0.099	0.07	0.07	0.02	1.06
Cu	0.048-0.680	0.324	0.30	0.22	0.06	0.30
Fe	36.00-47.28	41.80	41.86	3.43	0.88	0.01
K	12.80-25.66	18.74	18.65	4.21	1.09	0.15
Mn	0.089-0.860	0.439	0.45	0.24	0.06	0.17
Na	21.60-65.20	42.64	40.01	13.17	3.40	0.25
Ni	0.076-0.688	0.338	0.28	0.26	0.07	0.94

The mean values of Ca, Cr, Co, Cu and Na were found to be higher in the blood samples of men hepatitis C patients, while the average levels of Fe, K, Mn, Ni and Cd were higher in the blood samples of women hepatitis C patients (Fig. 1). Some of the elements like Ca, Fe and Na exhibited higher concentration levels in blood samples of controls (Fig. 2). However, Cd showed higher levels in the blood samples of women hepatitis C patients. Cobalt and chromium were significantly higher in men hepatitis C patients. The comparative study thus signified the role of specific elements in each class of the patients.

The data on element-to-element correlations in the blood samples of hepatitis C patients (men) Table-5, wherein the significant r-values are shown below at $p < 0.05$. In male hepatitis C virus patients, the positive strong correlations were observed between Fe-Cu (r = 0.546), Mn-Cr (r = 0.372), Ni-Mn (r = 0.363), Na-Ca (r = 0.324), Na-Mn (r = 0.309),

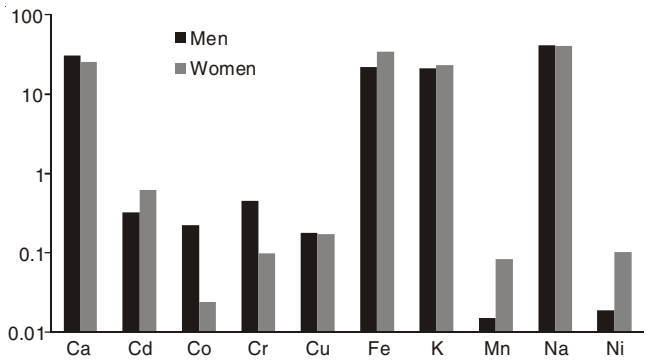

Fig. 1. Comparative evaluation of the average levels of the selected elements in the blood samples of men hepatitis C patients and women hepatitis C patients

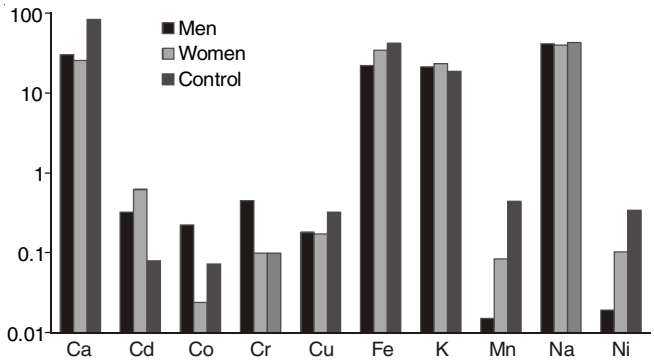

Fig. 2. Comparative evaluation of the average levels of the selected elements in the blood samples of men hepatitis C patients, women hepatitis C patients and healthy donors

Cd-Ca (r = 0.317), Ni-Na (r = 0.303), Mn-K (r = 0.277), Ni-K (r = 0.274), Na-Fe (r = 0.265), Cu-Ca (r = 0.254), K-Cr (r = 0.213), Ni-C0 (r = 0.195), Cu-Cd (r = 0.138), Ni-Cd (r = 0.167), Mn-Fe (r = 0.165), Na-Cd (r = 0.150), Na-Cu (r = 0.137), Na-K (r = 0.134), Ni-Fe (r = 0.129), Fe-Cd (r = 0.093) indicating their probable communal variation in the blood samples of men patients. The elements like K-Ca (r = -0.640), Co-Cd (r = -0.444), Mn-Ca (r = -0.427), K-Ca (r = -0.432), Cu-Cr (r = -0.389), K-Fe (r = -0.330), Cr-Cd (r = -0.329), Cu-Co (r = -0.270), Fe-Co (r = -0.262), Mn-Cd (r = -0.279), Na-Cr (r = -0.204), Ni-Cu (r = -0.234), Co-Ca (r = -0.198), Mn-Co (r = -0.139) and Ni-Ca (r = -0.142) revealed inverse relationship manifested by significantly negative correlation which showed the depletion or enrichment of specific elements at the cost of other.

The correlation study of blood samples of women hepatitis C patients (Table-6), revealed that very strong correlations were

TABLE-5
CORRELATION COEFFICIENT MATRIX OF SELECTED ELEMENTS IN THE BLOOD OF MEN HEPATITIS C PATIENTS

	Ca	Cd	Co	Cr	Cu	Fe	K	Mn	Na
Cd	0.317								
Co	-0.198	-0.444							
Cr	-0.058	-0.329	-0.003						
Cu	0.254	0.138	-0.270	-0.389					
Fe	-0.008	0.093	-0.262	**-0.599**	**0.546**				
K	-0.432	-0.198	-0.070	0.213	**-0.640**	-0.330			
Mn	-0.427	-0.279	-0.139	0.372	-0.003	0.165	0.277		
Na	0.324	0.150	-0.065	-0.204	0.137	0.265	0.134	0.309	
Ni	-0.142	0.167	0.195	-0.027	-0.234	0.129	0.274	0.363	0.303

noted between following elements pairs; Ni-Mn (r = 0.568), Mn-Cr (r = 0.546), Mn-Cu (r = 0.488), Mn-Cd (r = 0.456), K-Cu (r = 0.402), Na-Fe (r = 0.382), Mn-Co (r = 0.379), Ni-Cd (r = 0.329), Ni-Cu (r = 0.366), Co-Ca (r = 0.214), Cu-Cd (r = 0.241), Cu-Cr (r = 0.230), Fe-Co (r = 0.234), Mn-K (r = 0.180), Cu-Co (r = 0.168),Ni-Co (r = 0.138). The most important finding of the correlation study was the strong correlation between Mn-Cr and Ni-Mn pairs.

The correlation study was also applied on the elemental data of healthy donors blood (Table-7), to find out the possible similarities and differences. The following pair of elements exposed strong positive significant correlation Fe-Cr (r = 0.503) and Ni-Fe (r = 0.431). The week positive correlations were observed between Cd-Ca (r = 0.117), Cr-Ca (r = 0.365), Cr-Cd (r = 0.212), Fe-Ca (r = 0.051), Ni-Ca (r = 0.307) Ni-Co (0.315), K-Cu (r = 0.254), K-Fe (0.280) and Cr-Co (r = 0.131). The elements pairs like Cu-Cd (r = -0.301), Mn-Co (r = -0.117), Na-Cr (r = -0.415) and Na-Fe (r = -0.376) showed significant negative correlation, while other metals pairs exhibited either weak positive or negative relationship.

The multivariate cluster analysis (CA) of the selected essential and toxic metals in the blood samples of hepatitis C patients (men and women) and controls based on complete-linkage method is shown in Figs. 3-5. Four clusters of the metals were obtained in blood samples of the men hepatitis patients. The first cluster was composed of Ca-K-Na; the second was consisting of Co-Cu-Cr, while third cluster was constituted by Mn-Fe, whereas last cluster was represented by Ni-Cd. Four clusters of the selected elements were also identified in blood samples of the women hepatitis patients. A very strong cluster among Ca-Fe-Na, another prominent cluster consisted of Cd-Mn-Ni; third cluster including Cu-K and the fourth cluster was represented by Co-Cr.

In case of healthy donors three major clusters of the selected metals were observed. The first cluster was composed of Ca-Na-Cd; the second was consisting of Cu-Fe-Mn, while last cluster was constituted by Co-Cr-K-Ni. The cluster analysis includes total heterogeneity of every possible cluster formed by the linking two existing clusters. Therefore, mutual relationship of various trace elements is predicted.

The concentrations of selected elements in the blood samples showed statistical alterations in viral hepatitis C patients as compared to healthy subjects.

The present study evidenced that Co and Cr were significantly higher in the men hepatitis C virus patients as compared to women patients, thus indicating the higher exposure of these

TABLE-6
CORRELATION COEFFICIENT MATRIX OF SELECTED ELEMENTS IN THE BLOOD OF WOMEN HEPATITIS C PATIENTS

	Ca	Cd	Co	Cr	Cu	Fe	K	Mn	Na
Cd	-0.175								
Co	0.214	0.065							
Cr	-0.381	0.141	-0.250						
Cu	-0.083	0.241	0.168	0.230					
Fe	-0.293	-0.239	0.234	0.095	-0.095				
K	-0.037	-0.056	-0.226	0.053	0.402	-0.006			
Mn	-0.108	0.456	0.379	**0.546**	0.488	-0.328	0.180		
Na	-0.093	-0.047	-0.206	-0.431	-0.227	0.382	-0.054	**-0.789**	
Ni	-0.403	0.329	0.138	0.203	0.366	-0.008	0.112	**0.568**	**-0.521**

TABLE-7
CORRELATION COEFFICIENT MATRIX OF SELECTED ELEMENTS IN THE BLOOD OF HEALTHY SUBJECTS

	Ca	Cd	Co	Cr	Cu	Fe	K	Mn	Na
Cd	0.117								
Co	0.094	-0.185							
Cr	0.365	0.212	0.131						
Cu	-0.040	-0.301	-0.074	0.082					
Fe	0.051	0.205	0.345	**0.503**	-0.263				
K	0.388	-0.008	-0.082	0.109	0.254	0.280			
Mn	0.061	-0.073	-0.117	0.150	-0.174	0.010	-0.203		
Na	-0.001	-0.032	-0.111	-0.415	0.104	-0.376	**-0.185**	-0.201	
Ni	0.307	0.056	0.315	0.312	0.011	**0.431**	0.318	-0.195	-0.249

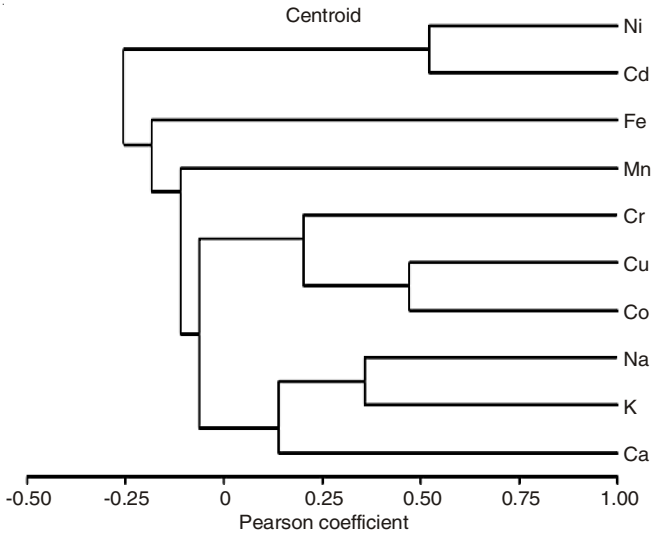

Fig. 3. Cluster analysis of selected elements in the blood of men hepatitis C patients

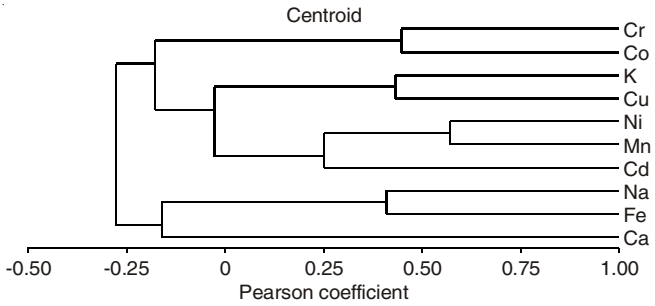

Fig. 4. Cluster analysis of selected elements in the blood of women hepatitis C patients

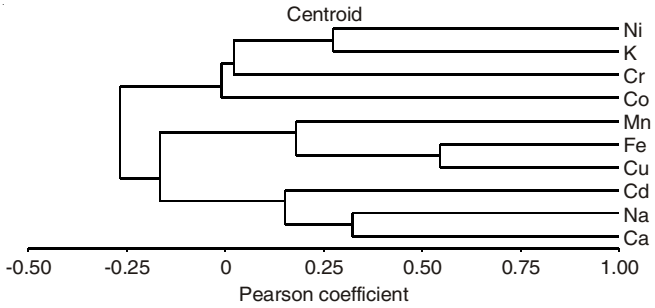

Fig. 5. Cluster analysis of selected elements in the blood of healthy subjects

metals in men. However, higher levels of Mn, Cd and Ni were observed in women patients in comparison with men hepatitis C patients. Relatively higher skewness values of K and Cr revealed the asymmetric distribution in blood of patients of both classes. It was concluded that marked divergences in the distribution of the trace elements in blood of hepatitis C patients happened.

The present investigation revealed that concentration of Co in hepatitis C virus patients was higher than healthy subjects. Some other studies [26] also reported higher levels of Co in hepatitis C patients. Low level of Co has been reported by Rashed *et al*. [27] among patients of hepatitis C. A low level of Fe was observed in hepatitis C virus patients in our study as compared to healthy donors. Analogous findings have been observed by Devrajani and Rahman [28]. The result of present study showed higher concentration of Mn in healthy subjects, compared to hepatitis C virus patients. Our results are coinciding with the findings of Nazir *et al.* [26].

A lower level of Cu in patients was observed as compared to healthy donors in our study as reported [29]. Gurjar and Mohan [30] recognized same pattern for Cd, Cr and Ni, as we observed in present study. In present study, a lower concentration for Ca, K and Na was observed in hepatitis C virus patients in comparison to healthy individuals. A decrease in concentration of these metals has adverse effect on metabolic pathways of body [31-33]. A gender based variation in concentration of trace metals was also observed in present study, which exposed that various factors like sex, age, nutrition and environmental contamination can influence trace element level in body [34-36].

Conclusion

In conclusion, Cd, Co and Cr levels in subjects suffering from hepatitis C were significantly high as compared to controls, whereas Fe, Cu, Mn, Ni relatively but Ca was significantly at low level in patients. Significant levels and variation of metals in analyzed samples specify the presence of these metals in the environment of Bhimber, as well as their role in progression of disease and hazards of these metals. The enhancement of Cd, Co and Cr collectively with decrease of Fe, Cu, Mn, Ni and Ca concentrations in blood samples of hepatitis C patients could be involved in disturbances of liver action and disruption of the antioxidant functions within the human body.

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