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Assessment of Blood Biochemistry and Hematological Parameters in Copper Pit Workers and Associated Employees

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ABSTRACT

The study describes the toxic health effects associated due to the chronic copper dust exposure to miners working in the actual mining area and its correlation with hematological and biochemical parameters. Total subjects (N=164) were selected and categorized according to their length and duration of exposure to copper dust. Blood metal concentration, biochemical and hematological parameters were assayed. Statistical analysis of results showed significant elevation in the levels of blood copper ($p<0.0001$), pack cell volume (PCV) and diminution in platelet count as well as mean corpuscular hemoglobin concentration (MCHC) ($p<0.05$) in chronically exposed Miners along with slight elevation in the counts of eosinophils as compared to control. Significant raise in serum total proteins (TP), albumin (A), globulin (G), Albumin/ globulin ratio (A /G ratio), cholesterol, triglyceride (TG), total bilirubin, urea, creatinine and random blood sugar along with raised activities of Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase were also recorded in chronically exposed miners (Miner-II) as compared to other groups. Significant positive pearson's correlationship of copper with such hematological parameters as pack cell volume ($r=18$) and negative correlationship with MCHC ($r = -0.21$) and platelet count ($r = -0.34$) were obtained. Such biochemical parameters as TP, A, G, cholesterol and TG, SGOT, SGPT, urea, creatinine and random blood glucose (RBG) were found to be associated with copper toxicity ($r = 0.22$; $r = 0.17$; $r = 0.27$; $r= 0.21$; $r= 0.25$; $r=0.18$; $r=0.17$; $r= 0.25$; 0.26 and $r=0.34$) and thus implicated. The odds ratio analysis validated that mean corpuscular volume (MCV), MCHC, TG, urea, creatinine, RBG and activity of SGPT were indeed affected by increase in the copper levels from normal to abnormal. Thus, the study suggests that chronic exposure to copper dust to pit workers does adversely affect certain hematological and biochemical parameters which may be used as guiding parameters for assessing health effects of such miners in future.
KEYWORDS: Biochemical parameters, Copper, Epidemiology, Hematological parameters, Metal Mine Toxicity, Occupational exposure and miner health.

INTRODUCTION

Copper has been mined all over the world since ancient time because of its essentiality in various spheres of life[1].Although, Copper as a micronutrient is necessary for good health, exposure in large doses adversely affect human health[2,3]. Infact, various ill effects on workers associated with such copper industries as smelting, brassing, wiring etc, have been reported earlier[4,5]. Prolonged occupational and environmental exposure to such ferrous group of minerals as manganese, chromite, iron[6,7,8] and nonferrous metallic minerals as aluminium, copper, lead & Zinc have been speculated to cause toxic impact on human health[9,10,11].Certain studies indicate that workers exposed to copper dust from smelters grow signs of hepatomegaly, digestive disorders and a range of respiratory distress[12,13,14]. The magnitude of these effects largely depended upon both level and length of exposure[15]. Deposition of copper in liver, brain, kidney and lung among dead workers at copper smelters have also been evidenced in Sweden[16]. Abnormal clinical symptoms pertaining to respiratory, nervous, skin and hematological status in copper fume exposed human have also been envisioned[17].It may be recalled that long term exposure to copper dust from copper smelters was found to cause irritation of nose, mouth and eyes accompanied with headache, dizziness, nausea and diarrhoea[5].Concerns have been expressed that exposure to chronic high levels of copper could enhance such risk factors as lung cancer and coronary heart diseases[12,13].It was found that studies represent demographic studies in copper smelters but systematic analysis of biochemical and hematological parameters were not investigated earlier and especially in copper mine workers. Surprisingly, reports are unavailable regarding the toxic effects of this metal in subjects working in open pit workers specifically with regards to the tenure of exposure in such mining operations. It may be recalled that chronically exposed miners from copper pit were first reported by us to suffer from high blood pressure, diabetes, Ischemic Heart disease (IHD) accompanied with bone and joint pain, chest pain, abdominal pain, backache and hair depigmentation[18]. It was also observed that office employees staying in the

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vicinity of mine were susceptible to diabetes and high blood pressure.[18]However, analysis of the risk associated with exposure of copper dust in chronically and mild (Acute) exposed miner as well as office employee's residing in the vicinity of mine were not examined earlier. Delineation of toxic effects of copper through copper dust exposure from a copper mine on the overall health status of miners therefore needs careful examination in order to evaluate their pathological overtone, if any. The present study attempts to obtain a cogent view regarding the variations in various biochemical and hematological parameters amongst the study groups as associated with metal covariates present in blood. A systematic evaluation of their probable association with blood biochemical parameters as random blood sugar, proteins and lipid profile, liver and kidney function tests as well as hematological parameters has been presented here by employing appropriate statistical tools.

MATERIAL AND METHODS

Selection of Subjects

A cross sectional study of mine workers (n=87) in the age group of 20-60 years were selected from copper mine workers from Malanjkhand area of Maydha Pradhesh (India). The health status of the employees was evaluated using a standard questionnaire.[18] Informed consent was obtained from all subjects prior to participation in this study. Information regarding exposure assessments included individual dust concentration monitoring, measurement of working condition and personal protective equipments which was obtained from the mining authorities as per their routine evaluation. Inclusion and exclusion criteria for subjects were fixed before selection of the study. Selected mine workers having exposure period to copper dust for more than 1 year were included in this study. All selected subjects were male working daily for 8 hours in the copper mine and female workers were excluded from this study. Miners who were engaged in mining activities (dozer, dumper, shovel operator and driver etc) and their duration of prolonged exposure to copper mine ranging from 1 to 40 years were considered as Experimental (EX) subjects. In order to assess the effect of copper dust exposure (which included copper and other associated metals such as manganese and zinc) at various duration, these subjects were further subdivided into two groups and classified according to their period of exposure such as 1-10 years as Mild or Miner-I (n=16) and >11-30 years as chronically exposed miners or Miner-II (n=71). For the purpose of comparison, a separate group of subject (n=30), considered as healthy individual were chosen from non mining area such as Nagpur region, which served as normal control after age and sex match. Office workers (n=47) not working in the mine but residing in the same mining environment were chosen as experimental control after age and sex match.

Sample Collection:

Blood samples were collected from all subjects using aseptic conditions. To minimize the possibility of blood sample contamination, workers were instructed to report for the collection before the start of shift and 5ml sterile syringes (metal free) were used for collection of blood. 3ml Whole Blood was collected in sterile tube for determination of metal concentration in blood and hematological parameters. Remaining blood samples were allowed to clot and centrifuged at 1000 rpm for 5 min. Aliquots of serum samples were allowed to freeze immediately and stored at -40ºC. The serum samples were then used for measurement of various biochemical parameters.

Analysis of Hematological Parameters

The various hematological parameters, such as counts of red blood cell, total leucocyte and platelet; contents of hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) as well as pack cell volume (PCV) and mean corpuscular volume (MCV) were determined by employing CBC analyzer.

Analysis of Biochemical Parameters

The estimation of Total Protein (ACCUREX – Autozyme), albumin (Erba Mannheim catalogue no. FBCER0045), glucose (ACCUREX –Eco-Pak Glucose), total cholesterol (ACCUREX – Autozyme cholesterol), triglyceride (ACCUREX – Autozyme Triglyceride), total HDL cholesterol (ACCUREX – Autozyme HDL- cholesterol) from serum were estimated by employing the methods of Henry *et al.*[19]; Doumas *et al.*[20]; Trinder *et al.*[21]; Gordon *et al.*[22]; Tietz *et al.*[23]; and Richmode *et al.*[24]respectively. All the above analysis was performed using various commercially available kits for respective assays employing semi autoanalyser. Analysis of alkaline phosphatase (ACCUREX – Autozyme), Serum glutamate oxaloacetate transaminase (ACCUREX – AutoZyme GOT UV Kinetic) and Serum glutamate pyruvate transaminase (ACCUREX – AutoZyme GPT UV Kinetic) were assayed by the method of Henry *et al.*[19]; Bergmeyer *et al.*[25]; and Tietz[26]respectively. Total bilirubin and direct bilirubin (ACCUREX – AutoZyme Bilirubin) were assayed according to Jendrassik *et al.*[27]; whereas urea (BEACON- Code No. Z-15, Z-15A) and Creatinine (ACCUREX – Autozyme UV kinetic) were estimated by the method of Henry *et al.*[19]and Fawcett *et al.*[28]respectively.

Quantitative estimation of Copper, Manganese and Zinc in blood by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES):

The metal content of the blood samples was analyzed through the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) by using multi element analysis technique. A detector measures the intensity of the emitted light and calculates the concentration of the particular element in the sample. Metal concentration was determined according to the methods of Bazzi et al and Henk[29,30]. Experimental set was prepared for determination of copper, Manganese and Zinc by using 2 ml blood sample with concentrated Nitric acid (30ml) in a glass beaker. This mixture was digested on hot

plate at 150^0C for 30 minutes. Digestion solution (upto 0.5ml) was filtered through whatman filter paper no. 40 and filtrate was diluted with nitric acid (0.5%) and volume was making up to 25-30 ml in marked graduated glass tube. Instead of blood sample, ultrapure water was used in another set of experiment followed by the same procedure. This set of experiment was used as blank. The final solution was aspirated in ICP-AES after Zeeman background correction. Each sample was checked after running separately from prepared mix standard in ICP-AES. Metal concentration of copper, Manganese and Zinc was calculated in PPM or mg/L.

Statistical Analysis

Data analysis was carried out using SPSS (version 19) and R-3.0.1 programming language with pre-validated programs. Analysis of covariance (ANCOVA) was carried out in all subjects amongst various study groups independently to adjust possible risk factors such as age, BMI and habitual characteristics (i.e. smoking, tobacco chewing and alcohol consumption) in order to assess the true effect of exposure in them. One way analysis of variance (ANOVA) was then used to calculate statistically significant difference in the overall mean adjusted values of various parameters across the study groups. This was followed by Tukey's post-hoc comparison analysis. P value of <0.05 was considered as statistically significant for all the analysis. Linear regression model was also undertaken using logistic regression analysis. The resulting model fitness was evaluated by referring to Hosmer-Lemeshow test. The odds ratios (OR) were obtained as a measure of effect of varying levels of copper, manganese and zinc concentrations as the risk factors. The normal and the abnormal ranges of each parameter were considered as two categories (dichotomous outcomes) dependent on the three metals, which were treated as independent predictors. The odds ratio (OR) more than 1.1 for corresponding metals was considered as risk factor (s).

RESULTS

A) Hematological Parameters:

Assessment of the hematological parameters amongst the study groups was examined by obviating the influence of such confounders as age, BMI and several habitual characteristics by employing analysis of *covariance* (ANCOVA). Post adjustment, ANOVA was performed to assess significant differences if any, in the above parameters across the study groups which has been presented in **Table 1**. It is apparent from **Table1** that both mild and chronically exposed miners showed statistically significant variations in the counts of lymphocyte, Eosinophil, MCHC and platelet counts and pack cell volume ($p<0.05$) as compared to either control subjects (normal and office employees) as verified through *one way analysis of variance. However*, the contents of Hemoglobin (Hb%) and MCH and counts of total leukocyte, neutrophil, monocyte, red blood cells (R.B.C) as well as MCV were found to remain unaltered.

On application of tukey's post-hoc pair wise comparisons, it was seen that there were statistically significant elevation in pack cell volume ($p<0.05$) along with significant diminution in MCHC and platelet count ($p< 0.05$) in Miner-II as compared to normal control. On the other hand, mildly exposed miner (Miner-I) did not experience any significant variation in all the hematological parameters except for platelet count which was significantly reduced ($p<0.05$) as compared to normal control. Concurrently, significant raise in the counts of eosinophil ($p<0.05$) along with significant diminution in MCHC ($p<0.05$) were noticed amongst Miner-II when compared to office employees. Interestingly, a significant drop in the platelet count ($p< 0.0001$) without any alteration in other hematological parameters was seen in office employees as compared to normal control.

Table 1: Hematological parameters in various study groups post-adjustment with confounder

Characteristics	Groups					Pair wise comparisons‡					
	Controls (n=30)	Office Employee (n =47)	Miner- I (n=16)	Miner-II (n=71)	P-value*	Control Vs Office employee	Control Vs Miner-I	Control Vs Miner-II	Office Employee Vs Miner-I	Office Employee Vs Miner-II	Miner-I Vs Miner-II
Hematological Parameters											
Hemoglobin (gm%)	13.44 ± 1.49	13.13 ± 1.53	12.86 ± 1.69	12.92 ± 1.57	0.4306	0.8166	0.6184	0.4072	0.9347	0.8928	0.9991
Total Leukocyte Count (/cumm)	7182.67 ± 1322.15	6516.32 ± 1795.89	7527.17± 1284.92	7065.75 ± 2906.2	0.3554	0.5876	0.9605	0.9952	0.4118	0.5677	0.8813
Neutrophils %	63.5 ± 10.34	58.21 ± 12.89	60.43 ± 9.56	62.07 ± 11.77	0.2003	0.2163	0.8304	0.9435	0.9133	0.2969	0.9567
Lymphocytes %	28.88 ± 10.38	35.05 ± 12.86	32.31 ± 8.63	29.73 ± 11.5	**0.0547***	0.1031	0.7702	0.9864	0.8433	0.0702	0.8494
Eosinophils %	4.03 ± 1.33	3.55 ± 1.39	3.62 ± 1.62	4.79 ± 2.38	**0.0036***	0.6984	0.8943	0.2628	0.9994	**0.0037***	0.1193
Monocytes%	2.94 ± 0.72	3 ± 0.86	3.41 ± 2.3	3.17 ± 1	0.4808	0.9948	0.5232	0.7825	0.5884	0.8607	0.8608
Red Blood Cell (Countmil/cumm)	4.51 ± 0.54	4.85 ± 0.59	4.76 ± 0.85	4.96 ± 1.02	0.1004	0.2814	0.7564	0.0656	0.9799	0.9135	0.8291
Pack cell volume%	40.37 ± 4.91	41.75 ± 5.8	43.17 ± 7.13	44.12 ± 6.13	0.0212	0.7509	0.4236	**0.0218***	0.8411	0.1511	0.9395
Mean Corpuscular volume (fL)	89.08 ± 9.38	86.71 ± 13.11	91.46 ± 12.83	90.04 ± 11.84	0.4027	0.8295	0.9181	0.9831	0.5171	0.4497	0.9733
MCH (Picogram)	28.51 ± 3.75	27.41 ± 4.11	27.85 ± 3.14	26.91 ± 3.72	0.2669	0.5957	0.9423	0.2144	0.9774	0.8986	0.8064
MCHC %	31.93 ± 3.53	31.95 ± 4.95	30.32 ± 1.9	29.59 ± 2.23	**0.0008****	0.9999	0.4322	**0.0115***	0.3592	**0.0021***	0.8719
Platelet Count(/cumm)	277.49 ± 43.62	191.74 ± 60.6	192.63 ± 34.74	171.63 ± 48.08	**0.0001*****	**0.0001*****	**0.0001*****	**0.0001*****	0.9999	0.1485	0.4337

†Adjusted for age, BMI, smoking, tobacco and alcohol; **P*-value obtained using one way ANOVA; ‡Tukey's post hoc comparisons; Bold numbers indicate statistical significance

*denotes p value less than < 0.05 as considered statistically significant

**denotes p value less than < 0.001 as considered highly significant

*** denotes p value less than < 0.0001 as considered extremely significant

B) Biochemical Parameters

Table 2 depicts the effects of short term and long term exposure to copper dust in pit workers on various biochemical parameters in serum. In order to obviate the influence of such confounders as age, BMI and several behavioral characteristics, a fresh assessment was made on the above biochemical parameters among the study groups by employing analysis of covariance (ANCOVA). Post adjustment, ANOVA was used to calculate the significant difference of the above said parameter if any, across the study groups which have been presented in **Table 2**. It was perceived that except for the levels of high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, Choleseterol/HDL cholesterol ratio and direct bilirubin, rest of the biochemical parameters such as total protein, albumin, globulin, cholesterol, triglyceride, total bilirubin, Urea, Creatinine & random blood glucose and activities of SGOT, SGPT, alkaline phosphatase all exhibited significant elevation ($p<0.0001$) in both the miner groups when compared to either control groups which was further substantiated by One way analysis of variance (ANOVA).

Protein Profile:

When tukey's post-hoc comparisons of serum protein profile were made between all the study groups, post adjustment with confounders, it was found that both miners having mild or Chronic exposure experienced significant elevation in the contents of total protein and globulin as compared to either controls respectively, though the degree of elevation was found to be much higher in chronically exposed ones ($p<0.05$; 0.0001, respectively). Elevation in the content of Albumin was observed in Miner-II ($p<0.05$) as compared to normal control, through it did not vary significantly amongst the miners when compared to office employees. Miner-II recorded a slight decrease in A/G ratio ($p<0.05$) as compared to normal control, but was found to be significantly diminished ($p<0.0001$) in either miner groups when compared to office employees. This ratio however was found to be unaltered when intercomparison were made between either miner groups or between control groups.

Lipid profile:

It is interesting to note that Miner-II was found to exhibit significant elevation in the contents of cholesterol and triglyceride when compared to either control groups (normal or office employees). Contrarily, except for a slight increase in triglyceride ($p<0.05$), in Miner I, other lipid parameters were found to be unaltered in them. Intercomparison of cholesterol/HDL cholesterol ratio between Miner-II with Miner-I group revealed that this ratio was significantly enhanced in Miner-II ($p<0.05$) as compared to Miner-I group in **Table 2**. Rest of the other lipid parameters did not show any significant variation on verification with Tukey's post-hoc pairwise analysis. Also, no significant variation was observed in all lipid parameters between normal control and office employees.

Liver function test:

On pairwise comparison, after adjustment with covariates total bilirubin was found to be significantly raised ($p<0.05$) in miner-II alone as compared to either control groups, though direct bilirubin was not found to be altered in **Table 2**. Although, a significant elevation in the activity of serum SGPT ($p<0.0001$) was observed in both the miner groups as compared to either controls, the activity of serum alkaline phosphatase was found to be significantly elevated in both the miner groups as compared to normal control alone but not with office employees. The activities of these liver enzymes however were not found to exhibit any perceptible variation between the miners. Further, the activity of serum SGOT was found to be significantly decreased only in Miner-II ($p<0.05$) but not in Miner-I when compared to normal control. However, there was no significant alteration in the activity of this enzyme when comparisons were made between either of the miner groups with office employees. Further, activities of serum SGOT and alkaline phosphatase were significantly elevated ($p<0.05$) along with slight decrease in the activity of SGPT in office employees when compared to normal control, though no significant variation existed in this activity between the miner groups **(Table 2)**.

Kidney Function Test:

Serum contents of urea and Creatinine were found to be significantly elevated in both the miners when compared to normal control, though the degree of elevation was found to be much greater in chronically exposed ones ($p<0.0001$). When the above contents were compared between office employees with either miners, it was observed that urea recorded significant elevation in Miner-II ($p<0.0001$) alone but not in Miner-I. Creatinine content was however found to be significantly higher in both the miner groups **(Table 2)**. It is relevant to note that these kidney function parameters did not vary significantly between miners as well as between control groups which was substantiated by tukey's post-hoc comparisons made after adjustment **(Table 2)**.

Random Blood Sugar:

When tukey's post-hoc comparisons were made after adjustment, it was seen that all the miners ($p<0.05$) as well as office employees registered significantly higher ($p<0.0001$) random blood glucose as compared to normal control. However, when Intercomparison of this parameter was made between the miner groups there was hardly any difference though office employees were found to show significantly higher random blood sugar levels ($p<0.05$) as compared to normal control**(Table 2)**.

Table 2: Biochemical parameters in various study groups post-adjustment with confounder

Characteristics	Groups					Pair wise comparisons‡					
	Controls (n=30)	Office Employee (n =47)	Miner- I (n=16)	Miner-II (n=71)	P-value*	Control vs Office Employee	Control vs Miner-I	Control vs Miner-II	Office Employee Vs Miner-I	Office Employee Vs Miner-II	Miner-I Vs Miner-II
B. Biochemical Parameters											
Protein Profile											
Total protein (g/dl)	6.84 ± 1.42	6.55 ± 0.9	8.25±1.6	8.39 ± 1.38	**< 0.0001**	0.7733	**0.003**	**< 0.0001**	**0.0001**	**< 0.0001**	0.9796
Albumin (g/dl)	3.63±0.64	3.83 ± 0.55	3.9±0.66	4 ± 0.57	0.0343	0.4783	0.4623	**0.0215**	0.9775	0.3926	0.9152
Globulin (g/dl)	3.2±1.04	2.73 ± 0.84	4.41 ± 1.54	4.73 ± 1.77	**< 0.0001**	0.4702	**0.0314**	**< 0.0001**	**0.0003**	**< 0.0001**	0.8446
A/G ratio (slightly above 1)	1.31 ± 0.67	1.62 ± 0.79	1.08 ± 0.4	1 ± 0.51	**< 0.0001**	0.1548	0.6446	**0.1132**	**0.0186**	**< 0.0001**	0.9672
Lipid Profile											
Cholesterol(mg/dl)	170.31 ± 25.09	176.3 ± 28.1	185.84 ± 27.58	198.65 ± 28.8	**< 0.0001**	0.7938	0.2766	**< 0.0001**	0.6385	**0.0002**	0.3468
Triglycerides(mg/dl)	104.92 ± 22.53	129.73 ± 34.26	158.16 ± 61.84	177.04 ± 50.1	**< 0.0001**	0.0729	**0.0006**	**< 0.0001**	0.1115	**< 0.0001**	0.3982
HDL cholesterol(mg/dl)	31.14 ± 6.97	30.8 ± 8.07	30.27 ± 4.54	29.72 ± 8.68	0.8255	0.9978	0.9849	0.8436	0.9958	0.8873	0.9943
Low Density Lipoproteincholesterol(mg/dl)	116.33 ± 29.22	119.31 ± 22.82	123.25 ± 21.97	126.24 ± 30.44	0.3308	0.9666	0.8477	0.3499	0.9599	0.5379	0.9793
Cholesterol/HDLcholesterol ratio(mg/dl)	7 ± 7.1	6.36 ± 7.17	2.15 ± 6.86	7.38 ± 7.6	0.0823	0.9826	0.1457	0.9952	0.1971	0.8826	0.0523
Liver Function Test											
Total Bilirubin(mg/dl)	0.67 ± 0.17	0.72 ± 0.22	0.96 ± 0.68	0.96 ± 0.52	**0.0023**	0.9418	0.1267	**0.0109**	0.2325	**0.0204**	0.9999
Direct bilirubin(mg/dl)	0.4 ± 0.16	0.33 ± 0.14	0.36 ± 0.17	0.39 ± 0.31	0.5662	0.6056	0.9421	0.9914	0.9795	0.6288	0.9776
SGOT (IU/L)	22.56 ± 6.37	28.14 ± 5.09	24.19 ± 6.51	25.67 ± 5.27	**0.0003**	**0.0002**	0.7785	0.0533	0.0712	0.0886	0.7713
SGPT (IU/L)	29.95 ± 7.75	36.19 ± 9.99	48.73 ± 12.98	55.5 ± 10.71	**< 0.0001**	**0.0434**	**0.0005**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.0753
Alkaline phosphatase (IU/L)	53.59 ± 19.5	67.81 ± 17.82	79.83 ± 27.41	75.54 ± 25.21	**0.0001**	**0.0383**	**0.0014**	**0.0001**	0.2584	0.2672	0.9019
Kidney Function Test											
Urea(mg/dl)	26.58 ± 3.66	31.17 ± 5.63	37.58 ± 15.9	39.73 ± 14.73	**< 0.0001**	0.3146	**0.0116**	**< 0.0001**	0.2154	**0.0006**	0.9046
Creatinine(mg/dl)	0.96 ± 0.25	1.04 ± 0.26	1.26 ± 0.32	1.42 ± 0.32	**< 0.0001**	0.6379	**0.0064**	**< 0.0001**	0.0525	**< 0.0001**	0.2082
Blood Sugar											
Blood Glucose(mg/dl)	83.31 ± 13.04	154.91 ± 50.8	119.8 ± 25.61	122.21 ± 47.18	**< 0.0001**	**< 0.0001**	**0.0314**	**0.0003**	**0.0251**	**0.0004**	0.9971

†Adjusted for age, BMI, smoking, tobacco and alcohol; **P*-value obtained using one way ANOVA; ‡Tukey's post hoc comparisons; Bold numbers indicate statistical significance

*denotes p value less than < 0.05 as considered statistically significant

**denotes p value less than < 0.001 as considered highly significant

*** denotes p value less than < 0.0001 as considered extremely significant

C) Metal Concentration in Blood

Examination of Blood copper and other associated metals in chronically exposed mine workers as well as office employees were performed but not in Miner-I on the basis of evidence obtained from alteration in hematological and biochemical parameters across the study groups. In order to obviate the influence of such confounders as age, BMI and several habitual characteristics by employing analysis of covariance (ANCOVA). Post adjustment, ANOVA was performed to assess significant differences if any, in the above parameters across the study groups which has been presented in **Table 3**. Significant alterations in the concentration of blood copper and manganese were found in Miner-II and office employees as compared to normal control which was substantiated through one way ANOVA.

Chronically exposed mine workers as well as office employees exhibited significantly higher contents of copper ($p<0.0001$) as compared to normal control as obtained from tukey's post-hoc comparisons. However, the content of Manganese was found to be significantly altered in Miner-II ($p<0.05$) alone as compared to either control groups (normal and Office employees). On the other hand, there was insignificant alteration in the level of blood zinc ($p>0.05$) across the study groups. Interestingly, there were hardly any significant differences found to exist in the levels of blood metal concentration between Miner-II and Office employees.

Table 3: Blood Metal Analysis in various study groupspost-adjustment with confounder

					Pair wise comparisons‡		
Biochemical Parameters	**Controls (n=30)**	**Office workers (n =47)**	**Miners- II (n=71)**	**P-value***	**Control Vs Office workers**	**Control Vs Miners- II**	**Office workers Vs Miners- II**
Copper (mg/l)	0.66 + 0.34	2.14 + 0.84	2.22 + 0.79	**0.0001*****	**0.0001*****	**0.0001*****	0.8206
Manganese (mg/l)	1.09 + 0.65	1.89 + 1.35	2.09 + 1.85	**0.0118***	0.0674	**0.0087***	0.7686
Zinc (mg/l)	7.31 + 1.19	8.44 + 2.46	8.1 + 3.01	0.1699	0.1482	0.3385	0.7618

†Adjusted for age, BMI, smoking, tobacco and alcohol; **P*-value obtained using one way ANOVA; ‡Tukey's post hoc comparisons Bold numbers indicate statistical significance

*denotes p value less than < 0.05 as considered statistically significant

**denotes p value less than < 0.001 as considered highly significant

*** denotes p value less than < 0.0001 as considered extremely significant

D) Correlation analysis of metals with Hematological and Biochemical Parameters:

Hematological Parameters: Table 4 represents the scatter plot of adjusted blood copper content which showed a positive pearson's correlation (r = 0.18) with pack cell volume and negative correlation with MCHC and Platelet count(r = -0.21; -0.34) with significance ($p<0.05$) as shown in **figure 1, 2 & 3**. Further, Correlation between blood manganese levels with platelet count bore significance ($p< 0.05$) but was negatively correlated (r= -0.16) **(figure 4)**.

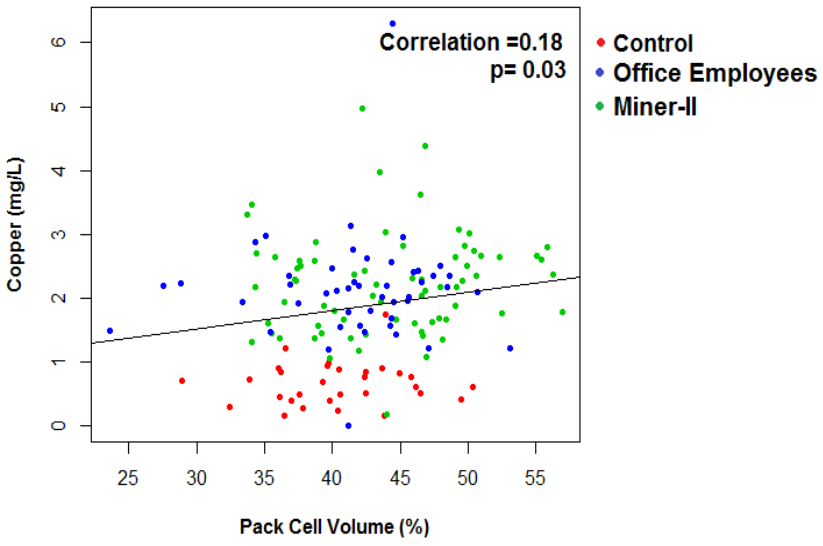

Figure 1: Showing Positive correlationship between Pack cell Volume &Copper

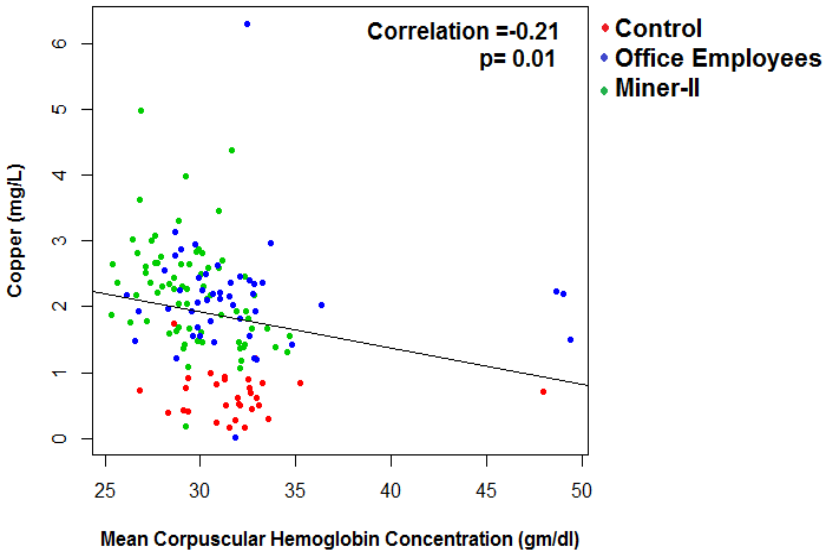

Figure 2: Showing Negative correlationship between Mean Corpuscular Hemoglobin Concentration & Copper

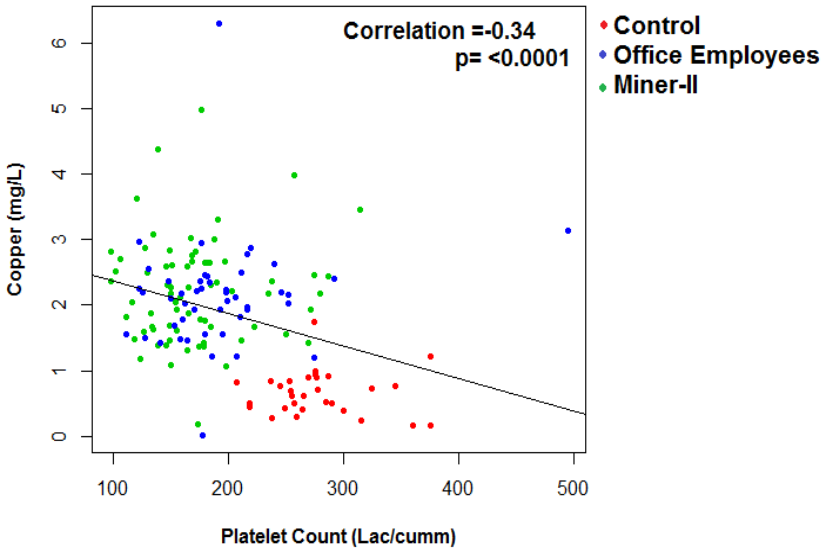

Figure 3: Showing Negative correlation between Platelet Counts& Copper

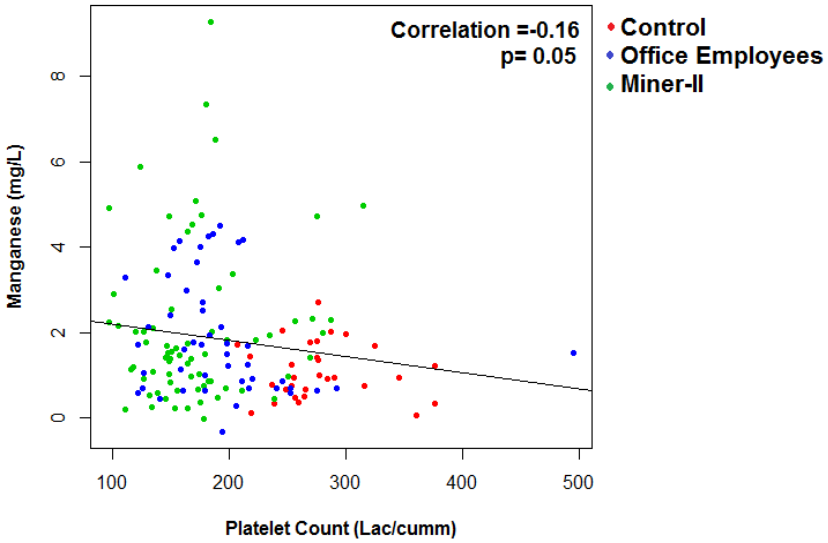

Figure 4: Showing Negative correlationship between Pack cell Volume & Manganese

Table 4: Pearson's correlation between different metals with hematological parameters

Hematological parameters	Correlation coefficient (*P*-value)		
	Metals		
	Copper	Manganese	Zinc
Hemogolobin	-0.10 (0.21)	0.02 (0.77)	-0.13 (0.12)
Total Leucocyte Count (TLC)	0.11 (0.18)	0.11 (0.17)	0.08 (0.32)
Neutrophils	0.11 (0.20)	0.08 (0.33)	0.05 (0.54)
Lymphocytes	-0.10 (0.24)	-0.08 (0.34)	-0.02 (0.79)
Eosinophils	-0.05 (0.54)	-0.05 (0.56)	-0.13 (0.10)
Monocytes	0.08 (0.31)	0.10 (0.21)	-0.05 (0.57)
Basophils	-0.11 (0.17)	-0.04 (0.64)	-0.04 (0.67)
R. B. Cs. Count	0.11 (0.19)	0.02 (0.84)	-0.01 (0.86)
Pack cell volume	**0.18 (0.03)**	0.15 (0.08)	-0.11 (0.20)
Mean Corpuscular volume (MCV)	0.05 (0.57)	0.12 (0.14)	-0.10 (0.23)
MCH	-0.15 (0.07)	0.01 (0.90)	-0.12 (0.16)
MCHC	**-0.21 (0.01)**	-0.12 (0.14)	-0.03 (0.75)
Platelet Count	**-0.34 (< 0.0001)**	**-0.16 (0.05)**	0.15 (0.07)

*denotes p value less than < 0.05 as considered statistically significant
**denotes p value less than < 0.001 as considered highly significant
*** denotes p value less than < 0.0001 as considered extremely significant

Biochemical Parameters:

When pearson's correlation analysis was carried out between Copper with either total protein, albumin, globulin, Cholesterol and Triglycerides, urea, creatinine and random blood glucose revealed positive correlationship (r = 0.22; r = 0.17; r = 0.27; r= 0.21; r= 0.25; r= 0.25; 0.26 and r=0.34 respectively), with statistical significance (p-values < than 0.01; 0.04; 0.001; 0.01; 0.002; 0.002; 0.001 and 0.0001respectively). The relationship has been depicted through scatter plot in **figure 5, 6, 7, 8, 9, 10, 11 & 12**. However, A/G ratio was perceived to bear a negative correlation (r=-0.11), though statistically insignificant (p>0.05). Further pearson's correlation study between metals with both the activities of SGOT and SGPT showed a significant positive correlation with Cu concentrations with coefficients (r= 0.18; 0.17) with p-value of 0.03 and 0.04 (p < 0.05) respectively. The scatter plots for these two parameters have been represented in **figure 13 &**

14 respectively. Manganese, the other associated metal also was found to show significant positive correlation (r= 0.16; r= 21) with Urea and creatinine levels significantly (p-value of 0.05 & 0.01) **(figure 15 & 16)**. However, Mn was found to be weakly correlated (r= 0.08) with glucose level with statistical insignificance (p > 0.05). On the other hand, Zn showed significant positive correlation (r= 0.22) with glucose levels with significance (p < 0.05) (Figure 17). However, Mn and Zn hardly demonstrated any significant correlation relationships with the rest of other biochemical parameters in **Table 5**.

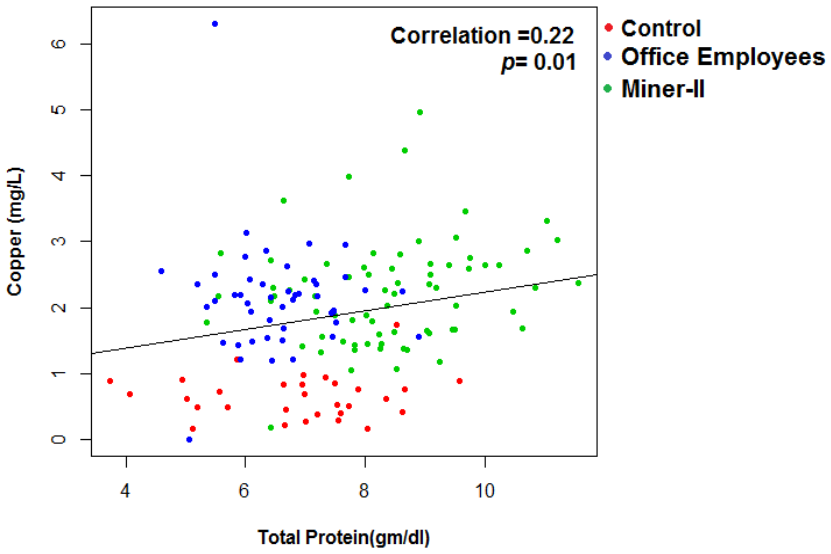

Figure 5: Shows positive correlationship between Total Proteins with copper

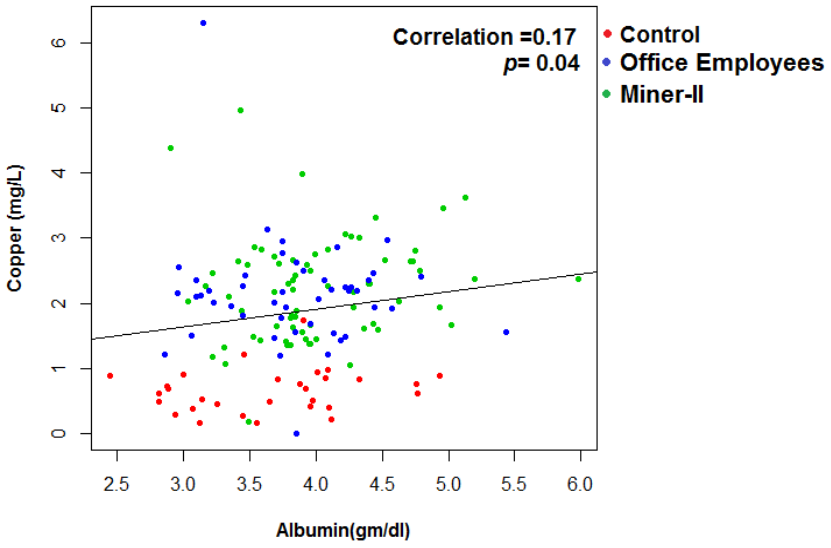

Figure 6: Shows positive correlationship between Albumin with Copper

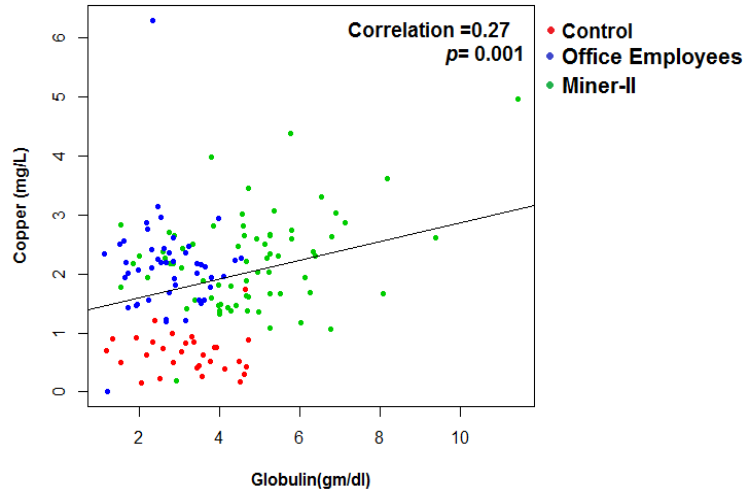

Figure 7: Shows positive correlationship between Globulin with copper

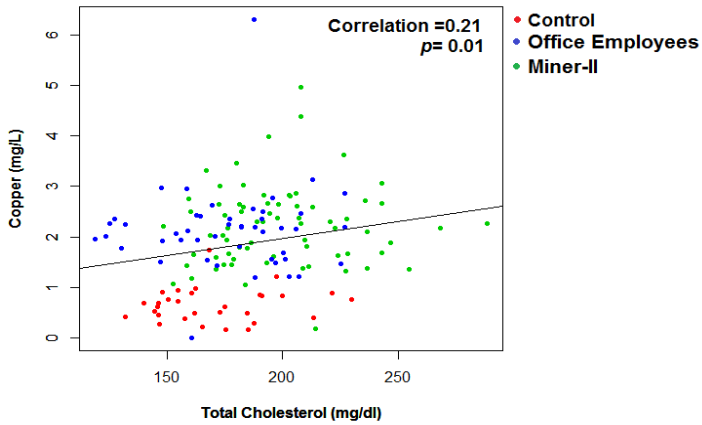

Figure 8: Shows positive correlationship between Total cholesterol with copper

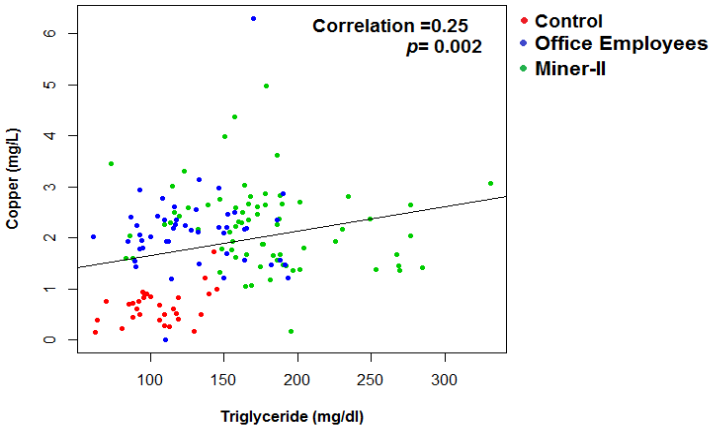

Figure9: Shows positive correlationship between Triglyceride with copper

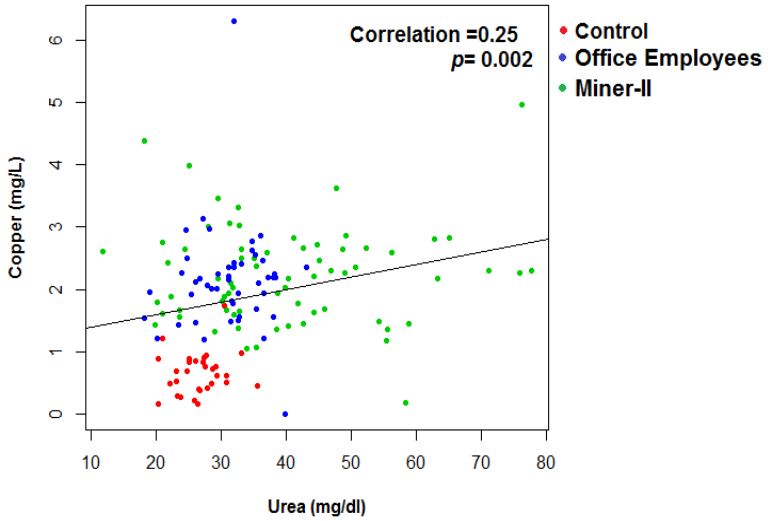

Figure 10: Shows positive correlationship between Serum Urea with Copper

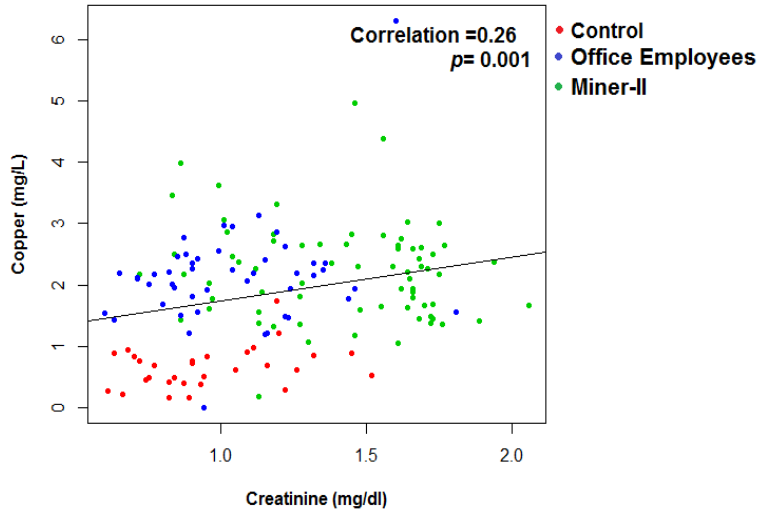

Figure 11: Shows positive correlationship between Serum Creatinine with Copper

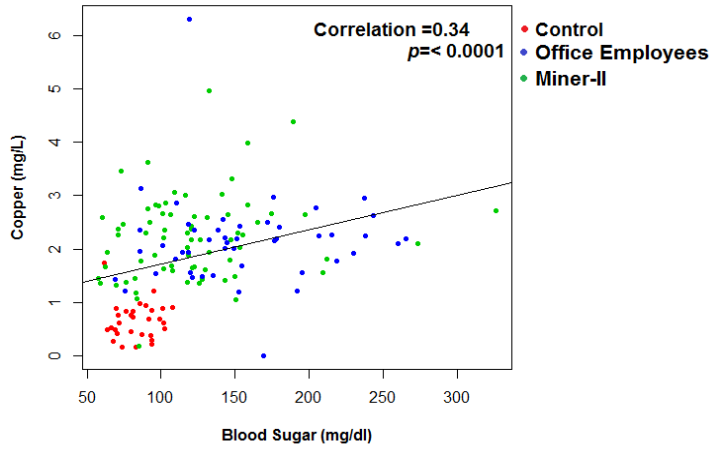

Figure 12: Shows positive correlationship between Random blood sugar with Copper

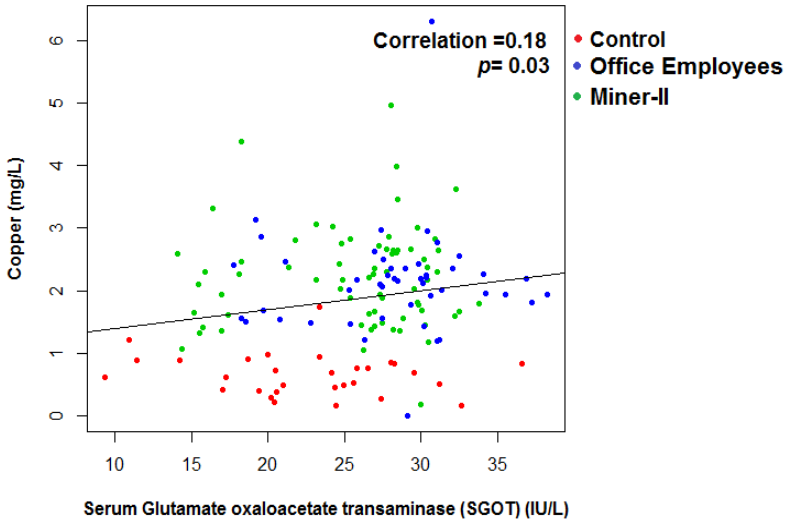

Figure 13: Shows positive correlationship between Serum Glutamate Oxaloacetate transaminase with copper

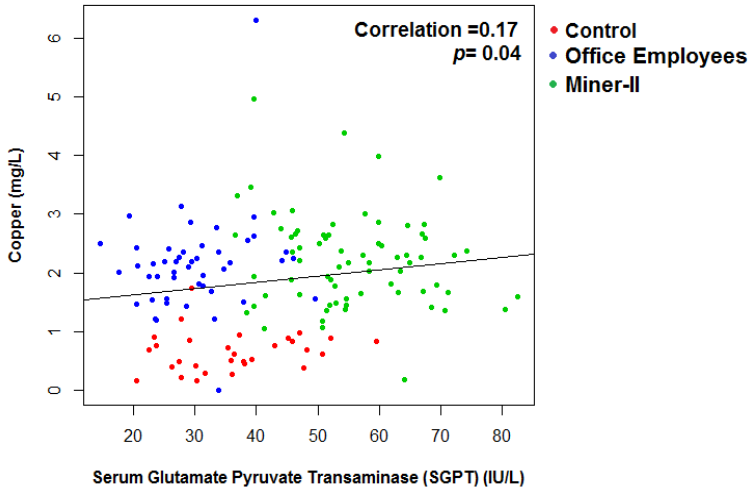

Figure 14: Shows positive correlationship between Serum Glutamate Pyruvate Transaminase with Copper

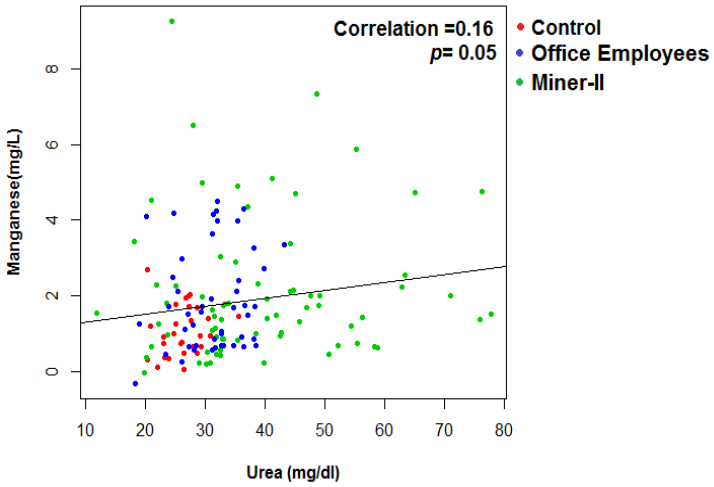

Figure 15: Shows positive correlationship between Serum urea with Manganese

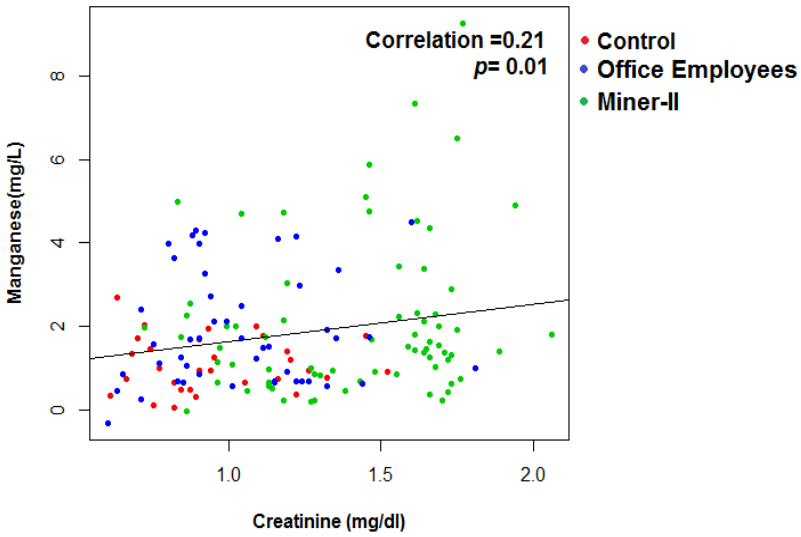

Figure 16: Shows positive correlationship between Serum Creatinine with Manganese

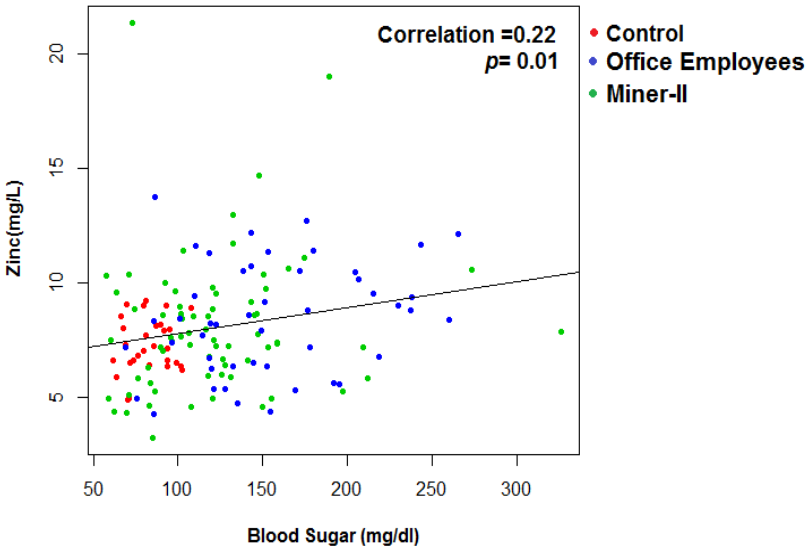

Figure 17: Shows positive correlationship between Random Blood sugar with Zinc

Table 5: Pearson's correlation between different metals with Biochemical parameters

B) Biochemical Parameters	Correlation coefficient (*P*-value)		
	Metals		
	Copper	Manganese	Zinc
I) Protein Profile			
Total protein (g/dl)	**0.22 (0.01)**	0.08 (0.30)	0.07 (0.39)
Albumin (g/dl)	**0.17 (0.04)**	0.10 (0.21)	0.08 (0.33)
Globulin (g/dl)	**0.27 (0.001)**	0.09 (0.30)	0.10 (0.25)
A/G ratio (slightly above 1)	-0.11 (0.20)	0.04 (0.66)	0.01 (0.92)
II) Lipid Profile			
Cholesterol (mg/dl)	**0.21 (0.01)**	-0.0004 (0.99)	-0.04 (0.64)
Triglycerides (mg/dl)	**0.25 (0.002)**	0.06 (0.47)	-0.09 (0.28)
HDL cholesterol (mg/dl)	0.06 (0.48)	0.06 (0.46)	-0.001 (0.99)
Low Density Lipoprotein cholesterol (mg/dl)	0.07 (0.39)	-0.05 (0.53)	0.02 (0.84)
Cholesterol/HDL cholesterol ratio (mg/dl)	0.07 (0.42)	-0.10 (0.23)	-0.08 (0.32)
III) Liver function Test			
Total Bilirubin (mg/dl)	0.07 (0.39)	-0.11 (0.20)	0.02 (0.79)
Direct bilirubin (mg/dl)	-0.05 (0.55)	-0.10 (0.21)	0.01 (0.95)
SGOT (IU/L)	**0.18 (0.03)**	0.08 (0.32)	-0.02 (0.77)
SGPT (IU/L)	**0.17 (0.04)**	0.09 (0.27)	-0.11 (0.17)
Alkaline phosphatase (IU/L)	0.10 (0.21)	0.09 (0.26)	0.08 (0.34)
IV) Kidney Function Test			
Urea (mg/dl)	**0.25 (0.002)**	**0.16 (0.05)**	-0.05 (0.52)
Creatinine (mg/dl)	**0.26 (0.001)**	**0.21 (0.01)**	-0.05 (0.52)
V) Random Blood Sugar			
Blood Glucose (mg/dl)	**0.34 (0.0001)**	0.08 (0.34)	**0.22 (0.01)**

*denotes p value less than < 0.05 as considered statistically significant
**denotes p value less than < 0.001 as considered highly significant
*** denotes p value less than < 0.0001 as considered extremely significant

E) Delineation of risk with hematological and Biochemical parameters:

Hematological Parameters:

It was found that except for MCHC and MCV, none of the hematological parameters were significantly influenced by an increase in copper content. It may be noted that for a unit increase in the copper levels, the odds in favour of increase in the MCHC [OR: 2.50; 95% CI: 1.47-4.27] and MCV [OR: 1.57; 95% CI: 0.99- 2.49] levels from normal to abnormal were highly significant (p < 0.0001; 0.05, respectively) as depicted in **Table 6** However, for a unit increase in Zinc (Zn) content,

a significant elevation in the levels of only MCH was observed but none with other parameters. The odds in favour of MCH alteration with increase Zink from normal to abnormal was found [OR: 1.24; 95% CI: 1.04-1.48] with a p value < 0.05. It may also be noted that none of the hematological parameters were found to be significantly influenced with increasing concentration of Manganese.

Table 6: Odds ratio for different hematological parameters after adjusting for covariates using logistic regression

Parameters	Odds Ratio [95% CI] (p-value)			Classification accuracy	HL test
	Copper (Cu)	Manganese (Mn)	Zinc (Zn)		
Hematological parameters					
Haemogolobin	0.99 [0.54, 1.80] (0.96)	1.31 [0.97, 1.75] (0.07)	1.13 [0.94, 1.37] (0.19)	87.84%	0.8667
Total Leucocyte Count (TLC)	1.46 [0.84, 2.54] (0.18)	1.09 [0.82, 1.44] (0.57)	0.88 [0.71, 1.08] (0.22)	83.78%	0.5340
Neutrophils	1.32 [0.84, 2.08] (0.22)	1.11 [0.88, 1.41] (0.37)	0.87 [0.74, 1.02] (0.08)	65.54%	0.7282
Lymphocytes	1.23 [0.80, 1.88] (0.35)	1.01 [0.81, 1.28] (0.90)	0.93 [0.80, 1.07] (0.31)	56.08%	0.3916
Eosinophils	1.26 [0.73, 2.20] (0.41)	0.87 [0.63, 1.20] (0.40)	0.76 [0.60, 0.97] (**0.03**)	82.43%	0.7414
Monocytes	2.05 [0.48, 8.78] (0.33)	0.62 [0.19, 2.09] (0.44)	0.62 [0.30, 1.28] (0.20)	97.97%	0.2896
Basophils	0.96 [0.63, 1.46] (0.84)	1.07 [0.85, 1.35] (0.55)	1.00 [0.87, 1.17] (0.90)	55.41%	0.2590
R. B. Cs. Count	1.43 [0.90, 2.28] (0.13)	1.01 [0.79, 1.30] (0.92)	0.90 [0.77, 1.04] (0.16)	63.51%	0.6824
Pack cell volume	1.12 [0.63, 1.99] (0.71)	1.09 [0.81, 1.48] (0.57)	1.04 [0.86, 1.25] (0.69)	85.81%	0.4045
Mean Corpuscular volume	1.57 [0.99, 2.49] (**0.04**)	0.91 [0.72, 1.16] (0.46)	0.87 [0.74, 1.03] (0.10)	62.84%	0.6604
MCH	1.21 [0.78, 1.88] (0.39)	0.93 [0.73, 1.18] (0.55)	1.24 [1.04, 1.48] (**0.02**)	62.16%	0.2843
MCHC	2.50 [1.47, 4.27] (**0.001**)	1.06 [0.81, 1.38] (0.69)	0.86 [0.73, 1.01] (0.07)	69.59%	0.3241
Platelet Count	0.66 [0.23, 1.91] (0.44)	0.73 [0.43, 1.25] (0.25)	1.06 [0.68, 1.66] (0.79)	97.97%	0.8984

*Cut-offs of respective parameters have been used to determine the odds ratio; HL: Hosmer-Lemeshow test

Biochemical Parameters:

The logistic regression analysis for biochemical parameters against the changing of copper concentration in blood showed that there were statistically significant increase ($p < 0.05$) in odds ratio corresponding to triglycerides from normal to abnormal [OR: 2.37; 95% CI: 1.35- 4.17], SGPT [OR: 1.68; 95% CI: 1.04-2.70], Urea [OR: 2.07; 95% CI: 1.11- 3.88], Creatinine [OR: 1.92; 95% CI: 1.17-3.15] and random blood glucose [1.57; 95% CI: 0.96 – 2.57]. Other parameters did not show any significant alteration due to change in copper levels. Manganese hardly showed any effect on changing concentration on KFT parameters. However, Zinc showed some influence on Creatinine. A unit increase in the Zn concentration depicted a lowering of Creatinine [OR: 0.85; 95% CI: 0.73, 1.00] and p-value of 0.04 ($p < 0.05$). Rest of the parameters did not exhibit any significant relevance with changing contents of either Mn or zinc levels in Table 7.

Table 7: Odds ratio for different Biochemical parameters after adjusting for covariates using logistic regression*

B) Biochemical Parameters	Odds Ratio [95% CI] (p-value)			Classification accuracy	HL test
	Copper (Cu)	Manganese (Mn)	Zinc (Zn)		
I) Protein Profile					
Total protein (g/dl)	1.17 [0.76, 1.82] 0.47	1.22 [0.95, 1.56] 0.13	0.97 [0.84, 1.12] 0.68	55.41%	0.2863
Albumin (g/dl)	0.72 [0.34, 1.49] 0.37	1.07 [0.74, 1.56] 0.71	1.05 [0.83, 1.34] 0.67	89.86%	0.2046
Globulin (g/dl)	1.24 [0.79, 1.94] 0.35	0.96 [0.76, 1.22] 0.74	0.91 [0.78, 1.05] 0.2	62.16%	0.0315
A/G ratio (slightly above 1)	1.34 [0.86, 2.08] 0.19	1.06 [0.84, 1.33] 0.64	0.89 [0.76, 1.03] 0.13	56.08%	0.0902
II) Lipid Profile					
Cholesterol (mg/dl)	1.84 [0.75, 4.52] 0.18	0.75 [0.38, 1.47] 0.41	0.77 [0.51, 1.17] 0.22	95.27%	0.8575

Triglycerides (mg/dl)	2.37 [1.35, 4.17] 0.003	0.98 [0.77, 1.26] 0.9	0.73 [0.60, 0.90] 0.002	69.59%	0.0304
HDL cholesterol (mg/dl)	0.95 [0.63, 1.45] 0.82	1.03 [0.82, 1.29] 0.81	0.93 [0.80, 1.08] 0.34	56.76%	0.1749
Low Density Lipoprotein cholesterol (mg/dl)	0.93 [0.42, 2.10] 0.87	0.82 [0.49, 1.38] 0.45	0.91 [0.67, 1.25] 0.57	91.89%	0.2133
Cholesterol/HDL cholesterol ratio (mg/dl)	1.30 [0.64, 2.64] 0.47	0.93 [0.65, 1.33] 0.7	1.12 [0.85, 1.47] 0.44	89.19%	0.747
III) Liver Function Test					
Total Bilirubin (mg/dl)	1.57 [0.83, 2.96] 0.17	0.56 [0.33, 0.95] 0.03	0.92 [0.73. 1.17] 0.5	85.81%	0.0522
Direct bilirubin (mg/dl)	0.87 [0.56, 1.33] 0.51	0.97 [0.77, 1.22] 0.77	0.98 [0.85, 1.14] 0.82	58.11%	0.8098
SGOT (IU/L)	1.05 [0.15, 7.50] 0.96	0.67 [0.15, 2.97] 0.6	1.12 [0.59, 2.09] 0.73	98.65%	0.5119
SGPT (IU/L)	1.68 [1.04, 2.70] **0.03**	1.14 [0.89, 1.47] 0.31	0.82 [0.70, 0.97] 0.02	62.84%	0.7458
Alkaline phosphatase (IU/L)	1.02 [0.64, 1.62] 0.95	1.12 [0.88, 1.44] 0.35	1.09 [0.93, 1.27] 0.29	70.95%	0.0456
IV) Kidney Function Test					
Urea (mg/dl)	2.07 [1.11, 3.88] **0.02**	1.08 [0.81, 1.44] 0.59	0.81 [0.64, 1.02] 0.07	84.46%	0.8111
Creatinine (mg/dl)	1.92 [1.17, 3.15] **0.01**	1.07 [0.84, 1.36] 0.59	0.85 [0.73, 1.00] **0.04**	60.81%	0.2574
V) Blood Sugar					
Blood Glucose (mg/dl)	1.57 [0.96, 2.57] **0.07**	0.95 [0.73, 1.24] **0.71**	1.09 [0.93, 1.28] **0.3**	70.95%	0.6048

*Cut-offs of respective parameters have been used to determine the odds ratio; HL: Hosmer-Lemeshow test

DISCUSSION

A wide range of mining activities associated with copper mine emanates dust from rocks and ore containing varied concentrations of copper which might impose adverse health effects in mine workers. However, no attempts have so far been made to provide a systematic health status of workers in a open copper mine without possessing any industrial setting especially with the tenure of exposure to such mining operations. This is probably the first ever attempt that has been made to screen the general health status of copper pit workers in the world in general and India in specific. Evaluation of the effects of long term exposure to copper dust (Miner-II) after adjustment for other predictor like age, BMI, smoking, tobacco chewing and alcoholism on systemic level of copper and other accompanying metals like zinc and manganese revealed that there were significant enhancement in the contents of copper in the blood of Miner-II and office employees as compared to normal control. Similar evidence of high levels of serum ceruloplasmin may be attributed to an increase in blood copper levels in the liver to evaluate the health status of residents from residential area near copper smelting complex with the possibility of health hazard risk based on the influence of mining and smelting activities, which can be explained by the delivery of copper in the non-metallothionein-bound form to ceruloplasmin outside the Golgi apparatus of the liver were observed in total 120 individuals during June to December 2011 [31]. Interestingly Manganese, one of the associated metals in copper mining ore was found to be raised in the blood of chronically copper dust exposed miners observed here is a new finding not hitherto reported. Incidentally, employees residing in the vicinity of mine did not register such a raise of manganese in their blood.

Our observation of significant diminution in MCHC along with elevation in pack cell volume in chronically exposed copper pit workers and not in mildly exposed workers suggests that this aberration so noticed were dependent on the tenure of copper dust exposure. This is furthered by the evidence of similar diminution in MCHC in office employees of the mine who were indirectly exposed to copper dust from the environment for a long period of time (25years). It would be proper here to speculate that such decrease in MCHC amongst chronically exposed workers would bear proneness towards microcytic hypochromic anaemia in them. It may be recalled that similar drop in MCHC in workers exposed to heavy metals was found to be diagnosed as microcytic – hypochromic anaemia[32]. The observed raise in PCV in chronically exposed copper pit workers could be attributed to slightly raised counts of eosinophil in them which could either cause asthma, rheumatoid arthritis or type I hypersensitivity (allergy). It is however difficult at the present juncture to ascribe if, the observed increase in eosinophil count alone in these workers would pose such above stated proneness.

The most promising hematological alteration was that of platelet count which was significantly diminished in all the miners irrespective of duration of exposure as well as in office employees. It may be relevant here to mention in the rabbit model of five copper overfeeding animals showed slump in platelet counts but exact mechanism of action beside its decrease levels in animals was not yet established[33].The repercussion of observed decrease in platelet counts in all miners

and associated office employees could be construed to precipitate towards either thrombocytopenia or disorder of aggregation (Thrombosis) or both, in them.

The present observation of positive correlationship of copper with such hematological parameters as pack cell volume and negative correlationship with MCHC and platelet count suggests that these parameters can be considered as potential biomarkers for copper toxicity besides ceruloplasmin. However, odd ratio analysis of these hematological parameters with copper denoted that only MCV and MCHC were affected by an increase in copper levels. Whereas, neither pack cell volume nor platelet counts were found to be influenced by changing levels of copper. Although, zinc out of the associated metals evinced some influence on the content of MCH, increase in levels of manganese did not cause any influence on any of the hematological parameters. Thus, copper from the dust seems to be the paramount risk factor for the observed alteration in such hematological parameters as MCV and MCHC.

The abnormal raise observed in total protein, albumin and globulin of chronically exposed pit workers probably arose as a protective mechanism against excessive copper dust exposure in order to facilitate transport of extra copper via albumin and meet immunological challenges via globulin[34]. Although, in mildly exposed miners some raise in the contents of total protein and globulin were seen albumin was found to be unaltered, which suggests that an induction of immune response occurred in them. The fact that Office employees did not show any such alterations in the serum protein profile indicates that a lesser effect was probably due to their indirect exposure to copper dust. Although a positive pearson's correlationship of copper with above serum proteins were obtained, the odd ratio analysis failed to confirm the above observations with an increase in copper levels, This suggests that though these parameters show some physiological anomalies through excessive copper exposure they may not be considered as potential biomarkers for copper toxicity.

A comprehensive examination of serum lipid profile amongst all miners as well as office employees reveal that the content of triglyceride was significantly enhanced in them with no alterations in such other associated lipid parameters as HDL cholesterol, LDL cholesterol and cholesterol/HDL cholesterol ratio. A similar enhancement in triglyceride level was earlier noticed amongst workers in zinc- Lead mine[35]. Total cholesterol on the other hand was found to be slightly elevated in chronically exposed as well as office employees group alone. Though a positive correlationship of copper with either cholesterol or triglyceride was obtained, the odd ratio however denoted that triglyceride alone was truly affected by an increase in copper levels. Thus, heightened serum triglyceride becomes a highlight for copper toxicity. However, the observed hypertriglycerdemia accompanied by raised serum total cholesterol in chronically exposed miner denotes their susceptibility towards cardiovascular and type 2 diabetic status. Although, office employees of the mine did not show any such alterations in lipid profile, proneness towards hypertension and type 2 diabetes was noticed in them as well earlier.[18] This morbidity of these office employees could be attributed alternatively to a stressful and sedentary life style in them.

Our present observation of a significant elevation in random blood glucose of both the type of miners as well as office employees as a result of copper dust exposure was found to be positively correlated with copper and validated through odd ratio analysis. Similar positive correlationship of fasting blood sugar with the concentration of such toxic elements, as Cadmium, lead, arsenic and selenium was noticed earlier by Akinloye *et al.* [36].

Total bilirubin and direct bilirubin were reported to be significantly elevated in manganese mine workers working for duration of 10-15 years[37]. Our observation of a significant elevation in the content of total bilirubin in chronically exposed copper pit miners are in partial agreement with the above study. The observation of unaffected total bilirubin in mildly exposed pit workers as well as in office employees connotes that the extent of liver anomaly mostly depended upon the term of exposure to copper dust. However, accentuated activities of such liver enzyme as SGPT and alkaline phosphatase in both the types of miners as well as office employees suggest an implicit hepatotoxicity in copper exposed miners. Strangely, the activity of SGOT was found to be elevated only in chronically exposed miners and office employees and not in mildly exposed miners. These alterations in hepatic enzyme activity are quite paraller to certain other observations obtained from manganese mine workers and copper based industries[37,4,38]. A positive correlationship of copper with either SGOT or SGPT suggests that these parameters could be considered as a hallmark of copper toxicity. The odd ratio analysis of liver function parameters however denoted that only SGPT was truly affected with increase in copper levels.

The observed elevation in serum contents of urea and Creatinine in mildly and chronically exposed miners depicts a renal malfunction in them through exposure of copper dust since these contents were found to denote show a positive correlationship with copper which was validated by odd ratio analysis. In fact, these parameters were found to be truly affected by increase in copper and manganese levels. It may be recalled that similar renal dysfunction was reported earlier in workers exposed to copper fumes, dust from a copper smelter and or feeding of sublethal daily dose of copper[39,40].At this juncture, it is difficult to ascertain, if the observed renal dysfunction in pit workers from a copper mine could unilaterally cause hypertension in them, since this pathophysiology is recognized to be largely dependent upon multiple factors. Chronically copper dust exposed miners were predominantly affected both hematologically and biochemically due to long term copper dust exposure in a copper mine whereas mildly exposed miners and office employees suffered by a lesser extent.

Conclusion:

The raise in PCV and diminution in platelet count as well as MCHC along with slight elevation in the counts of eosinophil were observed in chronically exposed pit workers as well as office employees when compared to normal control subjects.

A positive correlationship of copper with pack cell volume and negative correlationship with MCHC and platelet count suggest that these parameters could be considered as potential biomarkers for copper toxicity. The odd ratio analysis further validated that hematological parameters with an increase in copper levels truly affected MCV and MCHC. Whereas an increase in Zinc levels was found to offer some influence on the content of MCH.

Although, chronically exposed pit workers showed abnormal raise in the contents of serum total proteins, albumin and globulin, mildly exposed miners showed some elevations in the contents of total protein and globulin but without any alteration in albumin. Both the types of miners showed accentuated levels of triglyceride and activities of such liver enzymes as SGPT and alkaline phosphatase with a parallel raise in the level of total bilirubin. Total cholesterol on the other hand was found to be slightly elevated in chronically exposed as well as office employees. These groups also exhibited elevation in the contents of serum urea and Creatinine as well as random blood glucose which were confirmed by a significant positive correlationship of these parameters with copper. Odd ratio analysis suggested that triglyceride, urea, creatinine random blood sugar and activity SGPT were truly affected by increase in the level copper. These observations suggest that liver and renal dysfunction amongst affected miners could be attributed to copper toxicity in them.

Overall, our present observation suggests that in a long-term exposure to a copper mine can adversely affect human physiology and some of the parameters studied here may be considered as potential biomarkers for copper toxicity.

Conflict of interest

The authors state no conflict of interest.

Authors Contributions:

1. Rajani G. Tumane: Participated in the designing of the study, performed biochemical and hematological analysis, statistical analysis, prepared manuscript and also participated in editing of the manuscript**.**

2. Dr. Nirmalendu Nath: Conceived of the study, participated in its designed and coordination and also participated in editing of the manuscript

3. Dr. Aqueel Khan: Participated in its designed and coordination and helped to draft the manuscript.

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Human Participant Protection

The research advisory boards of RTM University and Mining Management of Copper mine of India has approved study protocols.

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