

Distributive Variability of Selected Trace Elements in the Blood Samples of Leukemia Patients

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Abstract

The study focused on the distribution of trace elements in the blood samples of leukemia patients. The blood samples of 62 patients of leukemia and their age, gender and demographically matched controls were analyzed for Cu, Ni, Mg, Zn, Fe and Cr by FAAS. The study evidenced higher mean levels for all the trace elements except Zn in leukemia patients than healthy controls. Mg was the major contributor at mean levels and Cr was present at sub-ppm levels in patients. The order for mean trace element levels in patients was: Mg > Fe > Ni > Zn > Cu > Cr, in comparison to healthy controls where the order remained: Mg > Fe > Zn > Cu > Ni > Cr. In patients Ni-Mg was significantly positively correlated while Mg was significantly negatively correlated with Cu. In healthy donors no such significant correlation was observed. The age of donors was found to be significantly positively correlated with Ni depicting an increase in Ni levels with increasing age of patients. Age of patients was significantly negatively correlated with Cu and Cr and positively correlated with Mg. Cluster analysis showed an independent behavior of Mg in patients contrary to healthy donors. Gender based study evidenced different orders of mean levels of selected trace elements in the blood of male and female patients. These orders were also different from the controls. Different clustering behavior of trace elements was observed in male and female patients.

Keywords: Leukemia; Age; Gender; Correlation; Trace elements

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Introduction

Trace elements play important roles in human health by regularizing the immune system, membrane potential, nerve contraction and mitochondrial activity etc. [1]. Recently, a number of studies have focused on the relationship of various metal levels in blood with biological disorders such as lung cancer, breast cancer, gastrointestinal cancer, heart diseases, neurodegenerative disorders, thyroid dysfunction, sclerosis, schizophrenia, parkinsons disease and diabetes [2-11]. The biological effects of trace elements may be explained on the basis of their ability to catalyze the initiation of free radical reactions as pro-oxidants or anti-oxidants or the decomposition of peroxides and other unstable molecules. It is worth noting that numerous biological processes and pathological states involve these free radical reactions [12]. Thus, it is imperative to study the

distribution and role of various metals under conditions of different diseases. An overview of roles of selected metals in human body is presented in forth coming paragraphs.

Zinc is an essential element, involved in numerous cellular metabolisms [13]. It catalyzes the activity of more than 200 enzymes [14, 15]. As an essential part of carbonic anhydrase and carboxypeptidase enzymes, it regulates CO₂ and protein digestion respectively [16, 17]. It is involved in wound healing [15], DNA synthesis and cell division [18], protein synthesis and immune function [15, 18]. In a study lower Zn levels were reported in leukemia lymphocytes as compared to normal lymphocytes. It was also found that under Zn deficiency conditions, the recovery from leukemia was slow [19]. At higher levels, it may cause low Cu status, altered Fe function and reduced immune function [20].

Iron is an integral part of different classes of proteins such as Fe-heme proteins; Fe-sulfur enzymes; Fe storage and transport proteins and Fe-activated enzymes (e.g. succinate dehydrogenase, NADH dehydrogenase, alcohol cyclooxygenases, dehydrogenase) etc. In all these forms, it performs vital body functions but Fe overload may cause acute Fe poisoning. Repeated blood transfusions or excessive Fe intake may also cause chronic poisoning [12].

Cu is essential for maintaining the strength of skin, epithelial and connective tissues and blood vessels throughout the body. It maintains the normal functioning of thyroid glands and plays an important role in the production of hemoglobin, myelin, melanin. About 90% of Cu in blood is incorporated into ceruloplasmin that transport Cu to the tissues [21, 22]. As an antioxidant Cu scavenges or neutralize the free radicals and may reduce some of the damage they cause [23-25]. As pro-oxidant it may promote the free radical damage and thus contribute towards Alzheimers disease [26, 27]. Cu acts as a reductant in the enzymes superoxide dismutase, cytochrome oxidase, lysyl oxidase, dopamine hydroxylase, and several other oxidases that reduce molecular oxygen. Its deficiency may lead to normocytic, hypochromic anemia, leucopenia and neuropenia, and inclusive osteoporosis in children [28]. Excessive Cu may cause liver damage and gastrointestinal effects characterized by abdominal pain, cramps, nausea, diarrhea, and vomiting

Nickel is considered as an essential nutrient that is required in very small amounts, but has a number of cellular effects. It may activate or inhibit enzymes that usually contain other elements. It is also involved in the production and function of certain hormones such as adrenaline, noradrenaline, prolactin and aldosterone. Intracellularly, it can affect membrane properties, oxidation/reduction systems and ion channels [29]. Recent studies have shown that metals including Fe, Cu, Cr undergo redox cycling while Ni deplete glutathione and protein bound sulfhydryl groups resulting in the production of reactive oxygen species as superoxide ion, hydrogen peroxide and hydroxyl radical. As a result enhanced lipid peroxidation, and DNA damage may occur [30, 31].

Magnesium is mostly found in bones, muscles and non-muscular soft tissue [32]. It is a cofactor of more than 300 enzymatic reactions [33, 34] and critically stabilizes enzymes, including many ATP-generating reactions [35]. An interference with magnesium metabolism may influence ATP dependant processes such as glucose utilization, synthesis of fat, proteins, nucleic acids and coenzymes, muscle contraction, methyl group transfer [36]. Magnesium may be considered a natural 'calcium antagonist' [37] as it antagonizes the calcium-dependent release of acetylcholine at motor endplates [38].

In view of the important roles played by selected metals in various biological processes, the present study was formulated to determine the levels of selected metals in blood samples of leukemia patients in comparison with healthy donors, and to trace the relationships among the measured metals in terms of correlation coefficient matrix and cluster analysis.

Materials and Methods

Sampling and pretreatment

The blood samples were collected from a total of 100 diagnosed patients of leukemia belonging to both genders and ages between 3 to 60 years. The informed written consent of the subjects was obtained prior to sample collection. The protocol of the study conforms to the ethical guidelines of the "World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the 59th WMA General Assembly, Seoul, South Korea, October 2008. Moreover, the protocol of study was approved by the Advanced Studies and Research Board of Lahore College for Women University, Lahore.

A 5.0-mL portion of blood was drawn from the antecubital vein of each patient in pyrogen-free disposable syringe by trained phlebotomists and the samples were transported to the laboratory for analysis where these blood samples were refrigerated and stored in contaminant-free evacuated tubes. Each sample was processed within 72 h for the analysis of Co, Cu, Pb, Cr, Fe, and Mg. An equal number of age and gender matched healthy individuals belonging to same demographic origin were selected and included in the study.

Analysis of trace elements in blood

Atomic absorption spectroscopy was used for the determination of trace elements in blood samples after following a wet acid digestion method. For this purpose the blood samples were heated first gently and then vigorously with equi-molar quantities of HNO_3 and HClO_4 to obtain a clear solution that was subsequently diluted to 50 mL. The blanks were prepared the same way as the samples but without blood. The solutions thus obtained for samples and blanks were used for direct aspiration onto flame atomic absorption spectrophotometer (FAAS). All the chemicals and reagents used during the analysis were of spectroscopic grade with a certified purity of 99.99% obtained from E-Merck, Germany.

The processing and preparation of samples was carried out all in pyrex glassware. Hitachi AA spectrophotometer Model AA-5000, equipped with Zeeman background correction facility, was used for the estimation of selected trace elements. Standard optimum analytical conditions were maintained and periodically checked on AAS system for each trace element as per manufacturer's instructions (**Table 1**). To establish the precision of the results, triplicate analysis of each sample was carried out. Moreover, the blanks were used to avoid the contamination arising from matrix. These blanks were run after every ten samples to ensure the accuracy. Inter-laboratory comparison of the obtained data was conducted at the University of Punjab, Lahore, Pakistan. The data of two laboratories did not differ more than 4%.

Statistical analyses

The data obtained for the levels of various trace elements in the blood samples of leukemia patients were processed for descriptive statistics. In order to trace the trace element-to-trace element

correlations among various trace element pairs, the Pearson correlation coefficient matrix was evaluated. The data were also segregated on the basis of gender, and the individual groups were studied thoroughly. Cluster analysis of trace element data was carried out by using Statistica software. It provided information about the clustering behavior of studied trace elements and their dependence on each other.

Results and Discussion

The demographic data of the patients of leukemia and their age and gender matched healthy controls is depicted in **Table 2**. The leukemia patients belonged to an average age of 26.6 years, and 58.06% of these patients were males and the remainder was female. Among these patients 48.4% were inhabited in comparatively less polluted environment of rural areas while 51.6% were of urban origin. The controls were selected with the similar demographic characteristics. Urban areas appear to have high incidence of disease are compared to rural areas suggesting that pollution arising from various industries may be a risk factor.

Distribution of trace elements

The distribution of trace elements in blood of leukemia patients and their age and gender matched controls is presented in **Table 3** and a

comparison of the trace element levels in two groups is furnished in **Figure 1**. The data exhibited large variation in minimum and maximum values for all the trace elements. Mg among the studied trace elements was found as the major contributor at mean levels while Cr was present at sub- ppm levels. The mean levels observed for Ni and Fe were found to be 5.312 and 5.785 $\mu\text{g}/\text{mL}$ respectively. Except Zn, the mean levels of all the observed trace elements in leukemia patients were much higher than healthy controls. Zinc was observed at levels of 1.625 $\mu\text{g}/\text{mL}$. The order for mean levels of trace elements in blood of leukemia patients was found to be: $\text{Mg} > \text{Fe} > \text{Ni} > \text{Cu} > \text{Zn} > \text{Cr}$, in contrast to healthy controls, where the observed order was: $\text{Mg} > \text{Fe} > \text{Cu} > \text{Zn} > \text{Ni} > \text{Cr}$. A very significant difference in the mean level of Ni was observed in the two groups ($p < 0.05$). Ni compounds are known to induce DNA protein cross linking, DNA strand breaking, and chromosomal aberrations in isolated human peripheral lymphocytes [39]. Thus abnormal levels of these trace elements observed in leukemia patients in present study may affect the progression of disease. Cr, another culprit for DNA damage by oxygen radical production was also found to be present at higher levels in the blood of leukemia patients than controls [40, 41]. Increased levels of iron in the blood of patients may also be associated with increased risk of cancer by inducing the mutations of cells [42, 43].

Table 1 Optimum analytical conditions for selected trace element analysis using air-acetylene flame on atomic absorption spectrophotometer.

	Wavelength (nm)	HC lamp current (mA)	Slit Width (nm)	Detection Limit ($\mu\text{g}/\text{L}$)	Fuel-gas flow rate (L/min)
Cu	324.8	4.0	0.50	3	2.0
Ni	232	4.0	0.20	10	2.0
Fe	248.3	5.0	0.20	6	2.0
Mg	285.2	9.0	1.3	10	2.2
Zn	213.9	5.0	1.0	1	2.0
Cr	357.9	7.0	0.20	6	2.9

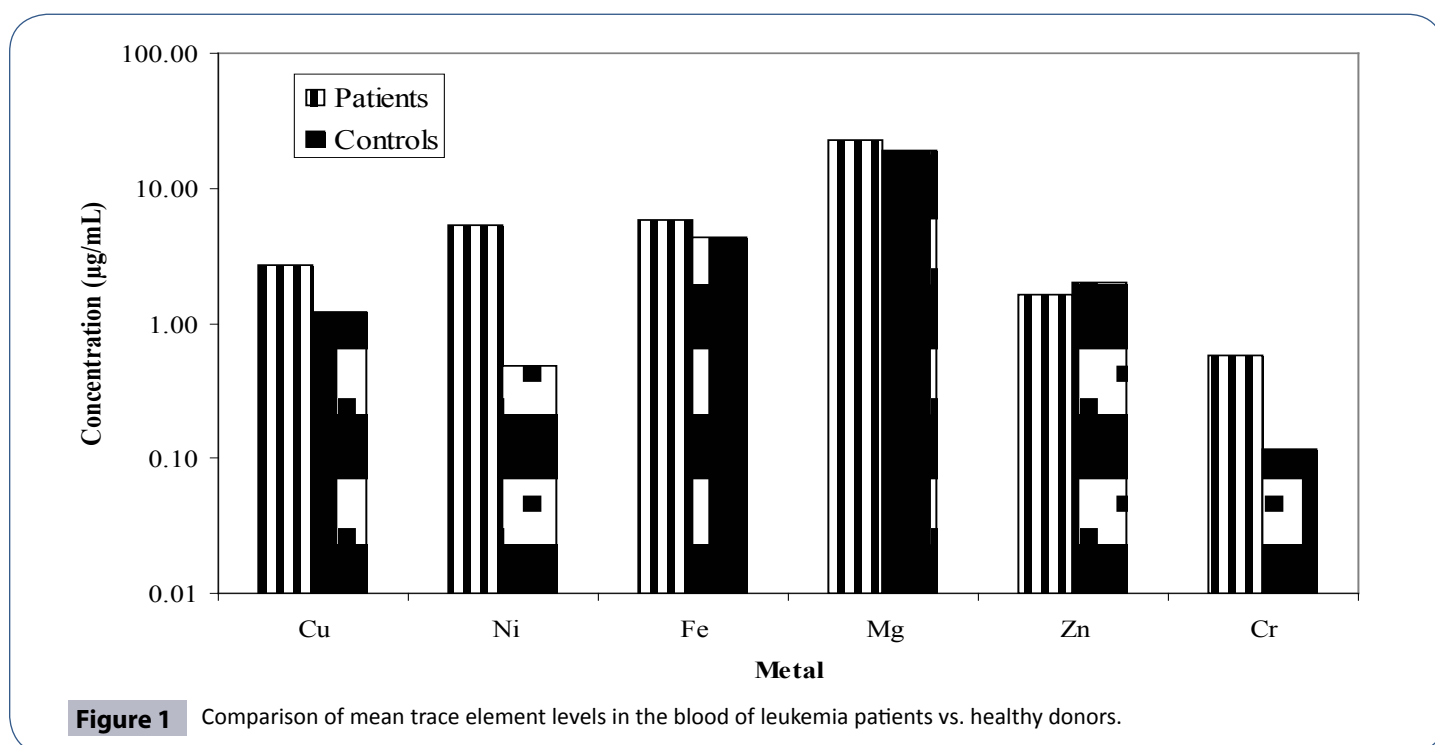


Figure 1 Comparison of mean trace element levels in the blood of leukemia patients vs. healthy donors.

The quartile distribution of trace elements in the leukemia patients and healthy controls is depicted in **Figure 2**. The figure also supported the data provided in **Table 3**. The relationships among different trace element pairs in the blood samples of leukemia patients and controls are provided in **Table 4** in terms of Pearson correlation coefficient matrix. The data for leukemia patients evidenced Ni-Mg pair to be significantly positively correlated with an r-value of 0.328. Magnesium was also found to be significantly negatively correlated with Cu. In case of healthy donors, Zn and Cr were the only trace elements that were found to be significantly correlated.

The age of healthy donors was found to be significantly positively correlated with Ni depicting an increase in Ni levels with increasing age of patients. In fact, the Ni excretion by the body depends on the immunological body conditions. Ni itself suppresses the immunological status of body [44]. So under leukemia conditions the urinary excretion of Ni is decreased leading to an increased retention of Ni in the blood that causes more oxidative DNA damage. Ni has also been considered as an etiologic factor for childhood acute leukaemia by oxidative DNA damage [45]. In leukemia patients age was found to be significantly negatively correlated with Cr; these results are consistent with the results of Davies et al., who reported age related decrease in blood Cr levels in diabetic patients [46]. With the increasing age, no mal-absorption of Cr was reported rather higher urinary excretions may be responsible for lower Cr levels in blood of patients [47].

The clustering behavior of the trace elements in blood of leukemia patients in comparison to healthy donors is depicted in **Figure 3**. In leukemia patients, the most significant cluster was formed by Cu, Zn and Cr within a linkage distance of 50. This primary cluster

was associated with another primary cluster formed by Ni and Fe and hence a secondary cluster was formed. In these leukemia patients Mg stood alone depicting its independent behavior. Healthy donors were characterized by the most significant primary cluster to be formed by Ni and Cr that were associated with in a linkage distance of 5. Another primary cluster was formed by Cu and Zn which joined the previous cluster with in linkage distance of 20. No independent role of Mg was observed here as in case of patients. On overall basis, the clustering behavior of trace elements in leukemia patients was quite different than in controls.

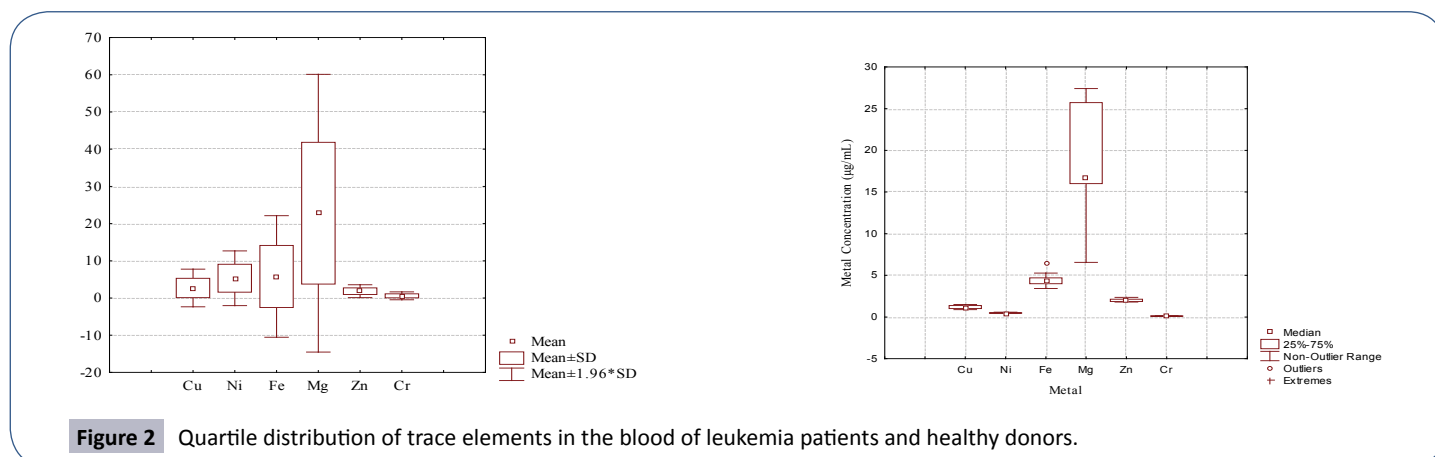
Gender based studies of trace elements in the blood of leukemia subjects

The trace elements observed in the blood of leukemia subjects were also segregated to study the behavior of trace elements in two genders. **Table 5** furnishes data on distribution of trace elements in thirty six male leukemia patients and 26 female patients in comparison to corresponding healthy donors. The data for male patients presented the highest mean level for Mg with the order for the selected trace elements being: Mg > Fe > Cu > Cr > Zn > Ni with their respective mean levels being: 28.49, 10.57, 3.032, 1.381, 1.248 and 0.713 µg/mL. The large variations in minimum and maximum values evidenced the non-normal distribution of the data. The order observed for the mean levels of trace elements in the blood of male healthy controls was: Mg > Fe > Zn > Cu > Ni > Cr which was quite different from the male patients. Also the levels observed for all the trace elements were significantly different in male patients than healthy donors (p<0.05).

For the female subjects the mean age was found to be 29.9 years (**Table 5**). Quite similar to the previous case, these patients exhibited the highest mean levels for Mg standing at 24.58 µg/mL. The highest level recorded for Cu was 13.4 µg/mL. Iron here was present at mean level of 5.372 µg/mL against the maximum concentration of 22.4 µg/mL. Zinc and Cu exhibited the mean levels of 1.491 µg/mL and 3.388 µg/mL respectively. Thus the order for the mean levels of selected trace elements in blood of female leukemia patients was: Mg > Fe > Cu > Zn > Ni > Cr. The counterpart data for the controls exhibited the order for mean levels: Mg > Fe > Zn > Cu > Ni > Cr. The levels of these trace elements were also found to be significantly lower than

Table 2 Demographic data of subjects.

Characteristics		Leukemia Patients	Healthy donors
Age	Mean	26.6	26.3
	Range	3-60	3-60
	Median	23	23
Gender	Male	36 (58.1 %)	36 (58.1 %)
	Female	26 (41.9 %)	26 (41.9 %)
Adobe	Urban	32 (51.6 %)	34 (54.8 %)
	Rural	30 (48.4 %)	28 (45.2 %)



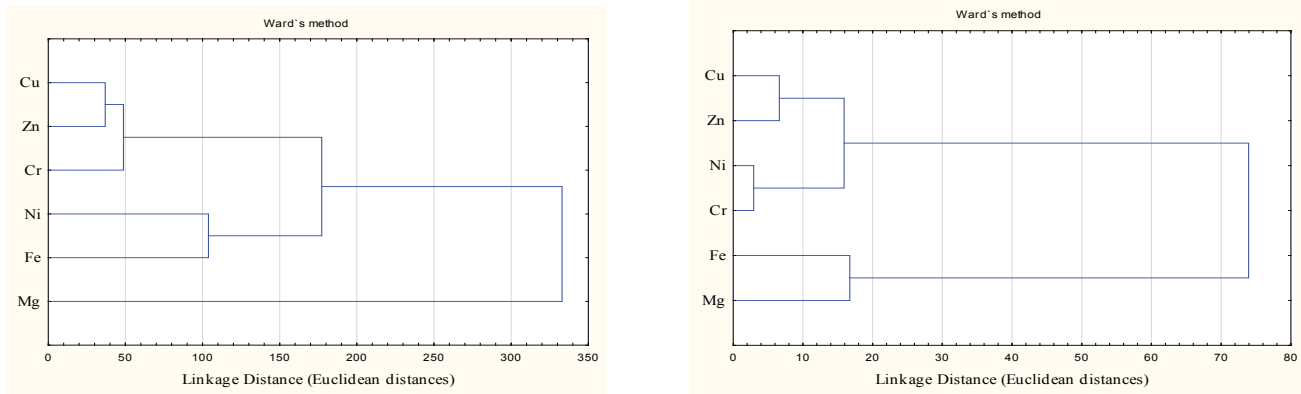


Figure 3 Cluster analysis of trace elements in the blood of cancer patients and healthy donors (n=62).

Table 3 Descriptive statistics for the distribution of trace element levels ($\mu\text{g/mL}$) in the blood of leukemia patients and healthy donors.

		<i>Cu</i>	<i>Ni</i>	<i>Fe</i>	<i>Mg</i>	<i>Zn</i>	<i>Cr</i>	<i>Age (year)</i>
Leukemia Patients	Min.	0.000	0.230	0.166	0.200	0.190	0.100	3.000
	Max.	13.40	28.00	38.70	75.90	3.68	3.500	60.0
	Mean	2.696	5.312	5.785	22.79	1.625	0.581	26.6
	Median	1.845	5.250	2.135	18.65	1.360	0.309	23.0
	S. D.	2.586	3.754	8.336	19.05	4.023	0.546	14.8
	S. E.	0.328	0.477	1.059	2.419	0.511	0.069	1.9
Healthy Donors	Min.	0.889	0.419	3.424	6.560	1.805	0.084	3.000
	Max.	1.507	0.586	6.357	27.42	2.366	0.146	60.00
	Mean	1.198	0.487	4.374	19.27	2.005	0.115	26.32
	Median	1.199	0.486	4.339	16.84	2.001	0.116	23.00
	S. D.	0.193	0.034	0.533	0.667	0.143	0.018	14.54
	S. E.	0.024	0.004	0.068	5.249	0.018	0.002	1.847

Table 4 Pearson correlation coefficient matrix for trace elements in the blood of leukemia patients and healthy donors (n=62).

		<i>Cu</i>	<i>Ni</i>	<i>Fe</i>	<i>Mg</i>	<i>Zn</i>	<i>Cr</i>	<i>Age (year)</i>
Leukemia Patients	Cu	1.000						
	Ni	0.062	1.000					
	Fe	-0.080	0.053	1.000				
	Mg	-0.318	0.328	0.051	1.000			
	Zn	0.089	0.074	-0.217	0.031	1.000		
	Cr	0.032	-0.132	-0.220	-0.185	0.040	1.000	
	Age (year)	-0.207	0.168	0.021	0.202	0.043	-0.219	1.000
Healthy donors	Cu	1.000						
	Ni	-0.097	1.000					
	Fe	0.004	0.104	1.000				
	Mg	0.098	-0.034	0.031	1.000			
	Zn	-0.010	0.186	0.201	0.038	1.000		
	Cr	0.099	0.049	-0.038	0.162	0.261	1.000	
	Age (year)	-0.133	0.211	-0.147	-0.045	0.094	-0.159	1.000

r-values are significant at ± 0.211 ($p = 0.05$)

the patients except for Zn that was present at level of 1.982 $\mu\text{g/mL}$ in controls in comparison to female patients where its mean

concentration was 1.49 $\mu\text{g/mL}$. In case of healthy donors, all the trace elements except Ni and Cu were present at higher levels in

Table 5 Descriptive statistics of trace elements in the blood of the leukemia patients and healthy donors of both genders.

		<i>Cu</i>	<i>Ni</i>	<i>Fe</i>	<i>Mg</i>	<i>Zn</i>	<i>Cr</i>	<i>Age (year)</i>
leukemia patients male (n=36)	Minimum	0.175	0.135	0.180	0.200	0.120	0.100	3.0
	Maximum	10.90	2.240	48.82	75.90	3.091	3.150	58.0
	Mean	3.032	0.713	10.57	28.49	1.248	1.381	24.3
	Median	2.210	0.611	2.625	28.80	0.675	1.044	20.5
	S. D.	2.622	0.431	15.38	22.02	1.711	0.861	14.3
	S. E.	0.437	0.072	2.564	3.671	0.285	0.143	2.381
Controls male (n=36)	Minimum	0.889	0.419	3.535	15.38	1.816	0.084	3.000
	Maximum	1.507	0.550	5.140	27.32	2.193	0.146	58.00
	Mean	1.174	0.483	4.352	20.78	2.022	0.119	24.31
	Median	1.117	0.481	4.523	17.15	2.092	0.120	21.50
	S. D.	0.203	0.035	0.457	4.923	0.138	0.017	13.99
	S. E	0.034	0.006	0.076	0.821	0.023	0.003	2.317
Leukemia female patients (n = 26)	Minimum	0.000	0.210	0.166	1.220	0.220	0.123	6.000
	Maximum	13.40	3.915	22.40	69.60	2.870	1.590	60.00
	Mean	3.388	1.022	5.372	24.58	1.491	0.732	29.9
	Median	1.775	0.555	1.825	24.60	0.858	0.666	26.50
	S D	3.829	0.991	7.201	18.05	1.312	0.481	15.12
	S E	0.751	0.194	1.412	3.539	0.257	0.094	2.965
Female controls (n = 26)	Minimum	0.954	0.448	3.424	15.71	1.805	0.086	6.000
	Maximum	1.498	0.586	6.357	27.28	2.366	0.140	60.0
	Mean	1.232	0.493	4.405	20.63	1.982	0.111	29.1
	Median	1.212	0.491	4.228	17.02	1.892	0.109	25.0
	S D	0.175	0.033	0.632	5.064	0.150	0.019	15.21
	S E	0.034	0.006	0.124	0.993	0.029	0.004	2.984

males than females. In leukemia patients in addition to Ni, Cu and Cr were also observed to be present at higher levels in females. The order observed for male patients was quite similar to one observed for female patients with only one difference, i.e., in male patients, Cr was present at least concentration levels where as in female patients, it was present at appreciably high concentrations. There is increasing evidence that trace elements differ in prevalence in prevalence in different genders and are manifested differently in two genders. However, the database is small. Differences in exposure, anatomy, physiology, biochemistry, and behavior between males and females are the dominant factors in deciding the effects and roles of different trace elements [48]. Higher levels of some of the metals such as Cd were reported to be associated with estrogenic effects in women [49].

The quartile distribution of the trace element data in male and female patients of leukemia in comparison to their healthy controls is furnished in **Figure 4**. The spread and SD of the data was more pronounced in male and female patients than the corresponding healthy donors. Cluster analysis (**Figure 5**) evidenced two main clusters of the trace elements in male patients. The first cluster comprised of Cu-Fe-Mg while the second cluster comprised of Ni-Zn-Cr. In case of controls again two major clusters were obtained; the first cluster comprised of Cu-Zn, while the second

cluster comprised of Ni-Mg-Fe-Cr (**Figure 5**). A different clustering behavior of the trace elements evidenced a change in trace element metabolism in patients as compared to healthy donors. The disturbed levels of one or more trace elements in patients as compared to controls in two genders may exert antagonistic influence on other trace elements thereby causing a change in etiology of disease.

In healthy females, the cluster analysis depicted three primary clusters of trace elements with the most significant between Fe-Zn, linked with in a Pearson linkage distance of 0.5 (**Figure 5**). Nickel and chromium formed other primary cluster. Within a linkage distance of 1.0, both these primary clusters were linked together to form a secondary cluster, there by evidencing the dependence of these trace elements upon each other. Cu and Mg were the trace elements that formed third primary cluster in female controls. A similar trend of formation of three primary clusters was also observed in female patients but with different sequence of trace elements. Here most significant primary cluster was observed between Mg-Zn pair that was associated with in a linkage distance of 0.7. With a slightly increased linkage distance, Fe-Ni formed second primary cluster that was joined with the third primary cluster formed by Cu-Cr thereby forming secondary cluster. Thus different clustering behavior of trace elements was recorded in female patients and controls.

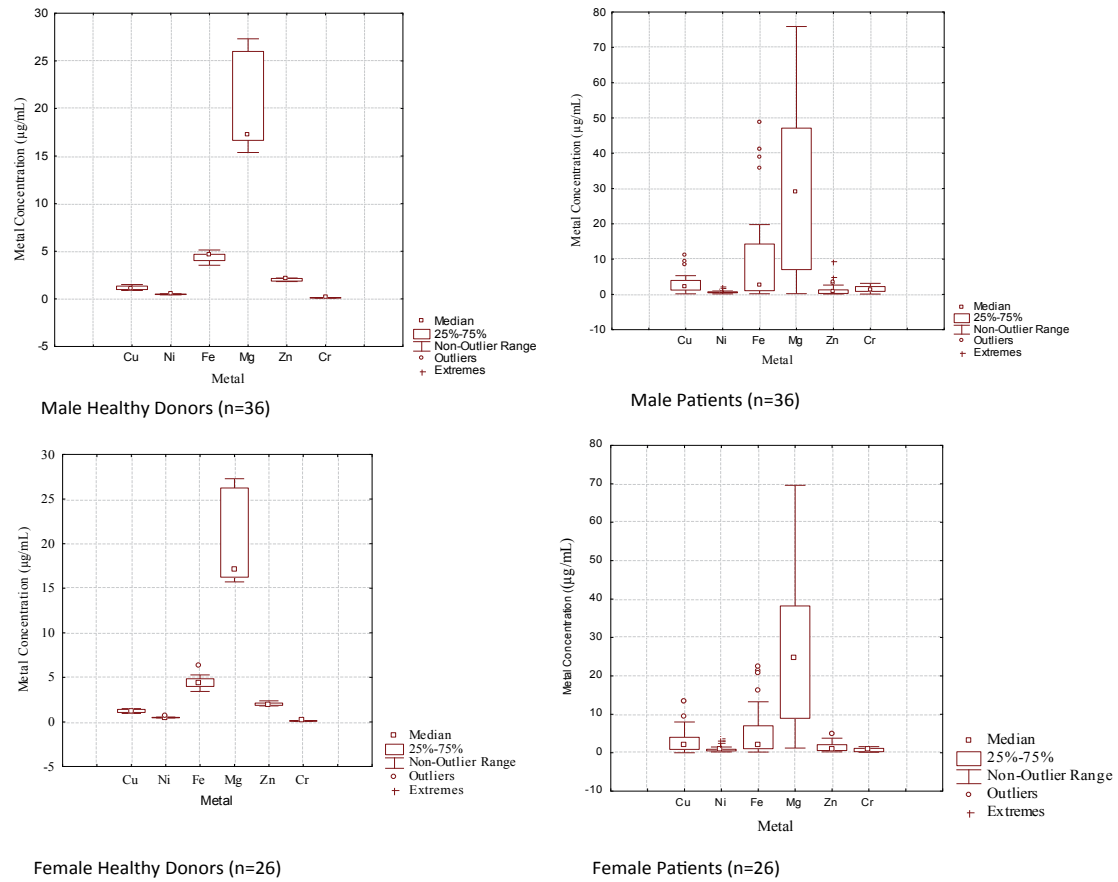


Figure 4 Quartile distribution of trace elements in patients of both genders vs. healthy donors .

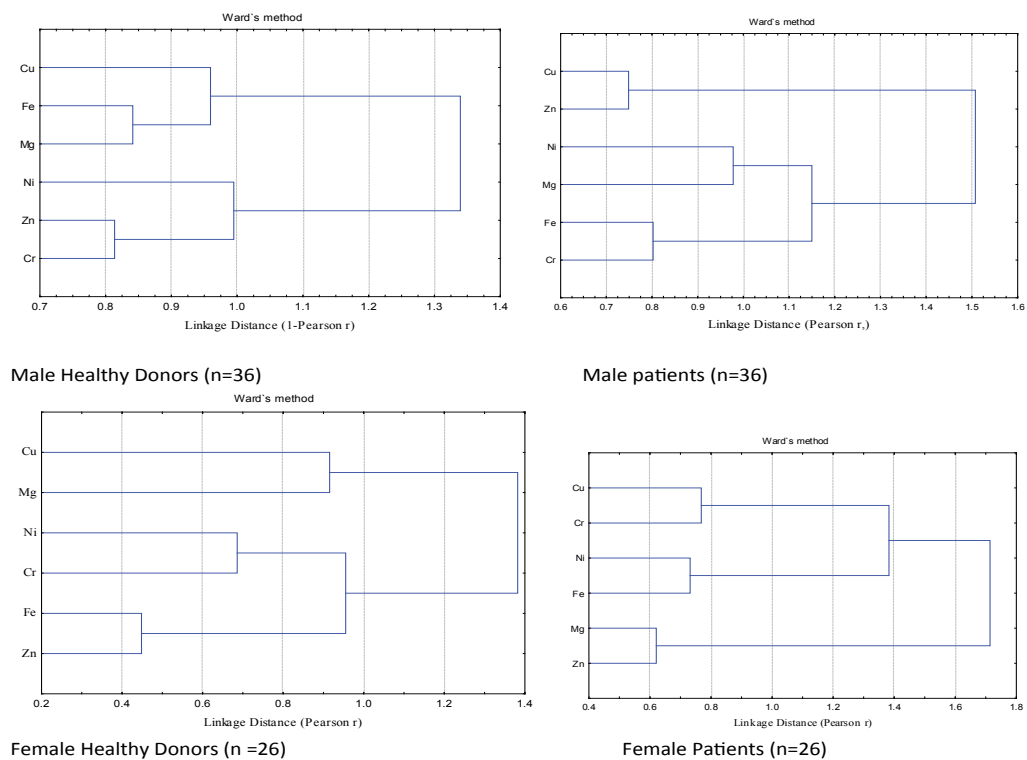


Figure 5 Gender based cluster analysis of leukemia patients vs. controls.

Conclusion

The present study has evidenced the abnormally high levels of trace elements such as Cr, Cu, Ni and Fe in the blood of leukemia patients than healthy controls. Zinc, that is known to form normal lymphocytes was recorded at lower levels in leukemia patients than healthy controls. Moreover, the clustering behavior of

trace elements in leukemia patients was quite different from the controls. The study thus evidenced that leukemia was associated with disorder of blood trace element levels. Gender was another factor that affected the distribution of trace elements in the blood of controls as well as patients. Cu and Zn were observed at higher levels in females of both controls and patients. Female leukemia patients were also characterized by higher Cr levels than males.

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