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Research Article

Assessment of diagnostic accuracy and optimal cut points of blood lead levels on serum proteins among workers exposed to Pb at a lead battery plant

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Abstract

Objectives: This study assessed the diagnostic accuracy and the optimal cut point of blood lead (Pb) level (BLL) in serum proteins (total protein, albumin, globulin, and albumin/globulin ratio) among workers with exposure to Pb at a Pb battery plant.

Methods: An examination of the diagnostic accuracy and optimal cut point value of BLL in serum proteins among workers exposed to Pb was performed using analysis of the area under the curve (AUC) and coordinates of receiving operating characteristic (ROC) curve. The study group consisted of 176 males who worked in a Pb battery plant. A control group consisted of 80 male office workers with no unusual occupational Pb exposure. The BLL of the patients was assessed using an atomic absorption spectrophotometer. The serum protein levels (total protein and albumin) were determined using diagnostic kits. The serum globulin was calculated from the total protein and albumin measures. The albumin:globulin ratio (A/G ratio) was calculated from the albumin and globulin levels.

Results: The serum total protein and globulin concentration values were lower and the A/G ratio was significantly higher in the study group compared with the control group. A negative and significant association was found between the BLL and serum total protein and serum globulin values. A positive and significant association was found between the BLL and the serum albumin level and the A/G ratio. The AUC value was 0.626 (95% confidence interval [CI]: 0.540–0.712; p=0.005) for serum total protein with a cutoff value of ≤6.9 g/dL. For serum albumin, the AUC value was 0.466 (95% CI: 0.344–0.587; p=0.574) with a cutoff value of ≤4.0 g/dL. The AUC value of serum globulin was 0.625 (95% CI: a0.541–0.708, p=0.005) with a cutoff value of ≤2.4g/dL. The A/G ratio had an AUC value of 0.606 (95% CI: 0.521–0.692; p=0.012) with a cutoff value >1.5g/dL. An optimal BLL cut point value of 25.5µg/dL was noted for serum total protein, with 56% sensitivity and 51% specificity. The BLL value of 24.5 µg/dL was determined to be the optimal cut point for serum albumin, with 44% sensitivity and 44% specificity. The use of 27.5 µg/dL as an optimal BLL cut point value for serum globulin and the A/G ratio demonstrated 55% sensitivity and 55% specificity.

Conclusion: The AUC values of serum total protein, globulin, and the A/G ratio were found to be significant when compared with the serum albumin. BLL optimal cut point values for serum proteins in Pb exposure ranged from 24.5 µg/dL to 27.5 µg/dL. The change in serum total protein, globulin, and A/G ratio suggested reduced liver function due to Pb-binding interactions.

Keywords: Area under the curve, blood lead level, coordinates of receiver operating characteristic curve, serum proteins

Lead (Pb)-binding proteins originate in the kidney, brain, erythrocytes, liver, and lung [1]. Proteins related to heme synthesis, calcium metabolism, and neurotransmission are associated with Pb poisoning [2]. Systemic effects of hypertension, frank anemia, cognitive deficits, infertility, immune imbalances, delayed skeletal and deciduous dental develop-

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ment, vitamin D deficiency, and gastrointestinal symptoms have been related to Pb exposure [3]. Pb intoxication in animals and workers exposed to Pb in the plastics industry resulted in significantly altered levels of serum aminotransaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), γ-glutamyl transpeptidase, and alkaline phosphatase, which was characterized as a hepatotoxic effect and was followed by intrahepatic cholestasis [4-7].

Animal models with acute and chronic Pb exposure have shown decreased total and protein-bound sulfhydryl (SH) groups in homogenates and synaptosomes of the brain [8]. Pb intoxication in animals has revealed a significant decrease in serum total protein, albumin, and the A/G ratio [9, 10]. The reduction of proteins was due to precipitation of soluble proteins, inhibition of protein biosynthesis through the specific enzymes in cell processes, and a decrease in free amino acid utilization for protein synthesis. Occupational exposure to Pb has also been reported to cause decreased protein SH groups [11] and elevated positive acute phase proteins [12]. Recent studies have reported decreased levels of serum total protein, albumin, globulin, and the A/G ratio in workers exposed to Pb in the automobile industry [13, 14], Pb battery manufacturing [15, 16], petrol filling stations, and traffic officers [17].

Measurement of diagnostic test accuracy can include measures of sensitivity and specificity, positive and negative predictive values, likelihood ratio, area under the receiver operating characteristic (AUROC) curve, Youden's index, and a diagnostic odds ratio. The AUROC is defined as a plot of sensitivity with the Y-axis plotted against the 1-specificity, or false positive rates, on the X-axis. It is an effective tool used for evaluating quality and performance of a diagnostic test. A test with an AUROC value of 1 is perfectly accurate. The lower limit for the AUROC of a diagnostic test is 0.5. The line segment from (0.0) to (1.1) has an area of 0.5. The coordinates of the ROC curve help to identify the optimal cutoff values of a diagnostic test [18].

Most of the animal and human occupational Pb exposure studies have reported decreased serum protein measures (total protein, albumin, globulin, and A/G ratio). However, the diagnostic accuracy and optimal cut point values have not been explored. The aim of this study was to evaluate the diagnostic accuracy and optimal cut point values for BLL in serum proteins for workers exposed to Pb through employment at a Pb battery plant using AUROC and coordinates of the ROC curve.

Materials and Methods

The study group consisted of 176 male subjects who worked at a Pb battery manufacturing facility located in Tamil Nadu, India. A control group comprised 80 healthy male subjects who worked in administrative jobs with no exceptional occupational exposure to Pb. The serum protein values (total protein, albumin, globulin, and A/G ratio) of the study and control groups were compared. This study evaluated diagnostic accuracy and optimal cutoff point values of BLL in serum proteins using analysis of AUROC and coordinates of ROC curves. Individuals with a history of diabetes; liver or renal disorders; fever; malignancy; or autoimmune, chronic inflammatory, hematological or rheumatological disorders were excluded from the study. The Institutional ethical committee of Regional Occupational Health Centre (Southern) approved this study on 3-12-2014 with letter no.142/6. The subjects were informed about the objectives of the study and consent was obtained before initiating their participation in the study.

Sample collection

Whole blood samples of 5 mL (2 mL in heparin tubes +3 mL in plain tubes) were collected from each subject. The 2 mL heparinized blood sample was used for BLL estimation. The 3 mL whole blood sample collected in plain tubes was centrifuged at 3000 rpm for 10 minutes at 4°C to separate the serum from red blood cells and used to examine serum proteins.

Serum total protein

The serum total protein concentration was determined using the biuret method. In this approach, alkaline copper reacts with the peptide bonds of proteins to form a characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the auto reduction of copper. The color intensity is directly proportional to protein concentration. The absorbance was measured at 546 nm. The concentration of serum total protein was expressed as g/dL.

Serum albumin

The serum albumin concentration was measured using the bromocresol green method described by Doumas et al. [19]. The albumin in a buffered solution reacts with anionic bromocresol green dye and produces a green color. The measurement was made at an absorbance at 628 nm. The intensity of the green color is proportional to the concentration of albumin present in the sample and was expressed as g/dL.

Serum globulin

The serum globulin level was calculated by subtracting the albumin value from the corresponding value of total protein. The concentration of serum globulin was expressed as g/dL.

Albumin and globulin ratio

The A/G ratio was calculated based on the measurements of serum albumin and globulin.

Blood lead level

The estimation of BLL was used as an indicator of Pb exposure. The BLL was measured using the method described by Barman et al. [20]. A 2-mL whole blood sample was prepared using a microwave digestion system (ETHOS-D, Milestone Srl,

Sorisole, Italy) with 2 mL of nitric acid (HNO_3) and 0.2 mL of hydrogen peroxide (H_2O_2). Triple-distilled water was used to create 5-mL samples, which were centrifuged. The BLL was measured using an atomic absorption spectrophotometer (Avanta; GBC Scientific Equipment, Braeside, Victoria, Australia). A standard solution was prepared from 20 µg/dL of the Pb solution and added to the lowest concentration of the sample. The analysis yielded 100% recovery with a relative standard deviation of <0.5% in 3 replicates.

Statistical analysis

The IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis of the data. The data were presented as mean± SD and proportions. A chi-square test was used to determine the difference in the frequency distribution of lifestyle factors (smoking and alcohol consumption) between the study group and the control group. An independent t-test was used to assess the difference in mean age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and serum protein (total protein, albumin, globulin, and A/G ratio) concentration between the 2 groups. Pearson's correlation coefficient (r) test was used to find the correlation between BLL and serum proteins. The AUC and coordinates of the ROC curve were used to assess the diagnostic accuracy and optimal cut point values to assess BLL effects on serum proteins. A probability value of ≤0.05 was considered significant.

Results

The demographic details of the study subjects are presented in Table 1. The average age, BMI, SBP, and DBP of the participants were suitably matched. The frequency distribution of smoking and alcohol consumption habits demonstrated no significant difference between the groups. The average BLL in the study group subjects was 1.6 times higher than that of the controls. It should be noted that the BLL was significantly greater in the study group compared with the control group. The World Health Organization (WHO) has identified a BLL threshold of 40 µg/dL for adults. The BLL in the control group subjects of this study was within that limit.

Table 1. Demographic details of the study and control groups

Variables	Study group (n=176)	Control group (n=80)
Age (years)	36.58±3.98	37.43±10.23
BMI (kg/m^2)	25.67±2.97	25.27±3.36
Length of exposure (years)	13.33±3.30	-
Alcohol consumption		
Yes	95 (54.0)	39 (48.8)
No	81 (46.0)	41 (51.2)
Smoking		
Yes	38 (21.6)	19 (24.0)
No	138 (78.4)	61 (76.0)
SBP (mmHg)	127.82±14.63	127.48±19.14
DBP (mmHg)	77.65±10.72	74.80±12.74
Blood lead levels (µg/dL)	32±12*	20±6.0
Hb%	14.4±1.3	13.9±0.9

*p<0.05. BMI: Body mass index; DBP: Diastolic blood pressure; Hb: Hemoglobin; SBP: Systolic blood pressure.

Table 2. Serum protein levels in the study and control groups

Variables	Study group (n=176)	Control group (n=80)
Serum total protein (g/dL)	7.5±1.30*	7.8±0.54
Serum albumin (g/dL)	4.09±0.54	4.10±0.42
Serum globulin (g/dL)	3.40±1.41*	3.70±0.57
Albumin/globulin ratio	1.41±0.87*	1.15±0.29

*p<0.05.

The mean level of serum total protein, albumin, and globulin, and the A/G ratio of the members of the study group and the control group are presented in Table 2. The levels of serum total protein and globulin were significantly lower and the A/G ratio was significantly higher in the study group compared with the control group.

Table 3 displays the correlation coefficient (r) between the BLL and serum proteins in the participants. A negative correlation coefficient was found between BLL and serum total protein and serum globulin. The correlation coefficient (r) was significant at p<0.05. A positive correlation coefficient was found between BLL and serum albumin and the A/G ratio. The correlation coefficient (r) between BLL and the A/G ratio was significant at p<0.05.

The 95% confidence interval (CI) AUROC values of the serum proteins examined are presented in Figures 1 to 4. The mean AUC values assess the diagnostic accuracy of test. The 95%CI is a range of values which we can be 95% certain contains the true value. Therefore, if the lower boundary of a 95% CI for the AUC for a test is >0.5, the test is statistically significantly better than making the diagnostic decision based on pure chance. The levels of serum total protein, globulin, and the A/G ratio were greater than the lower boundary 95% CI for AUC >0.5 and found to be significant. The cutoff values of serum proteins used were the reference intervals for healthy Indian males as reported by Sairam et al. [21].

The optimal cut point values for BLL in serum proteins are presented in Table 4. These were assessed using coordinates of a ROC curve. The optimal cut point value of 25.5µg/dL was noted for serum total protein with 56% sensitivity and 51% specificity. The BLL value of 24.5 µg/dL was found to be the optimal cut point for serum albumin with 44% sensitivity and 44% specificity. The use of 27.5 µg/dL as optimal cut point value for serum globulin and the A/G ratio demonstrated 55% sensitivity and 55% specificity.

Table 3. Correlation coefficient (r) between blood lead level and serum proteins in the study group

Variables	BLL (µg/dL)	STP (g/dL)	Alb (g/dL)	Glb (g/dL)	A/G ratio
Blood lead level (µg/dL)	1.000	--	--	--	--
Serum total protein (g/dL)	-0.157*	1.000	--	--	--
Serum albumin (g/dL)	0.015	0.136*	1.000	--	--
Serum globulin (g/dL)	-0.157*	0.901**	-0.307**	1.000	--
Albumin/globulin ratio	0.124*	-0.553**	-0.493**	-0.748**	1.000

*Correlation is significant at 0.05. **Correlation is significant at 0.01. A/G: Albumin/globulin; Alb: Albumin; BLL: Blood lead level; Glb: Globulin; STP: Serum total protein.

Table 4. Optimal cut points of blood lead level in serum proteins with sensitivity, 1-specificity, and specificity

Parameter	Optimal cut point BLL (µg/dL)	Sensitivity	1-specificity	(Specificity)
Serum total protein (g/dL)	25.5	0.556	0.490	0.510
Serum albumin (g/dL)	24.5	0.440	0.554	0.446
Serum globulin (g/dL)	27.5	0.545	0.453	0.547
Albumin/globulin ratio	27.5	0.557	0.446	0.554

BLL: Blood lead level.

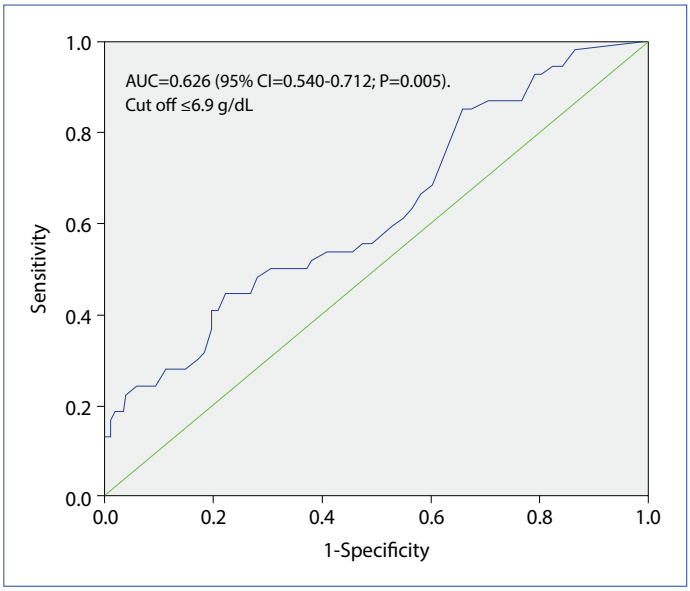

Figure 1. ROC curve of serum total protein.

AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.

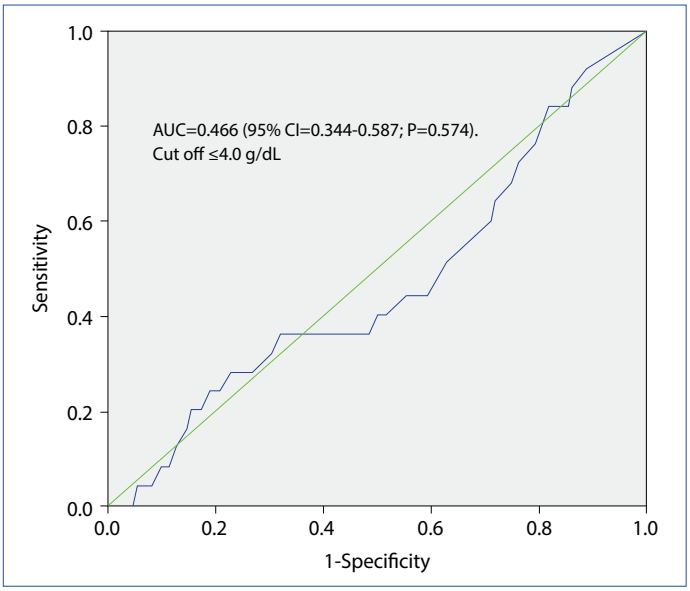

Figure 2. ROC curve of serum albumin.

AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.

Discussion

This study evaluated the diagnostic accuracy and optimal cut point values for BLL in serum proteins among Pb-exposed workers from a Pb battery plant using analysis of AUC and coordinates of ROC curves. Pb is a cumulative toxicant and disseminates in the brain, liver, kidney, and bones. Exposure has been assessed via blood Pb measurement [22]. The present study used the BLL as an indicator of the body burden of Pb. We observed significantly increased BLLs in Pb-exposed workers compared with the control subjects. The mean BLL in the study group was 1.6 times higher than that of the controls. Sudjaroen and Suwannahong [23] also recently reported a significant increase in the BLL in a group with a similar occupational exposure to Pb.

Dongre et al. [14] noted the greatest decrease in their study in the serum globulin level, followed by albumin, the A/G ratio, and total protein in automobile workers with a mean BLL of 47.32 µg/dL. The greatest decrease in the present study was also noted in the serum globulin measure, followed by total

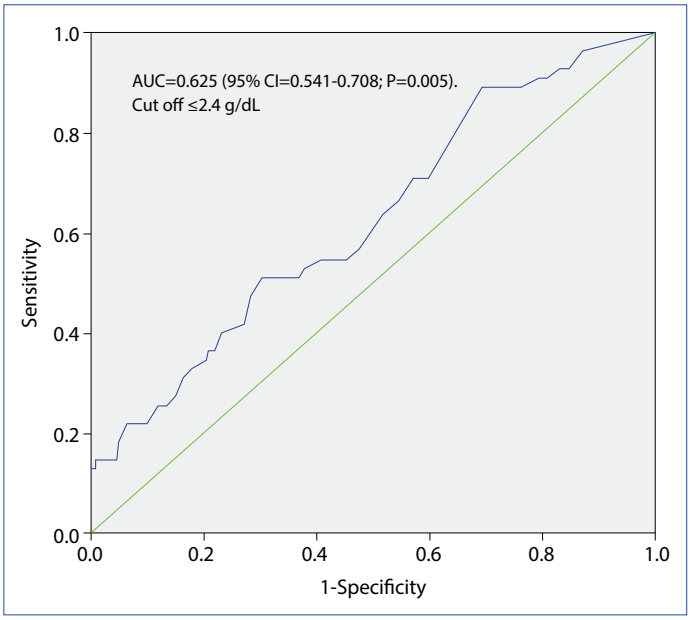

Figure 3. ROC curve of serum globulin.

AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.

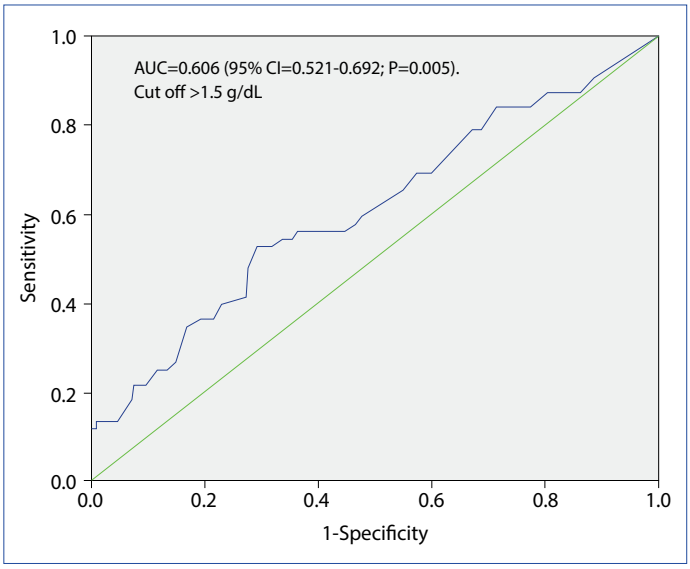

Figure 4. ROC curve for albumin/globulin ratio.

AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.

protein, with a mean BLL of 32 µg/dL. Both studies indicated that the serum globulin fraction was a principal target for Pb exposure compared with other protein fractions. Some other studies have found that Pb-treated lambs and Pb-exposed workers had reduced globulin levels without a significant change in serum albumin [24, 25].

We also assessed the hemoglobin percentage (Hb%) in the study group and control subjects. The comparison revealed no significant difference. A complete blood count was not assessed; our research was restricted to serum proteins and Hb%. Heidari et al. [26] reported low Hb% and low serum albumin levels in chronic hemodialysis patients. Watanabe et al. [27] observed a low Hb% with low serum total protein and albumin, but it was not significantly different when compared with normal Hb%. Our results revealed no significant association between Hb% and serum protein levels.

Pb toxicity primarily targets the SH groups [8], thiolate-rich sites, and small molecules of glutathione [28]. Sulfur-containing amino acids, such as homocysteine, cysteine, and cysteinylglycine have been reported to be present in a greater proportion in serum globulin compared with serum albumin [29]. In the present study, we observed no statistically significant alteration in serum albumin; however, a significant difference was observed in the total protein and globulin levels in the Pb-exposed workers. Reduced serum globulin and total protein (combination of albumin plus globulin) is related to the high proportion of sulfur-containing amino acids, which are more vulnerable to Pb exposure. The rationale for no significant change in serum albumin is due to the interaction of Pb ions with oxygen and nitrogen, rather than sulfur atoms, causing partial destabilization [30]. The interaction of Pb ions with albumin results in no secondary structure transformation, affecting mostly tyrosine and phenylalanine amino acids. The results of another study established that the positively charged Pb ion creates surface charge compensation of the protein molecule, after which albumin molecules start to experience dipole-dipole interaction, creating a favorable condition to form agglomerates [31].

Pachathundikandi and Varghese [13] reported a significant decrease in serum total protein in workers exposed to Pb in automobile workshops. We also observed a significant reduction in serum total protein. Bhagawat et al. [16] reported an increased A/G ratio among Pb battery workers. The results of the present study also indicated a significantly increased A/G ratio. The Pearson correlation coefficient (r) test was used in the current study to identify the association between BLL and serum proteins. The results revealed that the serum total protein and globulin parameters demonstrated a significant negative association with BLL, whereas the A/G ratio was shown to have a significant positive association with BLL.

Studies of Pb exposure have reported altered levels of serum proteins (total protein, albumin, and globulin, and A/G ratio). However, the diagnostic accuracy and optimal cut point values for BLL in serum proteins were not explored. The aim of the present study was to evaluate the diagnostic accuracy and optimal cut point values of BLL in serum proteins among workers exposed to Pb in a Pb battery plant using analysis of AUROC and coordinates of ROC curve. An AUC value of 0.626 (95% CI: 0.540–0.712; p=0.005) for serum total protein with a cutoff value of ≤6.9 g/dL was observed. For serum albumin, the AUC value was 0.466 (95% CI: 0.344–0.587; p=0.574) with a cutoff value of ≤4.0 g/dL. The AUC value for serum globulin was 0.625 (95% CI: 0.541–0.708; p=0.005) with a cutoff value of ≤2.4 g/dL. The A/G ratio produced an AUC value of 0.606

(95% CI: 0.521–0.692; p=0.012) with a cutoff value of >1.5 g/dL.

The ROC curve provides an optimal cut point value with sensitivity and specificity that are close to the AUC value [32, 33]. An optimal cut point value for BLL of 25.5µg/dL was noted for serum total protein with 56% sensitivity and 51% specificity. Serum albumin analysis led to an optimal cut value of 24.5 µg/dL BLL with 44% sensitivity and 44% specificity. An optimal cut point of 27.5 µg/dL BLL for serum globulin and the A/G ratio demonstrated 55% sensitivity and 55% specificity. The sensitivity and specificity obtained with the optimal cut point values were close to the AUROC.

Conclusion

AUROC values of serum total protein, globulin, and A/G ratio were significant when compared with serum albumin. BLLs ranging from 24.5 µg/dL to 27.5 µg/dL were noted as the optimal cut point values for serum proteins in cases of Pb exposure. Changes in serum total protein, globulin, and the A/G ratio likely indicate reduced liver function due to Pb-binding interactions.

Conflict of interest: None declared.

Ethics Committee Approval: The Institutional ethical committee of Regional Occupational Health Centre (Southern) approved this study on 3-12-2014 with letter no.142/6.

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References

1. Gonick HC. Lead-binding proteins: A review. J Toxicol 2011;2011:686050. [CrossRef]
2. De Souza ID, De Andrade AS, Dalmolin RJS. Lead-interacting proteins and their implication in lead poisoning. Crit Rev Toxicol 2018;48:375–86. [CrossRef]
3. Mitra P, Sharma S, Purohit P, Sharma P. Clinical and molecular aspects of lead toxicity: An update. Crit Rev Clin Lab Sci 2017;54:506–28. [CrossRef]
4. Abd Allah ES, Badary DM. Folic acid protects against lead acetate-induced hepatotoxicity by decreasing NF-κB, IL-1β production and lipid peroxidation mediataed cell injury. Pathophysiology 2017;24:39–44. [CrossRef]
5. Stavreva Veselinovska S. The activities of the aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase enzymes in the blood serum of rats in conditions of chronic lead poisoning. Section Biology 2016;1:149–57.
6. Offor SJ, Mbagwu HO, Orisakwe OE. Lead induced hepato-renal damage in male albino rats and effects of activated charcoal. Front Pharmacol 2017;8:107. [CrossRef]
7. Mazumdar I, Goswami K. Chronic exposure to lead: a cause of oxidative stress and altered liver function in plastic industry workers in kolkata, India. Indian J Clin Biochem 2014;29:89–92.
8. Dabrowska-Bouta B, Struzynska L, Rafalowska U. Effect of acute and chronic lead exposure on the level of sulfhydryl groups in rat brain. Acta Neurobiol Exp 1996;56:233–6.
9. Okediran BS, Kasali OB, Omotainse SO, Akinloye QA. Hematobiochemical alterations as biomarkers of lead induced toxicity in male wistar rats. Bangladesh J Vet Med 2016;14:227–32. [CrossRef]
10. Jaiswal R, Ali SL, Roy S, Sakya S, Dinani OP, Kumar S. Effect of induced lead intoxication on serological parameters without or with different antioxidants in broiler chicken. Journal of Entomology and Zoology Studies 2017;5:1020–3.
11. Kasperczyk S, Błaszczyk I, Dobrakowski M, Romuk E, Kapka-Skrzypczak L, Adamek M, et al. Exposure to lead affects male biothiols metabolism. Ann Agric Environ Med 2013;20:721–5.
12. Kasperczyk S, Dobrakowski M, Kasperczyk A, Ostałowska A, Birkner E. The administration of N-acetylcysteine reduces oxidative stress and regulates glutathione metabolism in the blood cells of workers exposed to lead. Clin Toxicol 2013;51:480–6. [CrossRef]
13. Pachathundikandi SK, Varghese ET. Blood zinc protoporphyrin, serum total protein, and total cholesterol levels in automobile workshop workers in relation to lead toxicity: our experience. Indian J Clin Biochem 2006;21:114–7. [CrossRef]
14. Dongre NN, Suryakar AN, Patil AJ, Rathi DB. Occupational lead exposure in automobile workers in North Karnataka (India): effect on liver and kidney functions. Al Ameen J Med Sci 2010;3:284–92.
15. Kshirsagar M, Patil J, Patil A, Ghanwat G, Sontakke A, Ayachit RK. Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests. Al Ameen J Med Sci 2015;8:107–14.
16. Bhagwat VR, Patil AJ, Patil JA, Sontakke AV. Occupational lead exposure and liver functions in battery manufacture workers around Kolhapur (Maharashtra). Al Ameen J Med Sci 2008;1:2–9.
17. Saeed HS, Abdellah AM, Abdalla FA, Abbas AR, Adam FA, ELgazali NA. Biochemical effects of lead toxicity on serum total protein, albumin and globulin levels in occupationally exposed workers in major Sudanese cities. Int J Emerg Technol Adv Eng 2015;7:132–8.
18. Simundic AM. Measures of diagnostic accuracy: basic definitions. Med Biol Sci 2008;22:61–5.
19. Dumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 1971; 258:21–30. [CrossRef]
20. Barman T, Kalahasthi R, Rajmohan HR. Effects of lead exposure on the status of platelet indices in workers involved in a

lead-acid battery manufacturing plant. J Expo Sci Environ Epidemiol 2014;24:629–33. [CrossRef]
21. Sairam S, Domalapalli S, Muthu S, Swaminathan J, Ramesh VA, Sekhar L, et al. Hematological and biochemical parameters in apparently healthy Indian population: defining reference intervals. Indian J Clin Biochem 2014;29:290–7. [CrossRef]
22. Lead poisoning and health. World Health Organization (WHO); 2018. Available at: https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health. Accessed Oct 09, 2019.
23. Sudjaroen Y, Suwannahong K. Biomarker related lead exposure of industrial battery's workers. Annals of Tropical Medicine and Public Health 2017;10:194–198. [CrossRef]
24. Hoffman LJ. Effects of oral lead on serum proteins and on the development of specific antibody response in young sheep. Theses and Dissertations 1974.
25. De Bruin A, Hoolboom H. Early signs of lead-exposure. A comparative study of laboratory tests. Br J Ind Med 1967;24:203–12.
26. Heidari B, Taheri H, Hajian-Tilaki K, Yolmeh M, Akbari R. Low baseline serum albumin as a predictor of anemia in chronic hemodialysis patients. Caspian J Intern Med 2015;6:161–4.
27. Watanabe T, Tanigawa T, Nadatani Y, Nagami Y, Sugimori S, Okazaki H, et al. Risk factors for severe nonsteroidal anti-inflammatory drug-induced small intestinal damage. Dig Liver Dis 2013;45:390–5. [CrossRef]
28. Cangelosi V, Ruckthong L, Pecoraro VL. Lead (II) Binding in Natural and Artificial Proteins. Met ions Life Sci 2017;17:271–317.
29. Hortin GL, Seam N, Hoehn GT. Bound homocysteine, cysteine, and cysteinylglycine distribution between albumin and globulins. Clin Chem 2006;52:2258–64. [CrossRef]
30. Belatik A, Hotchandani S, Carpentier R, Tajmir-Riahi HA. Locating the binding sites of Pb(II) ion with human and bovine serum albumins. PLoS One 2012;7:e36723. [CrossRef]
31. Saha A, Yakovlev VV. Structural changes of human serum albumin in response to a low concentration of heavy ions. J Biophotonics 2010;3:670–7. [CrossRef]
32. Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian J Intern Med 2013;4:627–35.
33. Unal I. Defining an Optimal Cut-Point Value in ROC Analysis: An Alternative Approach. Comput Math Methods Med 2017;2017:3762651. [CrossRef]