Effect of Lead Exposure on the Status of Reticulocyte Count Indices among Workers from Lead Battery Manufacturing Plant

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Earlier studies conducted on lead-exposed workers have determined the reticulocyte count (RC) (%), but the parameters of Absolute Reticulocyte Count (ARC), Reticulocyte Index (RI), and Reticulocyte Production Index (RPI) were not reported. This study assessed the effect of lead (Pb) exposure on the status of reticulocyte count indices in workers occupied in lead battery plants. The present cross-sectional study was carried out on 391 male lead battery workers. The blood lead levels (BLL) were determined by using an Atomic Absorption Spectrophotometer. The RC (%) was estimated by using the supravital staining method. The parameters, such as ARC, RI, and RPI, were calculated by using the RC (%) with the red cell indices (RBC count and hematocrit). The levels of RBC count and hematocrit were determined by using an ABX Micros ES-60 hematology analyzer. The levels of reticulocyte count indices - RC (%), ARC, RI, and RPI significantly increased with elevated BLL. The association between BLL and reticulocyte count indices was positive and significant. The results of linear multiple regression analysis showed that the reticulocyte count ($\beta = 0.212$, $P < 0.001$), ARC ($\beta = 0.217$, $P < 0.001$), RI ($\beta = 0.194$, $P < 0.001$), and RPI ($\beta = 0.208$, $P < 0.001$) were positively associated with BLL. The variable, smoking habits, showed a significant positive association with reticulocyte count indices: RC (%) ($\beta = 0.188$, $P < 0.001$), ARC ($\beta = 0.174$, $P < 0.001$), RI ($\beta = 0.200$, $P < 0.001$), and RPI ($\beta = 0.151$, $P < 0.005$). The study results revealed that lead exposure may cause reticulocytosis with an increase of reticulocyte count indices.

Key words: Blood lead levels, Reticulocyte count indices, Lead battery plant, Lifestyle factors

INTRODUCTION

Lead (Pb) is used as a corrosion-resistant metal. It is a bluish-white metal used in construction, soldering, cable sheathing, and in manufacturing of lead batteries, pesticides, glass, paints and ammunition (1). More than three-fourth of lead production is used for the manufacturing of lead batteries (2). Lead industries were considered hazardous as per the Indian Factories Act's first schedule under section 2 (cb) (3). The workers occupied in this process are exposed to Pb through inhalation and ingestion. The main targeted organs for Pb exposure are the brain, the blood forming system and the kidneys (4). Previous studies have reported high levels of oxidative stress (5), morbidities (6-9), biogenic amino acids and neurobehavioral functions (10), genotoxicity (11), decreased coagulation function (12), altered calcium metabolism indicating low bone-mineral density (13), toxic neuropathies (14), and dental erosion (15) among lead battery workers.

Immature red blood cells with the reticulum network of RNA and mitochondria are called reticulocyte, which is produced and matured in the bone marrow before being released into the blood. There are two types of reticulocyte such as aggregate and punctuate. The aggregate reticulocyte is larger in size and more immature with a coarsely clumped collection of reticulum; whereas, the punctuate reticulocyte is smaller in size and more mature with small sparse granules of residual RNA (16). The measurement of RC (%) is used to classify anemia into regenerative (reticulocytosis) and non-regenerative (reticulocytopenia). In epidemiological studies, reticulocytosis is associated with cerebrovascular diseases (17) and iron-deficiency anemia (18); whereas, reticulocytopenia is associated with malaria (19) and parasitic infections (20). Studies regarding occupational exposure to Pb have a reported higher RC (%) in

workers from traditional tile factories (21), lead battery plants (22), acute lead-intoxicated battery plants (female workers) (23), battery repair shops (24) and shops manufacturing mixtures of talismanic ash (25). A high reticulocyte count was reported in the chronic Pb intoxicated animals (26,27).

Anemia is accompanied with a high RC (%) but does not reflect the bone marrow response in anemia. The parameters of ARC, reticulocyte index (RI) and reticulocyte production index (RPI) are essential to identify the bone marrow response in anemia. The RC (%) was adjusted with the RBC count, and hematocrits, such as subjective hematocrit, normal hematocrit and hematocrit, with a maturation factor to attain ARC, RI and RPI, respectively (28). The assessment of RC (%) was used to evaluate the degree of the erythropoiesis activity. The ARC and RI parameters were used to measure the RBC production and the degree of anemia caused either due to the loss of RBC or inadequate production of RBC. In lead-exposed workers, only RC (%) was determined and other reticulocyte count indices, such as ARC, RI and RPI, were not assessed. Therefore, the present study was undertaken to investigate the effect of Pb exposure on the reticulocyte count indices such as RC (%), ARC, RI & RPI in workers who work in lead battery plants. Lifestyle factors were used as a mediating factors.

MATERIALS AND METHODS

This was cross-sectional study and investigations were carried out on 391 male subjects working in Pb battery plants located in Tamil Nadu, India. The status of reticulocyte count indices, such as RC (%), ARC, RI and RPI, among lead-exposed workers were compared with age, Body Mass Index (BMI), experience, alcohol consumption, smoking habits, BLL and job category. The ethical committee of the institution has approved the present study. Subjects were informed about the purpose of the study and their consent was obtained before their participation.

Demographic details, occupational history and habits of subjects (smoking and alcohol consumption) were collected by using a pre-designed questionnaire. BMI was calculated using subjective weight (Kg) and height (m), and were expressed as Kg/m^2.

Blood lead levels. Three ml of venous whole blood was collected in a heparinized vacuette from the study subjects and was stored at $-20^{\circ}C$ until the analysis. Using ETHOS-D, (Milestone Microwave Laboratory Systems, Sorisole, BG, Italy), 2 mL of whole blood was digested with 2 mL of nitric acid (HNO_3) and 0.2 mL of hydrogen peroxide (H_2O_2) while maintaining power, temperature and duration of process. The digested samples were made up to 5 mL using distilled water and centrifuged. The concentration of lead was measured using Atomic Absorption Spec-

trophotometer (GBC Avanta P, Dandenong, Victoria, Australia). A standard solution of 20 μg/dL of Pb was prepared from the stock standard solution obtained from the Merck (1.19776.0500) and added to the lowest concentration of the sample. The analysis found 100% recovery with % RSD at < 0.5 for three replicates and the BLL was expressed as μg/dL.

Reticulocyte count indices.

Reticulocyte Count (RC) (%): RC (%) in peripheral blood samples was determined using the supravital staining method. In this method, equal volumes of stain were added to EDTA-anti-coagulant blood and the diluted blood was used to prepare the smear to examine the RC present in the sample. The RC in the sample was expressed as percentage. The manual method of reticulocyte count is reliable and cost-effective for estimating reticulocytes, in addition to competently determining the high and low ranges of RC (%) (29).

Absolute Reticulocyte Count (ARC): ARC is a product of RC (%) multiplied with RBC count. The RBC count was assessed by using an ABX Micros ES-60 hematology analyzer.

ARC = RC (%) × RBC count (mill/μL) × 10.

Reticulocyte Index (RI): RI is a product of RC (%) multiplied with subjective hematocrit and divided by normal hematocrit. The hematocrit was determined by using an ABX Micros ES-60 hematology analyzer.

RI = RC (%) × subjective hematocrit/normal hematocrit

Reticulocyte Production Index (RPI): RPI is a product of RI divided by the maturation factor. The maturation factor is represented by 1 day when hematocrit values are between 36 and 45; 1.5 days, if hematocrit values are between 26 and 35; 2 days, if hematocrit values are between 16 and 25; and, 2.5 days, if hematocrit values are below 15.

RPI = RI/maturation factor

Statistical analysis. Statistical Package for the Social Sciences (SPPS Inc., Chicago, USA) version 16 was used for the statistical analysis of the data. The data are presented in proportions and adjusted means with standard error. One-way Analysis of Variance (ANOVA) was used to assess the effects of categorical variables on RC indices. One-way ANOVA with *post-hoc* tests was used to compare various categories within the categorical variables on RC indices. The spearman correlation coefficient test was used to evaluate the association between BLL and reticulocyte count indices. Multiple linear regression analysis was used to evaluate the association between reticulocyte count indices and BLL by controlling the influence of age, BMI,

smoking habits, alcohol consumption and experience. Probability (p) value less than 0.05 was considered significant.

RESULTS

Demographic characteristics of the subjects are presented in Table 1. The age distribution indicated that maximum number of workers was in the age group of 33~38 years and minimum was in the age group of 21~26 years. The BMI of the subjects revealed that 54.2% had normal BMI, 41.2% were overweight, 3.8% were obese and only 0.8% were underweight. A majority of the workers had more than thirteen years of lead exposure. Alcohol consumption and

Table 1. Demographic characteristics of lead exposed workers

Variables	N (391)	Percentage (%)
Age (years)		
21~26	18	4.6
27~32	91	23.3
33~38	233	59.6
≥ 39	49	12.5
BMI (Kg/m^2)		
≤ 18.5	3	0.8
18.5~24.9	212	54.2
25~29.9	161	41.2
≥ 30	15	3.8
Experience (years)		
≤ 4	19	4.9
5~8	54	13.8
9~12	114	29.2
≥ 13	204	52.2
Alcohol consumption		
No	246	62.9
Yes	145	37.1
Smoking		
No	315	80.6
Yes	76	19.4
BLL (µg/dL)		
≤ 20	137	35.0
21~30	110	28.1
31~40	81	20.7
41~50	49	12.5
≥ 51	14	3.6
Job categories		
Assembly	208	53.2
Casting	46	11.8
Pasting	41	10.5
Ball mill	9	2.3
Charging	25	6.4
Plate cutting	4	1.0
Formation	8	2.0
Acid filling	7	1.8
Engineer services	33	8.4
Other workers	10	2.6

smoking habits were recorded in 37.1% and 19.4% of the subjects, respectively. The frequency distribution of BLL among the subjects was 35%, 28.1%, 20.7%, 12.5% and 3.5% in ≤ 20, 21~30, 31~40, 41~50 and > 51 µg/dL, respectively.

The univariate analysis of variables that affect the reticulocyte count indices among lead-exposed workers are presented in Table 2. ANOVA was used to find out the effects of categorical variables, – such as age, BMI, experience, alcohol consumption, smoking habits, BLL and job category, – on reticulocyte count indices among lead-exposed workers. The results of the model indicated that the BLL and smoking habits were significantly associated with RC (%), ARC, RI and RPI. The One-way ANOVA with the *post-hoc* comparison method was used to evaluate the effects of various categories within the categorical variables on the status of reticulocyte count indices. The reticulocyte count indices, such as RC, RI and RPI, were significantly high in BLL categories, namely 21~30 and 41~50, and > 51 µg/dL in contrast to ≤ 20 µg/dL. Age, BMI, experience and job category did not have significant effect on reticulocyte count indices.

The results of Spearmen's correlation coefficients (r) between BLL and reticulocyte count indices among lead-exposed workers are presented in Table 3. A positive and significant correlation coefficient was noticed between BLL and reticulocyte indices such as RC (%) (r = 0.233), ARC (r = 0.232), RI (r = 0.227) and RPI (r = 0.227). Similarly, the association between RC (%) and ARC (r = 0.950), RI (r = 0.958) and RPI (r = 0.886) was positive and significant. The correlation coefficient between ARC and RI (r = 0.954), RPI (r = 0.849) and RC (r = 0.886) was positive and significant. The association between RPI and RI, ARC and RC was also found to be positive and significant.

Linear multiple regression analysis of variables that affect the reticulocyte indices among lead-exposed workers are presented in Table 4. In this model, each of the parameters of the reticulocyte count indices such as RC (%), ARC, RI and RPI were used as dependent variables, and factors such as BLL, age, BMI, smoking habits (yes = 1 and no = 0), alcohol consumption (yes = 1 and no = 0) and experience (years) were used as independent variables. The results of linear multiple regression analysis showed that the RC (%), ARC, RI and RPI were positively related with BLL and smoking habits. Age, alcohol consumption and BMI were not significantly related to the reticulocyte count indices.

DISCUSSION

Earlier studies on lead-exposed workers have reported that RC (%) and other indices such as ARC, RI and RPI are not studied. These parameters are essential to identify the bone marrow response in anemia. The present study assessed the effects of lead exposure on reticulocyte count indices in

Table 2. Univariate analysis of the variables that affect reticulocyte indices among lead exposed workers

Variables	N (391)	Percentage (%)	RC	ARC	RI	RPI
Age (years)						
21~26	18	4.6	0.99 ± 0.07	53.94 ± 4.29	1.02 ± 0.08	1.02 ± 0.08
27~32	91	23.3	1.07 ± 0.03	56.53 ± 1.71	1.07 ± 0.03	1.13 ± 0.03
33~38	233	59.6	1.03 ± 0.02	55.45 ± 1.12	1.03 ± 0.02	1.09 ± 0.02
≥ 39	49	12.5	1.00 ± 0.04	53.91 ± 2.33	0.99 ± 0.04	1.07 ± 0.05
P values for ANOVA test			P = 0.584	P = 0.821	P = 0.590	P = 0.588
BMI (Kg/m²)						
≤ 18.5	3	0.8	0.86 ± 0.29	48.00 ± 17.05	0.90 ± 0.32	0.96 ± 0.26
18.5~24.9	212	54.2	1.02 ± 0.02	54.93 ± 1.13	1.02 ± 0.02	1.09 ± 0.02
25~29.9	161	41.2	1.05 ± 0.02	55.95 ± 1.36	1.05 ± 0.02	1.10 ± 0.02
≥ 30	15	3.8	1.11 ± 0.07	58.66 ± 3.74	1.12 ± 0.07	1.15 ± 0.07
P values for ANOVA test			P = 0.435	P = 0.693	P = 0.521	P = 0.849
Experience (years)						
≤ 4	19	4.9	1.13 ± 0.07	60.57 ± 3.94	1.15 ± 0.07	1.15 ± 0.07
5~8	54	13.8	1.02 ± 0.04	55.64 ± 2.29	1.04 ± 0.04	1.15 ± 0.05
9~12	114	29.2	1.02 ± 0.02	54.07 ± 1.53	1.01 ± 0.03	1.05 ± 0.03
≥ 13	204	52.2	1.04 ± 0.02	55.67 ± 1.20	1.04 ± 0.02	1.10 ± 0.02
P values for ANOVA test			P = 0.493	P = 0.468	P = 0.350	P = 0.258
Alcohol consumed						
No	246	62.9	1.03 ± 0.02	55.26 ± 1.03	1.03 ± 0.02	1.09 ± 0.02
Yes	145	37.1	1.04 ± 0.03	55.74 ± 1.49	1.05 ± 0.02	1.10 ± 0.03
P values for ANOVA test			P = 0.945	P = 0.786	P = 0.604	P = 0.719
Smoking						
No	315	80.6	1.01 ± 0.01	54.02 ± 0.93	1.00 ± 0.02	1.07 ± 0.02
Yes	76	19.4	1.15 ± 0.03*	61.31 ± 1.96*	1.16 ± 0.03*	1.20 ± 0.03*
P values for ANOVA test			P < 0.001	P < 0.001	P < 0.001	P < 0.005
BLL (μg/dL)						
≤ 20	137	35.0	0.96 ± 0.02	51.36 ± 1.38	0.96 ± 0.02	1.02 ± 0.02
21~30	110	28.1	1.06 ± 0.03*	56.98 ± 1.56*	1.06 ± 0.03*	1.12 ± 0.03*
31~40	81	20.7	1.04 ± 0.03	55.22 ± 2.00	1.03 ± 0.03	1.11 ± 0.04
41~50	49	12.5	1.10 ± 0.03*	60.08 ± 2.22*	1.12 ± 0.04*	1.18 ± 0.04*
≥ 51	14	3.6	1.27 ± 0.07*	68.28 ± 3.70*	1.26 ± 0.06*	1.35 ± 0.11*
P values for ANOVA test			P < 0.005	P < 0.001	P < 0.005	P < 0.005
Job category						
Assembly	208	53.2	1.06 ± 0.02	56.95 ± 1.15	1.06 ± 0.02	1.13 ± 0.02
Casting	46	11.8	1.02 ± 0.04	54.19 ± 2.32	1.01 ± 0.04	1.04 ± 0.04
Pasting	41	10.5	1.02 ± 0.05	52.78 ± 2.63	1.01 ± 0.05	1.07 ± 0.05
Ball mill	9	2.3	1.20 ± 0.10	63.66 ± 5.75	1.18 ± 0.10	1.28 ± 0.10
Charging	25	6.4	0.98 ± 0.06	52.40 ± 3.65	0.98 ± 0.07	1.07 ± 0.07
Plate cutting	4	1.0	1.07 ± 0.07	58.00 ± 3.46	1.12 ± 0.06	1.12 ± 0.06
Formation	8	2.0	0.92 ± 0.11	48.87 ± 6.63	0.90 ± 0.12	0.96 ± 0.11
Acid filling	7	1.8	0.95 ± 0.06	49.71 ± 3.61	0.94 ± 0.06	1.04 ± 0.08
Engineer services	33	8.4	0.98 ± 0.06	53.06 ± 3.38	0.99 ± 0.06	1.02 ± 0.06
Misc	10	2.6	1.02 ± 0.09	56.90 ± 5.64	1.03 ± 0.10	1.15 ± 0.12
P values for ANOVA test			P = 0.614	P = 0.465	P = 0.572	P = 0.444

Values are mean ± standard error. * Indicate P < 0.05 and significant.

lead-exposed workers. Infant aged 27 months are reported to have higher RC (%)than a one-year old (30). People above 60 years are reported to have low RC (%) than the young, healthy adults (31). However, the findings of this study, i.e., both univariate and regression analyzes, observed no significant association between reticulocyte count indi-

Table 3. Spearmen correlation coefficient (r) between blood lead levels and reticulocyte count indices among lead exposed workers

Parameters	BLL (μg/dL)	Reticulocyte count (%)	Absolute reticulocyte count (× 10⁹/L)	Reticulocyte index (%)	Reticulocyte production index (%)
BLLs (μg/dL)	1.000	-	-	-	-
Reticulocyte count (%)	0.233**	1.000	-	-	-
Absolute reticulocyte count (× 10⁹/L)	0.232**	0.950**	1.000	-	-
Reticulocyte index (%)	0.227**	0.958**	0.954**	1.000	-
Reticulocyte production index (%)	0.227**	0.886**	0.849**	0.852**	1.000

**Correlation is coefficient significant at $P < 0.001$.

Table 4. Linear multiple regression analysis of variables that affect on reticulocyte count indices among lead exposed workers

Independent variables	Reticulocyte count (%)	Absolute reticulocyte count (× 10⁹/L)	Reticulocyte index (%)	Reticulocyte production index (%)
Age (years)	−0.004	−0.004	−0.030	0.029
Body mass index (Kg/m²)	0.036	0.029	0.039	0.000
Blood lead levels (μg/dL)	0.212**	0.217**	0.194**	0.208**
Duration of exposure (years)	0.009	0.014	0.006	−0.002
Alcohol consumption	−0.084	−0.070	−0.064	−0.053
Smoking	0.188**	0.174*	0.200**	0.151*

Linear multiple regression model included age, BMI, Blood lead levels, duration of exposure (years), alcohol consumption and smoking. Standardized regression coefficient β is significant *$P < 0.05$ and **$P < 0.001$.

ces and age groups. A reduced BMI was associated with decreased RC (%) and RPI (32). In this study, both univariate and regression analyzes showed positive and no significant association between reticulocyte count indices and BMI. Earlier studies report high levels of hematocrit and RC (%) in smokers as compared to non-smokers (33). Smoking is also associated with high levels of hemoglobin (Hb%), RBC count and hematocrit (34). In this study, a positive and significant association between the smoking habit and reticulocyte count indices were found.

Anemia in alcoholics may be due to multiple factors such as poor nutrition, prolonged inflammation, blood loss, liver dysfunction and ineffective erythropoiesis (35). About 37% of chronic alcoholics were reported with reticulocytosis (36). In the present study, the reticulocyte count indices showed no significant difference between the group that consumed alcohol and the group that did not consume alcohol. The pattern of occasional alcohol consumption was also observed among the study subjects.

High levels of RC (%) with low levels of Hb%, hematocrit and RBC count were observed in populations exposed to lead from cooking utensils, indigenous medicinal powder (37), lead poisoning cases (38) and lead batteries (23). The literature reports a positive and significant association (> 30 μg/dL) between BLL and RC (%) in lead battery workers (22). In the current study, the correlation coefficient between BLL and the reticulocyte count indices (RC, ARC, RI and RPI) was found to be positive and significant. Lead exposure has been associated with induced anemia due to short lifespan of erythrocytes, inhibition of hemoglo-

bin synthesis, reduction of erythroid progenitor cells and inappropriate erythropoietin production (39). The association between BLL and erythropoietin concentration was reported to be negative (40). Pb exposure on human RBC could increase micro-vesicle generation and hemolysis (41), the severity of which is positively associated with reticulocyte count (42). The result of the study shows that the BLL and smoking habits have a significant effect on the reticulocyte count indices (RC, ARC, RI and RPI) among lead-exposed workers. The variables of age, BMI, experience, alcohol consumption and job category did not predict any significant association with reticulocyte count indices. The results of the study indicate that Pb exposure may cause reticulocytosis with increased reticulocyte count indices.

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