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RESEARCH ARTICLE

ERYTHROCYTE PROTOPORPHYRIN AS AN INDICATOR TO LEAD EXPLOSURE AROUND A LEAD MINE AREA OF ODISHA

[1]Ashok Ku. Khatua, [2,*]Prafulla Ch. Rout and [3]Bijaya N. Naik

[1]Department of Zoology, M.P. Mahavidyalaya, Erakana, Cuttack, Odisha, India 754206
[2]Department of Zoology, S.B. Women‟s College, Cuttack, Odisha, India 753001
[3]Group Head Biotechnology and Biomedical Engineering Group, R&D Centre, Sophitorium Engineering College, Khurda, Odisha, India

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ABSTRACT

Lead poisoning was studied in a mining area of odisha. The study area taken is in between the radius of 5km. from the mining site. Males of different age groups living in different distances are considered for observation. The PbB level increases with increase in age up to 55years and slight decrease in PbB level ins seen in the people aged above 56. The EP level also increases with increase in age and distance from the mining site. The level of EP increases beyond normal above PbB level 10µg/100ml. A considerable increase in EP level is seen in PbB level>30µg/100ml. In most of the cases the co-relastion co-efficient r value is above 0.5 with P<0.05. Increase in PbB level increases EP level which shows lead interferes significantly with the formation of haem from protoporphyrin in blood, thus clearly indicates its toxic effects.

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INTRODUCTION

Lead is one of the heaviest and softest of the common mental available. So far, no functional significance has been ascribed to lead in any biological system yet it is found in human body & a trace amount causes adverse health effect. PbB is a good indicator of the current level of lead on human Body (Ahemed *et al.,* 1990). Leadimpair the haematopoietic system and is usually related to deficiency in cognitive functioning. Metabolic disturbance in human due to body lead burden includes inhibition to haem biosynthesis (Moore, *et al.,* 1980) and the resultant increase in erythrocyte protoporphyrin (EP) in blood have been found to generally precede. Clinical manifestation of lead poisoning (Piomelli *et al.,* 1980). The measurement of (EP) level provides a better indicator of long term lead exposure than will blood lead level (Cavalleri *et al.,* 1981). Increase in blood lead levels cause exponential increase in EP level (Piomelli, 1982). The Present study reflects the PbB levels in human around the mining area and its relation to EP levels to assess the exposure of human being to toxic metal. Though increase in EP decreases haem synthesis which control the Oxygen carrying capacity of blood and oxidation of food stuff to release energy, so as to affect all the body tissue, therefore in the present study we tried to co-relate the level of PbB with EP.

Corresponding author: Prafulla Ch. Rout ,
Department of Zoology, S.B.Women"s College, Cuttack, Odisha, India 753001.

MATERIALS AND METHODS

The present study area (Fig.1) is the sargipali lead mining area located in sundargarh district of odisha, India (22’ 02’30” N :83’55’17”E). It is a rocky area having highest level attaining area is 381 meters & lowest elevation of 220 meters approximately. This area is surrounded by scanty out crops & patches of villages in which villages are mainly tribes. It is a tropical area having maximum temp. of 45^0c & 09^0c & an average rain fall in 1500mm. In the study different villages around 5 kms radius in different directions from mines were taken for sampling. 2-3 villages are taken as study area in the radius of 1km, 2kms, 3kms, 4kms, 5kms approximating. The control area Manguspur village is approximately 10kms away from mining site.

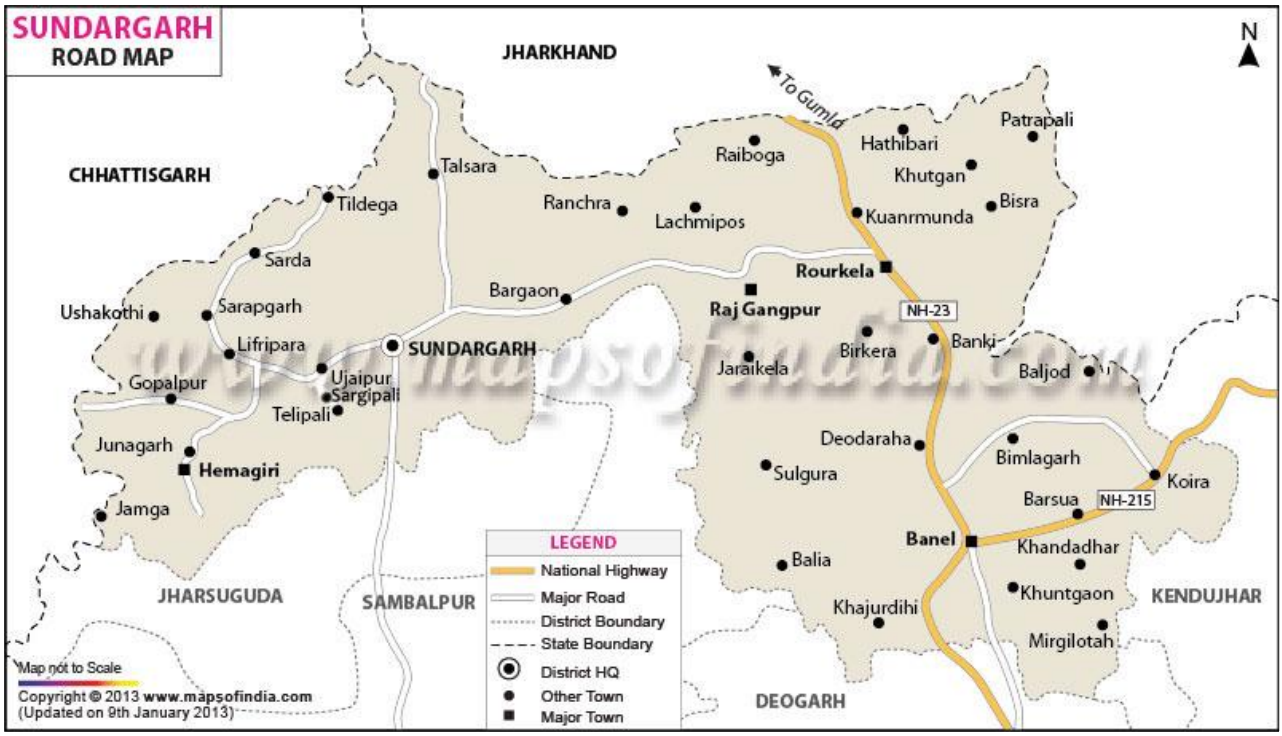

Fig. 1. Location of the study area

Sample analysis

All blood samples were collected with the help of local medical personals from the people who donated the blood voluntarily. Blood samples were collected from vein in small glass bottle by adding dipotassium salt of EDTA (1mg/ml) and Neomycin sulphate (100µg/ ml) & Preserved at 4^0c. Out of this sample 3ml of blood way used for a single analysis. The lead in blood was complexed with APDC (Ammonium pyrrolidine dithiocarbamate) by adding 0.5ml of 2% ADDC to it and extracted into 3ml of n-butyl acetate by proper shaking. Lead was than determined In organic phase by flames AAS following Australian standard method (AS-2411,1980). Protoporphyrin determination were carried out on 10 ml blood specimen collected in anti-coagulant. The blood was centrifuged, the plasma withdrawn, and distilled water added to restore the original volume. 5 ml of this solution of lysed erythrocytes was added to 50 ml of acetone / concentrated HCl (50:1).

The resulting mixture was centrifuged and the supernatant was removed. The residue was washed once with 10 ml of acetone / HCl solution. To the combined acetone / HCl extract was added an equal volume of 1.5N HCl. The hemin precipitate formed was centrifuged and washed with 10 ml of 1.5N HCl. 10 ml of saturated sodium acetate was added to the extract & the pH was adjusted to 3-5 with 10% KOH. The porphyrins were extracted into 50 ml of anhydrous ethyl ether. The ether extract was washed twice with 10 ml of 10% Sodium acetate, once with 10 ml of water & twice with 5 ml of 0.1 N HCl. Finally protoperphyrin was extracted with small aliquots & of 3 N HCl. To remove impurities, the HCl solution of protoperphyrin was vigorously shaken for 2-3 min with on equal volume of ethyl acetate. The lower HCl layer contained the protoporphyrin, its volume was noted.

Protoporphyrin concentration was then measured in a spectrophotometer & calculated according to the method of Rimington & sveinsson.

$[2A_{407}-(A_{380}+A_{430})]\times1.226\times5\times HCt/100\times100 = \mu g$ protoporphyrin/100ml packed cell.

1.226 represent the correction factor, 5 the blood volume used, HCt → hematocrit.

The blood samples analysis for PbB & EP in males by considering age and distance from mining. The age groups taken in years as 6-14, 15-35, 36-55 & ≥56. The concentration of PbB & EP is expressed in µg/100 ml of blood. All the apparatus used are thoroughly washed many times by HNO_3 for low blank value & to avoid contamination. The co-relation co-efficient between PbB & EP is worked out By Parsons method. The P values are also calculated to establish high co-relation.

RESULTS AND DISCUSSION

The blood lead content PbB is observed in different age groups considering the distance around mining area (Table 1). The recommended maximum level (RML) for PbB in adult is 40µg /100ml & that children is 30µg /100ml, WHO, (1995), but current acceptable PbB level is less than 5µg/100ml for children (ACCLPP, 2012) and 25µg/100ml for adult (Wu, 2006). The children living in between 1kms radius of mining site show higher level of PbB, about 70% of the children PbB>RML. The present study show PbB level is inversely proportional to distance in case of children. The mean PbB level for children is more living in between one km radius than the mean of 33µg/100ml for children of an acid factory working in London (Elwood *et al.,* 1977).

The mean PbB value for adults living in between 2km radius shows more than RML, but about 34% of people living in between the distance of 4km shows greater value than RML. 6% of the children living of a distance of 4-5 kms shows PbB value above RML, but Only 4% of the adults living above 4km radius shows greater value than RML. The PbB level increases in adults up to age of 55years then slightly decreases may due to low exposure in old age, but in all adults PbB level decreases when distance increase

Table 1. Blood lead level (µg/100ml) in human male around Sargipalli lead mine area

Distance in KM	Age in year			
	6-14	15-35	35-55	≥56
Control 10	7.36 ±2.15	10.13 ±3.21	9.81 ±3.19	8,25 ±4.06
≤1	36.31 ±7.36	50.87 ±8.21	45.45 ±9.85	38.96 ±8.79
›1-≤2	24.06 ±9.93	40.13 ±11.37	40.23 ±10.73	33.63 ±9.94
›2-≤3	24.36 ±10.75	36.57 ±7.14	37,83 ±8.57	31.08 ±12.80
›3-≤4	24.82 9.30	34.63 ±12.21	34.17 ±10.53	25.67 ±8.97
›4-≤5	20.05 ±8.91	24.92 ±9.67	25.31 ±6.24	17.96 ±5.96

Table 2. Erythrocyte protoporphyrin (EP) µg/100ml of blood in human male around Sargipalli lead mine area

Distance in KM	Age in year			
	6-14	15-35	35-55	≥56
Control 10	26.43 ±14.23	34.39 ±12.67	37.81 ±22.21	40.06 ±18.65
≤1	123.86 ±20.76	199.66 ±21.72	187.03 ±25.03	197.71 ±23.61
›1-≤2	102.98 ±24.30	145.15 ±22.13	159.33 ±21.07	107.46 ±23.23
›2-≤3	83.39 ±16.27	125.57 ±20.46	121.66 ±16.21	95.84 ±19.83
›3-≤4	78.33 ±18.99	129.18 ±24.41	109.26 ±20.84	91.23 ±29.02
›4-≤5	67.59 ±15.33	97.64 ±18.57	86.06 ±16.93	83.91 ±14.41

Table 2 shows mean EP level for children and adult living around mining area. The people living in control area shows lower EP level than normal (16-65 µg/100ml). The EP level in both children and adult living in the radius of 1 km from mining site shows highest values and almost all people of the study area shows higher EP value than recommended. Except 27%, all other people living in between radius of 4-5 kms shows higher EP level than normal, but mean level is nearby to boarder line value for EP. Hutten (1987) shows observable elevation of EP even in low PbB value of 15µg/100ml in adult.

This study shows the threshold at which an increase of EP occurs is PbB above 10µg/100ml in children, which agree with the fact advocated by Piomelli *et al.* (1982) is in the range of 100-200µg/lit. The mean EP level in people of control area is in between the range of 26.43-40.06 where as the mean EP level in study area is in the range of 67.59-199.66. In some cases people living in between 3-4 km shows higher values than those are living in between 2-3 km may be due to one of the village out of three is nearby to tailing dam.

It was also observed that the old men shows higher EP value than the young even in the same PbB level.

Table 3. Co-relation co-efficient 'r' in between PbB and EP of human male around Sargipalli lead mine area

Distance in KM	Age in year			
	6-14.	15-35.	35-55.	≥56.
Control 10	0.235	0.214	0.398	0.342
1≤	0.729*	0.852*	0.796*	0.667 Ω
›1-≤2	0.833*	0.785*	0.713↑	0.725*
›2-≤3	0.738*	0.582↑	0.649 Ω	0.858*
›3-≤4	0.529 Ω	0.543↑	0.642*	0.737↑
›4-≤5	0.392	0.445 Ω	0.359	0.457 Ω

*-P<0.001 ↑-P<0.01 Ω- P,0.05

The Fig. 2 shows that increase in PbB level increases EP level. From PbB level 15µg/100ml the EP level increases significantly but it increases considerably when EP level goes above 30µg/100ml.

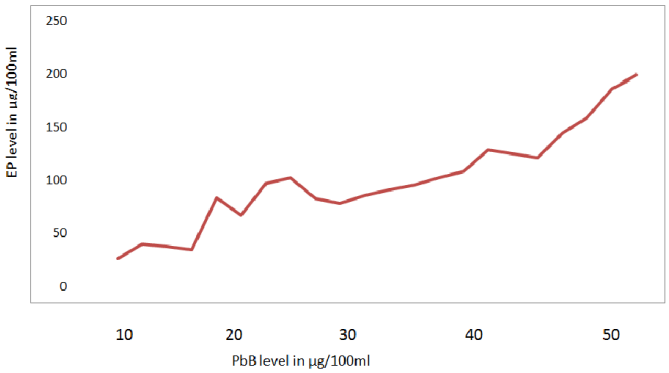

Fig. 2. PbB and EP level in human male around Sargipalli lead mine area

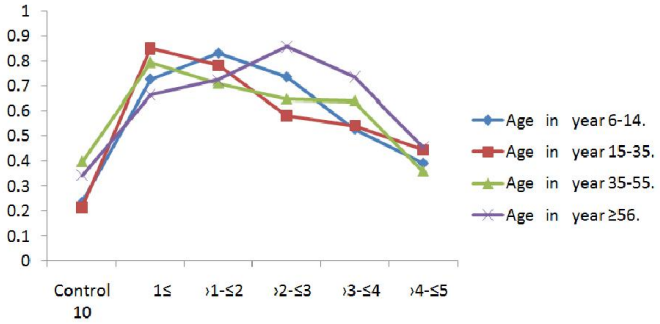

Fig. 3. Co-relation co-efficient 'r' in between PbB and EP of human male around Sargipallilead mine area

Table 3 shows co-relation co-efficient in between PbB and EP (Fig.3). A significance co-relation is observed for PbB and EP in most of the cases with P value < 0.05. The co-relation co-efficient in between PbB and EP in age group 6-55 living inside the radius of 1km and in the age group of 6-35 living inside the radius of 1-2 km shows significant relation, r>0.7 and P<0.001. The people in age group 35-55 living in between radius of 1-2km, 15-35 living in between 2-4km and 35-55 living in between 3-4km show P value less than 0.01, which indicates a higher relation in between PbB and EP level. Increase in PbB level increases EP level (Fig. 2) which shows lead interferes significantly with the formation of haem from protoporphyrin in blood, thus clearly indicates its toxic effects.

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