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Evaluation of Serum Levels of Trace Elements in Myeloproliferative Neoplasms: A Case-Control Study

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ABSTRACT

The lack or excess of some of trace elements or heavy metals could be associated with the risk of hematologic malignancy. This study aimed to compare the patients with myeloproliferative neoplasm with the serum concentrations of Zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), iron (Fe), cadmium (Cd) and cobalt (Co) in healthy individuals.

Healthy individuals in the control group and newly diagnosed patients with myeloproliferative neoplasm were involved in the research. The patient and control groups were similar in terms of socioeconomic status and eating habits. The serum levels of the trace elements were determined via Atomic Absorption Spectrophotometry.

The myeloproliferative neoplasm group consisted of 60 patients while the control group consisted of 20 volunteers. In the patient group, serum Zn and Fe concentrations were significantly lower than the control group ($p=0.001$ and $p=0.001$). Serum Cu, Pb, Cd, Co, Ni and Mn concentrations were higher in the patient group ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$, $p=0.027$, respectively). When a comparison was made between Mn serum level and the control group, Mn serum level was determined to be low in PMF ($p>5\%$). Serum Cd and Ni levels were higher in PMF, ET, CML and PV ($p=0.005$). Pb serum level was higher in ET, CML and PV ($p=0.001$, $p=0.001$, $p=0.001$, respectively). Serum Fe and Zn levels were lower in the PMF, ET, CML and PV groups ($p=0.001$ and $p>0.5$, respectively, for Fe).

Serum Zn and Fe levels were lower and Cu, Pb, Cd, Co, Ni and Mn levels were higher in patients with myeloproliferative neoplasm. In particular, Zn is a very strong antioxidant element, and the fact that Zn is low in patients suggests that it could be an important marker in PV, ET, CML and PMF cases.

Keywords: Trace elements, myeloproliferative neoplasm, zinc, iron

Introduction

Myeloproliferative neoplasms (MPN) are clonal diseases that are characterized by uncontrolled proliferation of one or more myeloerythroid cells in the bone marrow and the increase in the number of mature and immature cells in the peripheral blood and that can make progress to hemostasis and thrombosis anomalies and acute leukemia. Polycytemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are also in this disease group (1).

Trace elements serve as cofactors in enzymatic reactions in the execution and maintenance of many metabolic events in our body. Trace elements are also involved in the preservation and synthesis of the structure of DNA. In addition, trace elements are also involved in the nerve conduction, the transportation of substance in the cell membrane, muscular contraction, antioxidant effect, and especially the maintenance of mitochondrial functions. Although minerals constitute only 5 % of the human diet, they are necessary for the continuation of human health and functions (2).

The studies on the function of trace elements in the etiology of myeloproliferative neoplasm are limited and their results are not sufficient. In this study, it was aimed to compare the levels of some trace element in patients with myeloproliferative neoplasm with the levels in healthy individuals and to evaluate their possible effects in myeloproliferative neoplasm.

Materials and Methods

60 patients (26 male, 34 female) who were admitted to Van Yüzüncü Yıl University Faculty of Medicine Dursun Odabaş Medical Center Hematology policlinic and were new diagnosed with myeloproliferative neoplasm were included in the study. Of these 60 patients, 5 had primary

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myelofibrosis (PMF), 15 had essential thrombocytosis (ET), 20 had chronic myelogenous leukemia (CML), and 20 had polycythemia vera. The bone marrow biopsy and the flow cytometric analysis was performed for the diagnosis and to determine the type of myeloproliferative neoplasm. The healthy control group was composed of 20 healthy individuals. The two groups had similar socioeconomic status and eating habits. Informed consent was received from all participants. The ethical approval decision for the study was received from the local ethics committee. Recent history of blood transfusion, and the non-use of any medicine and mineral supplement for therapeutic purposes were determined as the inclusion criteria of the study for the patients and control group. A complete physical examination was conducted in all patients for the diagnosis of a disease such as chronic liver disease.

Venous blood samples (5 millilitres) were taken from all individual on an empty stomach in the morning using metal-free tubes. Blood samples with hemolysis were not included in the study. The blood samples taken were centrifuged at 5000 rpm for 10 minutes, and then serum samples were separated. Serum samples taken were stored in the deep-freezer in deionized polyethylene tubes and at -80 centigrade degree until the working day. For biochemical analysis, serum Cu, Zn, Ni, Mn, Fe, Pb, Cd and Co concentration were measured both from patients and healthy individuals using the Atomic Absorption Spectrophotometric method (Unicam-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK).

Statistical Analysis: Descriptive statistics for the characteristics analyzed were expressed as Mean, Standard Deviation, Minimum, and Maximum values. One-way analysis of variance was performed for the comparison of group averages for these features. Following the variance analysis, the Duncan multiple comparison test was used to identify different groups. Pearson correlation coefficients were calculated separately in the groups to determine the relationship between the features. During the calculations, according to Kolmogrov-Smirnov and Shapiro-Wilk normality tests, independent t-test was applied to the data due to normal distrubition of the data. The statistical significance level was taken as 5 % and IBM SPSS v21 statistical package program was used.

Results

The myeloproliferative neoplasm group was composed of 60 patients. 26 of the patients were female. The mean age of the patients was 51.3±15.16. The healthy control group was composed of 20 people including 10 female. The mean age of the healthy control group was 50.50±15.47. There was no statistically significant difference between groups in terms of age ($p=0.833$). The patients with and the serum cadmium (Cd), nickel (Ni), manganese (Mn), iron (Fe), zinc (Zn), cobalt (Co), copper (Cu) and lead (Pb) concentrations in the control group are presented in table 1.

Serum zinc (Zn) and iron (Fe) levels were significantly lower in the patient group ($p=0.001$, $p=0.001$, respectively). Serum copper (Cu), lead (Pb), cadmium (Cd), cobalt (Co), nickel (Ni) and manganese (Mn) concentrations were higher in the patient group compared to the control group ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$, $p=0.027$, respectively). Comparison of PMF-ET-CML-PV and serum Mn, Cd, Cu, Pb, Fe, Ni, Co, Zn levels in the control group was shown in Table 2.

Serum Mn level was lower ($p<0.001$) in patients with PMF and higher ($p=0.001$) in with PV. No statistically significant difference was found between the control group and ET and CML ($p>0.5$). Serum Cd level was higher in patients with PMF, ET, CML and PV ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.001$, respectively). Serum Cu level was higher in patients with PMF, CML and PV ($p=0.001$, $p=0.001$, $p=0.001$, respectively). A statistically significant difference was not determined between the control group and ET ($p>0.005$). Serum Pb level was higher in patients with ET, CML and PV ($p=0.001$, $p=0.001$, $p=0.001$, respectively). Serum Fe level was lower in patients with PMF, ET, CML and PV ($p=0.001$, $p<0.001$, $p<0.001$, $p<0.001$, respectively). Serum Ni level was higher in patients with PMF, ET, CML and PV ($p=0.001$, $p=0.001$, $p<0.001$, $p=0.001$, respectively). Serum Co level was higher in patients with PMF, ET and CML ($p=0.001$, $p<0.001$, $p=0.001$, respectively). Serum Zn level was lower in patients with PMF, ET, CML and PV ($p=0.001$, $p<0.001$, $p=0.001$, $p<0.001$, respectively).

Pearson correlation coefficients and probability values are tabulated in Table 3. The p-values are found to be as Fe-Mn (0.191), Co-Mn (0.207), Co-Cd (0.435), Co-Pb (0.255) and Co-Ni (0.206) and statistically significant difference was found

Table 1. Serum Zn, Co, Cu, Fe, Ni, Mn. Pb and Cd levels of the participants with independent t test

Parameter	Patient (n=60)	Control (n=20)	Min	Max	p
	Mean±Std Dev.	Mean±Std Dev			
Zn (µg/dL)	0.484 ± 0.269	1.173 ± 0.294	0.102	1.779	0.001
Cu (µg/dL)	0.857 ±0.16	0.647 ± 0.157	0.344	1.324	0.001
Co (µg/dL)	0.065 ± 0.077	0.004 ± 0.002	0.002	0.507	0.016
Fe (µg/dL)	0.844 ± 0.140	1.386 ± 0.430	0.409	2.176	0.001
Ni (µg/dL)	0.671 ± 0.235	0.219 ± 0.083	0.111	1.255	0.001
Mn (µg/dL)	0.429 ± 0.347	0.247 ± 0.166	0.021	0.998	0.001
Pb (µg/dL)	0.642 ±0.391	0.004 ± 0.002	0.001	1.231	0.001
Cd (µg/dL)	0.054 ± 0.027	0.013 ± 0.015	0.001	0.099	0.001

Table 2. Comparison of PMF-ET-CML-PV and serum Mn, Cd, Cu, Pb, Fe, Ni, Co, Zn levels with the control group

Parameter	Control(n=20)	PMF (n=5)	ET (n=15)	CML (n:20)	PV (n=20)	p
Mn (µg/dL)	0.247±0.166bc	0.039±0.012 a	0.113±0.161 ab	0.367±0.228c	0.826±0.116d	0.001
Cd (µg/dL)	0.026±0.154a	0.035±0.007b	0.031±0.006b	0.044±0.013b	0.086±0.020c	0.001
Cu (µg/dL)	0.647±0.157a	0.893±0.144b	0.776±0.137ab	0.881±0.207b	0.884±0.106b	0.001
Pb (µg/dL)	0.004±0.002a	0.054±0.013a	0.504±0.365b	0.536±0.337b	0.996±0.109c	0.001
Fe (µg/dL)	1.386±0.431c	1.015±0.113b	0.806±0.185a	0.785±0.101a	0.888±0.091ab	0.001
Ni (µg/dL)	0.219±0.083a	0.636±0.097c	0.671±0.128c	0.469±0.201b	0.880±0.167d	0.001
Co (µg/dL)	0.004±0.002a	0.068±0.028bc	0.101±0.144c	0.075±0.013bc	0.026±0.007ab	0.001
Zn (µg/dL)	1.173±0.294d	0.635±0.195c	0.431±0.197b	0.757±0.126c	0.212±0.086a	0.001

[a,b,c,d] Means in the same line not sharing the same superscript are significantly different ($p < 0.05$).

($p>0.05$). Moreover, relation between other variables is statically insignificant ($p<0.05$).

Discussion

Essential metals such as copper, iron, magnesium and zinc are necessary for various biological processes in the form of functional groups (like hem in hemoglobin) for enzymatic cofactors or proteins. In the studies conducted, metal ions may cause cell cycle modulation, carcinogenesis or apoptosis by interacting with cell components, like DNA and nuclear proteins (3). Studies reported that some trace elements and heavy metals are effective in hematopoietic and solid cancers. Until the 1950s, whether serum zinc and copper levels in cancer patients would function as markers in cancer activity had been discussed in detail (4).

Although copper is rarely found in pure form in nature, it is mainly found in combination with other chemicals. It is a basic micronutrient that is necessary for growth, bone/connective tissue, the development and protection of the brain, heart and various other organs (5). Several studies reported that tumoral and serum copper levels of cancer patients were significantly higher compared to healthy controls. It has been reported that serum copper levels and copper / (zinc, selenium, iron) ratios are significantly higher in solid organ malignancies such as breast, cervical, ovarian, lung, prostate and stomach cancer, and leukemia (6). In the study carried out by Demir C. et al. on patients with acute leukemia, they found that serum copper level was higher in cases with acute leukemia (7). In the study carried out by E. O. Akanni et al. on chronic myeloid leukemia, serum selenium, calcium, copper, iron, manganese and zinc levels were found to be significantly higher in the patient group, and they reported that there was a need for further studies for the prognostic values of serum trace levels in CML patients in the survival of the disease (8). Tadmor et al (9) also found that the gene expressions of lysyl oxidases (LOX), that are copper-bound amino oxidases, in myeloproliferative diseases were increased and that there was no expression in the bone marrow of normal healthy controls. In this study, expression was detected in all LOX including LOX,LOXL1 (only in PMF),LOXL2,LOXL3 in PMF, LOX expressions were detected in CML, ET and PV, and it was reported that these

Table 3. Pearson correlation coefficients for serum Zn, Co, Cu, Fe, Ni, Mn, Pb and Cd

Pearson correlation coefficients		Mn	Cd	Cu	Pb	Fe	Ni	Co
Cd	Pear_Cor.	0.773						
	p	0.001						
Cu	Pear_Cor.	0.338	0.348					
	p	0.002	0.002					
Pb	Pear_Cor.	0.696	0.672	0.464				
	p	0.001	0.001	0.001				
Fe	Pear_Cor.	-0.106	-0.265	-0.256	-0.394			
	p	0.191	0.013	0.016	0.000			
Ni	Pear_Cor.	0.488	0.604	0.265	0.606	-0.335		
	p	0.001	0.001	0.013	0.001	0.002		
Co	Pear_Cor.	-0.099	-0.146	-0.020	0.080	-0.271	0.100	
	p	0.207	0.114	0.435	0.255	0.012	0.206	
Zn	Pear_Cor.	-0.460	-0.619	-0.284	-0.644	0.389	-0.763	-0.139
	p	0.001	0.001	0.009	0.001	0.001	0.001	0.126

observations could be targeted in the treatment of bone marrow fibrosis in these diseases. In this study, similar to the results of the literature studies, serum copper level was found to be significantly higher in patients with myeloproliferative neoplasm. When PMF, CML and PV patients were compared with the control group, serum copper levels were found to be high in the patient group while no statistically significant difference was found in ET groups. In the literature studies conducted, we did not find any study showing the relationship between ET and copper serum levels.

In the ecologic study carried out by Spangler et al., it was found that there was a correlation between cancer and environmental manganese levels (10). The anticarcinogenic effect of manganese on tumor induced by nickel subsulphide was reported by Sunderman et al. (11), and Marklund et al. found that manganese showed this anticarcinogenic effect by activating the superoxide dismutase (12). In the study carried out by Wen CG et al. on the importance of trace elements in patients with acute leukemia, they found that serum copper and nickel levels were high and serum zinc and manganese levels were low (13). In the study carried out by Wang YH et al. with human acute myeloid leukemia cells, they showed that manganese superoxide dismutase regressed the proliferation of HL-60 human leukemia cells in relation to concentration and time (14). In the study carried out by Vydyborets et al. on the relationship between PV and manganese level, abnormalities were found in manganese and zinc levels in erythrocytes (15). In the literature reviews performed, we have determined that there is no study on correlation between serum manganese level and ET, CML and PMF; and in these current 34 studies, unlike existing studies, we found that serum manganese level was low in PMF and high in PV compared to the control group. No statistical difference was found between serum manganese levels in ET and CML compared to the control group. Although cadmium does not have any basic function in the human body, it may cause toxic effects even at very low doses (16). The correlation between high serum cadmium levels and cancers such as prostate, kidney, lung and stomach cancer has been revealed in various studies (17). In contrast to these studies, Mahmoud A. Ghandour et al. reported that serum cadmium levels were low, in their study on patients with acute leukemia (18). In the study of Demir et al. on acute leukemia, serum cadmium levels in leukemia patients were reported to be high. As far as we know, no literature study has been reported on the correlation between serum cadmium levels and PV, ET, PMF and CML. Therefore, herein, it was focused that determination of serum cadmium levels was investigated for PV, CML, PMF and ET patients and the control group.

It has been demonstrated in both in vitro and in vivo studies that lead compounds can lead to genetic damage with various indirect mechanisms, like the inhibition of DNA synthesis and repair, oxidative damage, the interaction between tumor suppressor proteins and DNA-linked proteins.

Lead-associated cancer cases revealed by experimental rat models have been published (19). In the study carried out by Cooper and Gaffey on workers exposed to lead in the United Nations, they revealed that there was an increase in the mortality of respiratory and digestive system neoplasms (20). In the study in which Demir C. et al. investigated the serum levels of some elements in acute leukemia in Turkey, serum lead levels were revealed to be significantly higher compared to healthy controls. Although we were not able to find a literature study showing the correlation between myeloproliferative neoplasms such as PMF, ET, PV and CML and serum lead levels, we found in our current study that the serum lead level was statistically significantly high in ET, CML and PV compared to the control group. No statistically significant difference was found between the PMF group and healthy control group. Iron is the most abundant metal in the crust of the earth. In experimental mouse and rat studies related in the study carried out by Adronikashvili and Mosulishvili on human leukemic cell DNA, they found that the ratio of iron in leukemic cell DNA was lower than normal (21). In the literature, there are several studies on the relationship between chronic myeloproliferative neoplasms and iron. Strati P. et al. suggested that microcytosis is a common symptom in PMF cases and that this could be due to the dysregulation of the iron balance (22). In the study in which Pardanani et al. investigated the prognostic effects of serum ferritin, hepcidin, and inflammatory cytokines in PMF, they found that increased serum hepcidin and ferritin levels were the markers of poor prognostic and shortened life expectancy (23). Tarkun P. et al. found that serum GDF-15 (growth differentiation factor-15) levels significantly increased in PV and ET patients and that serum hepcidin levels were not statistically different from the control group. In this study, the fact that hepcidin levels were not suppressed despite increased erythroid activity and high GDF-15 levels was shown, and it was suggested that this could prevent the increase in erythropoietic activity that may result from the reduced iron utilization (24). Although no literature study showing the relationship between CML patients and iron was found, we found that serum iron levels were low in PV, ET, CML and PMF patients in this present study. Nickel is an element that occurs naturally in the earth and is found in the soil, sediment layer, water resources, plants and animals. There are many epidemiologic studies showing the relationship between exposure to nickel and increased nasal and lung carcinogenesis (25). Wen CG et al. found that serum nickel level was high in patients with acute leukemia and argued that this high level could be the indicator of inadequate response to the treatment and poor prognosis (13). In a study conducted in Chinese children with acute leukemia, urine nickel levels of the patient group were found to be high and it was suggested that nickel could be an etiological factor of childhood acute leukemia through DNA damage (26). No study showing the correlation between myeloproliferative neoplasms and nickel was found in the literature; in the present study, it was determined that serum nickel level was be significantly higher in PV, ET, PMF and CML patients.

Cobalt may cause DNA damage through the formation of hydroxyl radicals in the presence of hydrogen peroxide in in vitro human lymphocytes and isolated DNA (27). Sheppard et al. reported that serum cobalt levels are high in childhood acute leukemia patients (28). No study regarding the interaction between cobalt and PMF, ET and PV was found in the literature; in the present study, we determined that serum cobalt level was high in PMF, ET and CML. We did not find any statistical difference in terms of serum cobalt levels between PV patients and control group.

It has been shown that DNA synthesis increases with zinc addition and is inhibited by the decrease in zinc level (29). Although there is no study showing that zinc is carcinogenic for humans, there are some indirect epidemiological evidences regarding the fact zinc deficiency increases susceptibility to esohageal carcinogenesis (30). Simmer K et al. reported that zinc levels in the neutrophils of PV patients were lower compared to the control group and suggested that this could lead to some nonspecific symptoms in polycytemia (31). Georgia Metzgeroth et al. suggested that zinc protoporphyrin (ZPP) is produced instead of hem in PMF and ET in cases where there is impaired iron support to erythropoiesis and reported that is unlikely that ZPP concentration would be above 60 μmol/mol of hem in essential thrombocythemia after the exclusion of iron deficiency and that ZPP could be useful in monitoring the response of PMF patients to the treatment (32). In the present study, serum zinc levels were lower in PV, ET, CML and PMF patients. Because Zn is a very strong antioxidant element, and the fact that Zn level is low in patients gives rise to thought that it could be an important marker in PV, ET, CML and PMF cases.

In this study, it was determined that Zn and Fe levels were low and Cu, Pb, Cd, Co, Ni and Mn levels were increase in the patient group with myeloproliferative neoplasm. Cu, Pb, Cd, Co, Ni, Zn, Fe and Mn elements can play a significant role in the pathogenesis of myeloproliferative neoplasms. Moreover, there is a need for further studies on myeloproliferative neoplasms.

References

1. Abkowitz JL, Catlin SN, Guttorp P. Evidence that hematopoiesis may be a stochastic process in vivo. Nat Med 1996; 2: 190.
2. Zuo XL, Chen JM, Zhou X, Li XZ, Mei GY. Levels of selenium, zinc, copper, and antioxidant enzyme activity in patients with leukemia. Biol Trace Elem Res 2006; 114: 41-53.
3. Chang LW, Magos L, Suzuki T. Toxicology of Metals. Boca Raton. FL, USA: CRC Press; 1996.
4. Aldor Y, Walach N, Modai D, Horn Y. Zinc and copper levels in plasma, erythrocytes, and whole blood in cancer patients. Klin Wochenschr 1982; 60: 375-377.
5. Olivares M, Uauy R. Copper as an essential nutrient. Am J Clin Nutr 1996; 63: 791-796.
6. Gupte A, Mumper RJ. Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. Cancer Treat Rev 2009; 35: 32-46.
7. Demir C, Demir H, Esen R, Sehitogullari A, Atmaca M, Alay M. Altered serum levels of elements in acute leukemia cases in Turkey. Asian Pac J Cancer 2011; 12: 3471-3474.
8. Akanni EO, Onuegbu AJ. Assessment of Some Selected Trace Metals in Chronic Myeloid Leukemia Patients in a Tertiary Health Facility in South West Nigeria. Asian J Med Sci 2013; 5: 71-75.
9. Tadmor T, Bejar J. The expression of lysyl-oxidase gene family members in myeloproliferative neoplasms. Am J Hematol 2013; 88: 355-358.
10. Spangler JG, Reid JC. Environmental Manganese and Cancer Mortality Rates by County in North Carolina: An Ecological Study. Biol Trace Elem Res 2010; 133: 128-135.
11. Sunderman FW, Lau TJ, Cralley LJ. Inhibitory Effects of Manganese Upon Muscle Immunogenesis by Nickel Sulfide. Cancer Res 974; 34: 92-95.
12. Marklund S. Purification and Characterization of a Manganese Containing Superoxide Dismutase from Bovine Heart Mitochondria. Int J Biochem 1978; 9: 299-306.
13. Wen CG, Pang GY, Huang SQ. Significance of the serum level of Cu, Zn, Mn, Cr and Ni in acute leukemia. Zhonghua nei ke za zhi 1989; 28: 734-736.
14. Wang YH, Xu XJ, Zhang LF, Li HL. Mimic of manganese superoxide dismutase induces apoptosis in human acute myeloid leukemia cells. Leuk Lymphoma 2014; 55: 1166-1175.
15. Vydyborets SV, Tishchenko LM. The chemical element content of the erythrocytes in polycythemia vera. Vrachebnoe Delo 1989; 10: 80-81.
16. Rose J. (Ed.). Trace elements in health: A review of current issues, Butterworth-Heineman, 1983.
17. Reddy BS, Charles JM, Raju NJG, Vijayan V, Reddy SB, Kumar RM, Sundareswar B. Trace elemental analysis of carcinoma kidney and stomach by PIXE method. Nucl Instr Methods Phys Res B 2003; 207: 345-355.
18. Ghandour MA, Thabet AF, Mohamed R. Determination of Zinc, Copper, Cadmium and Lead in Serum of Patients with Acute Leukemia. J Environ Sci Toxicol Food Technol 2013; 5: 66-76.
19. IARC. Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 23, IARC, Lyon, in press.
20. Cooper WC, Gaffey WR. Mortality of lead workers. J Occup Med 1975; 17: 100.
21. Andronikashvili EL, Mosulishvili LM, Belokobil'skiy AI, Kharabadze NE, Shonia NI. Human leukaemic cells. Determination of trace elements in nucleic acids and histones by neutron-activation analyses. Biochem J 1976; 157: 529-533.
22. StratiP, Pemmaraju N, Estrov Z. Cardenas-Turanzas M, Pierce S, Newberry KJ, Daver N, Cortes J, Kantarjian H, Verstovsek S. Clinical significance of microcytosis in patients with primary myelofibrosis. Leuk Res 2014; 38: 1212-1216.
23. Pardanani A, Finke C, Abdelrahman RA, Lasho TL, Tefferi A. Associations and prognostic interactions between circulating levels of hepcidin, ferritin and inflammatory cytokines in primary myelofibrosis. Am J Hematol 2013; 88: 312-316.
24. Tarkun P, Mehtap O, Atesoğlu EB, Geduk A, Musul MM, Hacihanefioglu A. Serum hepcidin and growth differentiation factor-15 (GDF-15) levels in polycythemia vera and essential thrombocythemia. Eur J Hematol 2013; 91: 228-235.
25. Anttila A, Pukkala E, Aitio A, Rantanen T, Karjalainen S. Update of cancer incidence among workers at a copper/nickel smelter and nickel refinery. Int Arch Occup Environ Health 1998; 71: 245-250.

26. Urinary level of nickel and acute leukaemia in Chinese children.Yang Y1, Jin XM, Yan CH, Tian Y, Tang JY, Shen XM.. PMID:19106127 [PubMed - indexed for MEDLINE]
27. Lloyd DR, Phillips DH, Carmichael PL. Generation of putative intrastrand cross-links and strand breaks in DNA by transition metal ion-mediated oxygen radical attack. Chem Res Toxicol 1997; 10: 393-400.
28. Sheppard PR, Speakman RJ, Ridenour G, Witten ML. Temporal variability of tungsten and cobalt in Fallon, Nevada. Environ Health Perspect 2007; 115: 715-719.
29. Fujii T. Presence of zinc in nucleoli and its possible role in mitosis. Nature 1954; 174: 1108-1109.
30. Woo YT, Lai DJ, Arcos JC, Argus MF. Chemical Induction of Cancer, Structural Bases and Biological Mechanisms 1988.
31. Simmer K, Pearson TC, Wheeler MJ, Thompson RP. Zinc status in polycythaemia. Eur J Hematol 1987; 38: 433-436.
32. Metzgeroth G, Kanders EM, Erben P, Hofmann WK, Hastka J. The role of zinc protoporphyrin measurement in the differentiation between primary myelofibrosis and essential thrombocythaemia. Ann Hematol 2011; 90: 389-394.