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Hematological and hepatic alterations among copper mine workers and office employees in a copper mine in the west of Iran, 2015

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Abstract

Background: Workers in different occupational positions experience significant Cu exposures, however, Cu toxicity has not been fully studied as compared to other heavy metals. In the present study, hematological and hepatic alterations have been investigated among copper mine workers.
Materials and Methods: This descriptive study was conducted in a copper mine in west of Iran, on 402 copper mine workers (study group) and 52 office employees (control group) during winter 2015. 5ml blood samples were provided from each subject and hematological and hepatic parameters including white blood cell (WBC), platelet (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH), lymphocytes, neutrophils, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum Cu levels have been determined using their commercial kits. The results were analyzed in the SPSS software using t-test and regression method.
Results: ALT, AST, HGB, MCV, MCH and plasma Cu levels among the workers were significantly higher than the office employees (p<0.050). WBC and RBC counts among the workers were significantly lower than the control group (p= 0.049 and 0.024, respectively). Serum Cu levels of 215 of the workers were higher than its normal recommended range (120 µg/dl). For the subjects with serum Cu levels above 150 µg/dl, increases in AST and ALT were in compliance with serum Cu levels increase.
Conclusions: Significant hepatic and hematological alteration were observed among copper miners compared to control group. Employment of workers with background hematological and hepatic disorders in copper industries must be accompanied with great caution.

Keywords: Copper, Toxicity, Liver, Hematologic Diseases, Miners

Introduction

Copper (Cu) is an essential trace element found in all living organisms. Numerous physiological functions have been reported for Cu. Cu is necessary for structural and catalytic activity of a variety of important enzymes, including cytochrome *C* oxidase, tyrosinase, dopamine beta hydroxylase (DBH) and Cu-zinc super oxidase dismutase (Cu, Zn SOD). Cu supplements were used to treat anemia among animals

since 1920s and Cu deficiency was documented among humans in the 1960s, respectively (1).

Sources of environmental exposure to Cu include water pipes, cookware, contraceptive pills, vitamin and minerals supplements and fungicides with Cu is added for swimming pools. Plumbers, welders and machinists occupationally expose to Cu and are at risk of Cu toxicity (2). High levels of Cu exposure were reported among individuals working in copper-smelting areas and copper mines (3, 4).

Cu acute poisoning is a rare condition and most reports on Cu toxicity among human were related to its chronic and sub-chronic exposures. Cu chronic toxicity has been reported among patients undergoing dialysis treatment who receive dialysis fluid via Cu tubing and workers in pesticides industry (5).

After gastrointestinal or respiratory absorption, Cu mainly deposits in liver and its chronic toxicity primarily affects the liver. Cu toxicity is typically manifested by the development of liver cirrhosis with episodes of hemolysis and damage to renal tubules, brain and other organs. Symptoms can progress to coma, hepatic necrosis, vascular collapse and death. Cu poisoning may result in weakness, lethargy and anorexia in the early stages in addition to erosion of the epithelial lining of the gastrointestinal tract, hepatocellular necrosis and acute tubular necrosis (6). Background disease like anemia and hepatic disorders could exacerbate toxic effects of Cu excess. Women and elderlies are more sensitive to copper toxicity (7).

Experimental studies demonstrated that Cu excess induces dose and time dependent changes in hepatic enzymes activities and fatty liver syndrome among fish (8, 9). Among rats, chronic Cu exposure at sub-toxic level results in liver and kidney dysfunctions. Cu induced oxidative stress among fish liver suggests the involvement of differential mechanisms in Cu uptake and toxicity (9, 10). Two studies on sheep showed that sub-acute Cu exposure induce jaundice, anorexia, excessive thirst, hemoglobinuria and dramatic reductions in blood hemoglobin and glutathione concentrations. Transient increases have been reported in blood methemoglobin (11, 12).

Despite of these evidences about copper toxicity, there are a few studies on Cu hepatic and hematological effects among humans in the literature (7). Therefore, the present descriptive study was conducted with the aim to assess hematological and hepatic alterations among workers of a copper mine (in west of Iran) and office employees of this mine in winter 2015.

Material and Methods

This descriptive study was conducted in a copper mine in west of Iran, during winter 2015. 402 copper mine worker and 52 office employees were participated in this study as study and control groups, respectively. These exposed and un-exposed groups had the same conditions in terms of age, gender, race, working location and living areas.

All workers of the mine were participated in the study and were divided in two groups including, copper mine workers from different areas of the mine (as exposed group, n = 402) and office employees of this mine working in an office building, 5 km far away from the mine (as un-exposed group, n = 52). All subjects were men and two study groups were in the same conditions in terms of age, smoking status and living area. Necessary information like demographic characteristics (age, race, gender and occupational roles), smoking, medical and occupational history, special diet, residence location and secondary jobs were collected through a questionnaire before sampling, then the questionnaires were reviewed by the investigators for

identifying confounding factors. To avoid confounding factors, subjects were included only if they were with the lack of a history of smoking, hepatic or hematological diseases and background exposures to Cu. The proposal of the study has been approved by Islamic Azad University-Ahar Branch ethics committee. All of the subjects were provided with an informed consent.

Blood samples (venous blood, 5 ml) were collected aseptically using sterile 5 ml syringe. Serum was separated by centrifugation (Hettich Zentrifugen, Tuttlingen, Germany) of blood at 3000 × *g* for 10 minutes at room temperature, then the sera were transferred into 1.5 ml sterile microtubes and hematological and hepatic markers were determined. The selected markers included: red blood cell (RBC) counts, white blood cell (WBC) counts, platelet (PLT) counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH), lymphocyte counts (LYM), neutrophil percent (NEUT), serum concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). All samples were analyzed in a qualified central laboratory by a biochemical automatic analyzer (Autolab, AMS Corporation, Rome, Italy) using their commercial kits (Pars Azmoon, Esfahan, Iran). Serum copper was analyzed by its commercial kit (Zist-shimi, Tehran, Iran) according to manufacturer's instructions. All laboratory tests were performed in an approved medical lab in Tabriz, Iran, during winter 2015.

All measurements were repeated in triplicate and the data were presented as mean ± standard deviation (SD). Prior to statistical analysis, Shapiro-Wilk test was applied to determine the normal distribution of data. For assessment of the correlation between serum Cu levels and hematological/hepatic changes, Pearson and Spearman's correlation coefficient analysis was performed for data with normal and non-normal distribution, respectively. Student t-test was used for detection of differences in hematological and hepatic markers among exposed and unexposed groups. Statistical analysis was performed using the SPSS (version 20, IBM Corporation, Armonk, NY, USA) statistical software package. A probability of $P < 0.050$ was considered statistically significant.

Results

Demographic characteristics of study and control groups are summarized in table 1. No statistically significant difference was observed between the ages of exposed and the control group with mean ± SD of 36.00 ± 5.87 and 34.00 ± 6.65, respectively ($P = 0.017$). Mean ± SD of each hematological and hepatic parameter for copper mine workers and office employees of this mine are presented in table 1. Shapiro-Wilk test confirmed normal distribution of data for all parameters ($P = 0.037$).

ALT and AST levels among the miners were significantly higher than office employees ($P < 0.500$). WBC and RBC count decreased significantly among the miners ($P = 0.049$, 0.024) compared to control group. MCH increased significantly ($P < 0.0001$). HCT, PLT, LYM and NEUT levels showed no significant differences between study and control groups ($P > 0.050$). Significant decrease of hemoglobin levels was observed among the mine workers compared to office employees ($P = 0.003$). Linear regression showed no significant relation between increase in serum Cu levels and increase of AST and ALT ($P > 0.050$).

Serum Cu levels of workers in some areas of mine reached the rate of 230 µg/dl and its mean concentrations were significantly higher than recommended reference range (90-120 µg/dl).

Table 1: Hematological and hepatic parameters for workers and office employees of a copper mine in west Iran, winter 2015

Parameter / Study group	Copper mine workers (n = 402)		Office employees (n = 52)		Reference interval	P of T-test(95%)
	Mean	SD	Mean	SD		
AST (IU/l)	22.59	9.17	20.56	6.46	Up to 41	0.045
ALT (IU/l)	31.60	13.23	27.20	12.18	Up to 47	0.036
WBC count ($x10^3$/µl)	7.00	1.86	7.40	7.71	4-11.3	0.049
RBC count ($x10^6$/µl)	5.34	0.40	5.47	0.41	4.5-5.9	0.024
HGB (g/dl)	15.27	1.18	15.82	1.26	13.5-17.5	0.003
HCT (%)	46.29	3.04	46.14	3.16	42-50	0.756
MCV (fl)	86.72	3.01	84.32	6.63	80-96.1	0.011
MCH (pg)	29.64	1.26	27.96	2.67	27-33.2	< 0.001
PLT ($x10^3$/µl)	227.76	51.24	222.76	48.05	140-250	0.472
LYM (%)	39.77	9.38	40.15	7.65	25-45	0.724
NEUT (%)	48.32	9.45	47.49	8.20	35-75	0.533
Cu (µg/dl)	123.21	27.24	94.96	15.06	70-120	< 0.0001

* AST: Aspartate aminotransferase; ALT: Alanine transaminase; WBC: White blood cells; RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean cell volume; PLT: Platelet; LYM: Lymphocytes; NEUT: Neutrophils; Cu: Copper

Discussion

Cu in its ionic form rapidly becomes toxic to a variety of cells including hepatic and hemal systems. Excessive intracellular accumulation of Cu promotes formation of reactive oxygen species (ROS), catalyzing the reaction between the superoxide anion and hydrogen peroxide and production of hydroxyl radical. Furthermore, Cu can bind directly to free thiols of cysteines (Cys), leading to oxidation and crosslinks between proteins, thus inactivating enzymes or impairing structural proteins (13). Cu induced ROS generation initiate apoptotic/necrotic processes or other pathologies like cancer, neurological diseases, and aging (14). It was found that, multiple sclerosis (MS) subjects had increased levels of Cu in their fluids or brain cells (15).

The results of the present study showed that serum AST and ALT levels among workers were significantly higher than the office employees. In the case of AST, this increase was not very serious and only 12 miners showed elevated levels (above 41 IU/l) of AST while serum ALT levels of 48 miners were in alarming way, above its reference level (47 IU/l). As alcoholic beverages are forbidden in Iran, this increase cannot be due to the distractive effects of alcohol on hepatic cells. The AST/ALT ratios for these workers were below or equal to 1 indicating non-alcoholic hepatic injuries.

As mentioned above, in the initial analysis of the results, linear regression showed no significant relation between increase in serum Cu levels and increase of AST and ALT, however, the detailed analysis showed that among subjects with serum Cu levels above 150 µg/dl, increase in AST and ALT is in compliance with serum Cu levels increase (Figure 1A and 1B). A study by Sakhaee et al. also showed significant increase in serum ALT and AST levels among the individuals living in residential areas near the copper smelting complex (16). Another study by Kumar et al. showed that Cu mainly concentrated in liver (29 folds higher than kidney) and Serum ALT, AST and bilirubin correlated with liver Cu among rats (11). As, increase in AST and ALT levels is an important indicator for hepatic injury, routine monitoring of serum AST and ALT levels among the copper miners is necessary.

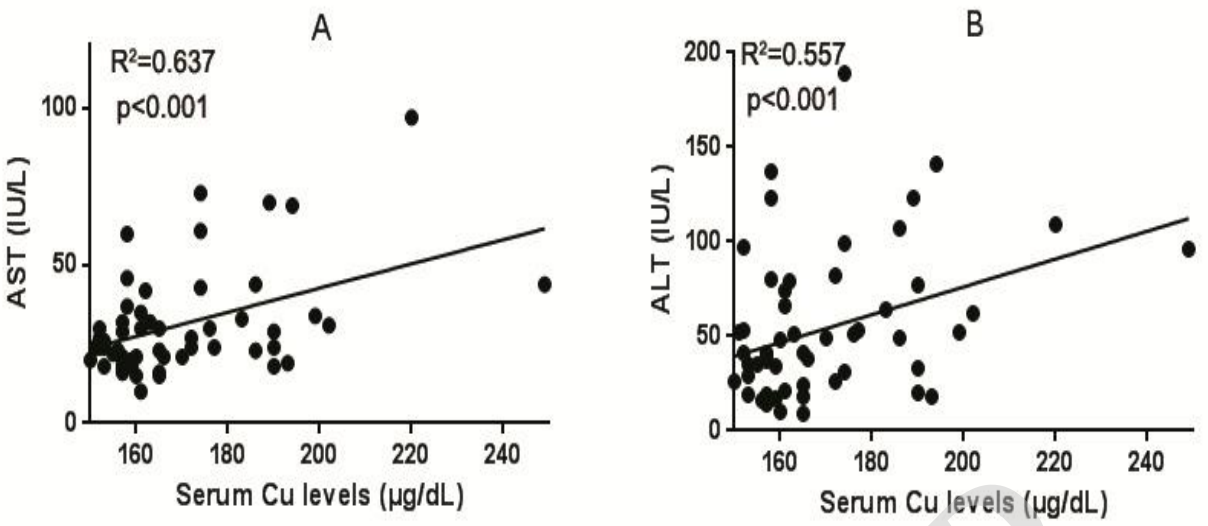

Figure 1: Correlation between serum Cu levels and hepatic injury indexes (ALT and AST) among workers of a copper mine in west of Iran, winter 2015

As seen in table1, RBC and WBC counts and hemoglobin content decrease significantly among the miners compared to the control group. Two previous studies on toxic effects of Cu on hematological system of the fish confirmed decrease of RBC count and hemoglobin content due to Cu toxicity, however, increase in WBC counts was reported in both studies (17, 18). This finding is in conflict with the results of the present study, which may be due to the different mechanisms of Cu toxicity among humans and fish. Further studies are required for better understanding of exact effects of Cu toxicity on WBC count. Furthermore, no direct correlations were found between increase in serum Cu levels and changes in hematological parameters, however, it is obvious that Cu toxicity could impose potent hematological consequences and continuous monitoring of these parameters among the exposed individuals is recommended.

Cu is sequestered in blood by the ceruloplasmin and its serum Cu level reflects recent exposures (19). In the literature, various levels were reported for serum copper concentrations among different human populations. Some of the recent studies in this filed are summarized in table 2. As expected, mean serum Cu levels of the office employees showed no significant difference with Iranian general population, however, its levels among the mine workers were significantly high. Serum Cu levels among 215 and 74 of workers were higher than its normal recommended range (120 µg/dl) (20) and above 150 µg/dl, respectively. It seems that copper mine workers experience intensive Cu exposures which can cause considerable health effects for them.

It should be noted that the number of subjects in control group (office employees of copper mine, n = 52) was lower than the study group (n = 402). It was a limitation for this study and could affect the study results. However, the office employees of this mine were the best match cases for control group of this study but unfortunately only 65 persons worked in this position. We suggest further studies on Cu-exposed workers with larger control groups.

Table 2: Mean serum Cu levels among different populations

Populations	Mean serum Cu levels (µg/dl)	Reference
Italian (general population)	122.50	(21)
Brazilian (general population)	89.00	(22)
Chinese (general population)	108.10	(23)
German (general population)	102.00	(24)
Swedish (general population)	85.50	(25)
Indian and Pakistani (general population)	103.00	(26)
Jordanian (Smokers)	232.80	(27)
Iranian (living near copper smelting complexes)	94.80	(16)
Iranian (general population)	93.63	(20)

Conclusion

The findings of the present study showed significant hepatic and hematological alteration among copper mine workers compared to the control group. These effects were in correlation with serum Cu levels. Employment of workers with background hematological and hepatic disorders in copper industries must be accompanied with great caution. In periodic medical checkup of workers, the physician should have a greater focus on the hematological and hepatic symptoms.

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Conflict of interest: None declared.

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