Assessment of δ-Aminolevulinic Acid in relation with Lead levels in Urine samples of Lead Exposed people Living Around Lead mine area of Odisha State, India

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Abstract- Among the heavy metals lead plays no known role in the human body with no deficiency syndromes and a trace of it impairs body physiology. According to WHO health criteria (1995) mining smelting and refining as well as the manufacturing of lead obtaining compounds and goods, can give rise to lead exposure. Health hazards of the human environment include undue exposure to lead remains serious public health concerns (WHO, 1995). Mining and smelting activities of lead are well known to create health problems in local area. 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. Villages taken for study are located approximately one Km distance of each other from core mining area. People of different age groups and sex living in different distances are considered for observation. It was found that the incidence of body lead burden is significantly higher in exposed area than the control area which is 10Km away from mining site. In this study the level of PbU and δ-ALAU is considered. The level of δ-ALA was measured to observe interference of lead in haem biosynthesis. The PbU level increases with increase in age up to 35years and slight decrease in PbU level is seen in the people aged above 35. The δ-ALAU level also increases with increase in age and decrease with increase in distance from the mining site. The level of δ-ALAU increases beyond normal above PbU level 10µg/lit.. A considerable increase in δ-ALAU level is seen in PbU level >30µg/lit.. In most of the cases the co-relation co-efficient 'r' value is above 0.5 with $P<0.01$. Increase in PbU level increases δ-ALAU level which shows lead interferes significantly with the formation of haem from protoporphyrin in blood, thus clearly indicates its toxic effect on human health..

Index Terms- Blood lead level (PbB), δ-Amino Levulinic Acid (δ-ALA), δ-Amino Levulinic Acid in Urine (δ-ALAU), Atomic absorption spectrophotometer (AAS), Recommended maximum level (RML), Maximum acceptable level (MAL).s)

I. Introduction

Among the heavy metals lead plays no known role in the human body with no deficiency syndromes and a trace of it impairs body physiology. Use of lead and its adverse health effects resulting from lead exposure have occurred for thousands of years. During last few years understanding of the range of human health effects due to lead exposure has expanded markedly. According to WHO health criteria (1995) mining smelting and refining as well as the manufacturing of lead obtaining compounds and goods, can give rise to lead exposure. Health hazards of the human environment include undue exposure to lead remains serious public health concerns (WHO, 1995). Mining and smelting activities of lead are well known to create health problems in local area. The effect of mining and concentrating plant on the surrounding air, water and soil depends to a large extent on the height of the stack, position of tailing dam, climatic condition, topography and other local features. Through air, water and soil lead enters into the food chain of human being and create serious health hazards (Dash et al. 1996). Lead impairs 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. M.R et al. 1980).

The PbU level substitute the PbB level as an indicator of recent exposure to lead (Goyer, 1985).. Even normal PbB level that is < 20µg/100ml affect the human biochemistry that is inhibit the action of ALA dehydrase (Environmental chemistry, 1998). ALA is regarded as being the earliest and most sensitive biological indicator of lead exposure in human population at very low PbB level (Telisman.S et al,1986). Nakao et al.(1968) suggested that estimation of ALA in urine should give an early indication of lead poisoning. Estimation of ALA is reliable and rather more specific for lead, the result co-relate well with metabolically active lead in the body and have a linear relationship with body lead (Selander et al., 1970).

The Present study reflects the PbU levels in human around the mining area and its relation to δ-Amino Levulinic Acid in Urine to assess the exposure of human being to toxic metal. Though increase in δ-ALA decreases haem biosynthesis which control the Oxygen carrying capacity of blood and hampers energy liberation by oxidation of food stuff, so as to affect all the body tissue. Therefore in the present study we tried to co-relate the level of PbU with δ-ALAU and its hazardous effect on human health.

II. MATERIALS AND METHODS

The present study area 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 and minimum temperature of 45^0C & 09^0C & an average rain fall in 1500mm. In the study different villages around 5 km radius in different directions from mines were taken for sampling. 2-3 villages are taken as study area in the radius of 1km, 2km, 3km, 4km, 5km approximately. The control area Manguspur village is approximately 10km away from mining site.

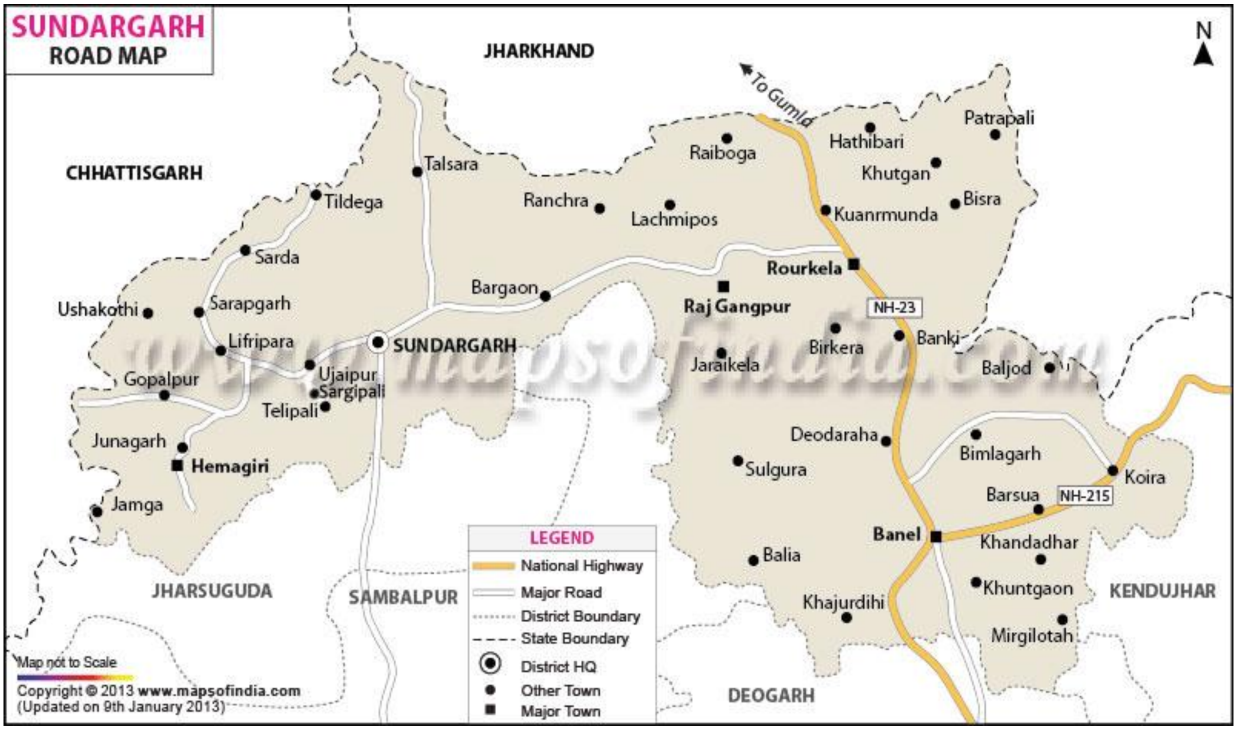

Fig -1 Location of the study area

Sample analysis

The urine samples were collected in sterilized glass bottles thoroughly washed with dilute nitric acid for 2-3 time. The glass bottles are labelled with marker pen for identification. Of this 20ml of sample is taken in the test tube and add 2ml of concentrated HNO_3. Keep on hot plate at 80-120^0C up to dryness in a pyrex evaporating disc. Ignite in a muffle furnace at 450^0C to get white ash. Take the ash in 1:1 nitric acid and perchloric acid to digest, evaporate to dryness. Take the aliquot in 25% nitric acid to a known volume for determination of lead concentration by AAS and calculate by using standard curve (Telisman et.al, 1985). The concentration of lead in urine is expressed in µg/lit.. The RML and MAL for urine is 50µg\lit. and 80 µg\lit. respectively.

Analysis of urinary δ-Aminolevulinic acid followed K. Tomocuni and M. Ogata (1972). 1.0ml of urine was pippeted into each of the two 10 ml. glass stoppered tubes and adds 1.0ml of acetate buffer (pH 4.6) to one of the tubes add 0.2 ml of ethyl acetoacetate and mix with a vibration mixture for about 5 seconds. Ethyl acetate was omitted from the other tube which was run as blank. The tubes were placed in a boiling water bath for ten minutes, cooled and 3 ml of ethyl acetate was added and shaken thoroughly by hand. Then it was centrifuged for 3 minutes at 1500- 2000rpm. 2 ml of the ethyl acetate was pipetted out from the supernatant into another glass tube and 2 ml of Ehrlich's reagent was added mixed thoroughly for ten minutes and OD measured at 553 nm vs. an equal volume of the blank. For calculating the concentration of urinary ALA, the absorbance of urine with (A) and without ethyl acetate (B), subtracting B from A and reading the concentration of ALA from a standard curve prepared by the aqueous solution of ALA. The acceptable level for δ-ALAU is 1-7mg\day.

1) The urine samples analysis for PbU & δ-ALA in both the sexes by considering age and distance from mining. The age groups taken in years as 6-14, 15-35, 36-55 and ≥ 56. All the apparatus used are thoroughly washed many times by HNO_3 for low blank value and to avoid contamination. The co-relation co-efficient between PbU and δ-ALA is worked out by Parsons Method. The P values are also calculated to establish high co-relation.

II. RESULTS AND DISCUSSION

In the earlier studies it has been seen that absorption and accumulation of lead in blood (PbB) is both dose dependant as well as depends on period of exposure (Rout et al. 2013). The body lead burden in people living around mining and smelting area may due to different grades of exposure for prolonged period (Telisman. et al.,1989). The PbU level substitute the PbB level as an indicator of recent exposure to lead (Goyer, 1985).

The collected urine samples are analyzed and interpreted in relation to age, sex and distance from mining site (table-1 & 2). The level of PbU in both the sexes of control area shows lower values than RML, but the all PbU level of exposed area shows higher values than the control area. In males the mean PbU level of different age groups in the villages living in between 1km radius are in the order of 6-14 years < 15-35 years < 36-55 years >age group ≥56 years , but in females, this values are in the order of 6-14 years <15-35 years >36-55 years > ≥56 years (table-1 and 2). The higher Pb excretion (mean 104.39 μg/lit.) in male is seen in age group 36-55 years living in between 1km radius , but its level decreased in the age group ≥ 56 years may due to lower exposure in old age. In female highest Pb excretion is seen in the age group 36-55 years, may due to higher exposure, because the females are more active in this age group. The mean PbU level in both the sexes of age group 15-35 years shows higher level than any other age group in the villagers living in between 5km radius. In relation to distance, the PbU level is inversely proportional to the distance from mining site in all age groups, but the mean PbU level in villagers living in between 3-4 km radius shows slightly higher PbU level than villagers living in between the distance of 2-3 km may due to one of the village jhimirmoul is present nearby to the tailing dam.

Table-1 Lead level (μg/lit.) in Urine of human males around Sargipali lead mine area.

Distance in KM	Age in year			
	6-14	**15-35**	**36-55**	**≥56**
Control 10	13.63±5.91 n=23	21.61±8.43 n=47	23.03±6.52 n=59	17.12±6.54 n=53
≤1	83.32±8.63 n=39	103.06±16.54 n=44	104.39±14.95 n=53	94.02±16.51 n=42
>1-≤2	61.91±11.26 n=43	71.31±19,59 n=56	68.30±12.06 n=71	62.72±18.54 n=63
>2-≤3	48.81±10.55 n=37	63.43±11.93 n=66	61.34±9.64 n=49	59.03±12.56 n=61
>3-≤4	43.59±12.53 n=40	65.64±15.59 n=62	63.59±10.93 n=79	56.36±9.67 n=81
>4-≤5	33.75±10.12 n=31	46.63±12.50 n=45	41.08±19.39 n=51	38.18±13.74 n=60

Data:-Mean±SD

Table-2 Lead level (μg/lit.) in Urine of human females around Sargipali lead mine area.

Distance in KM	Age in year			
	6-14	**15-35**	**36-55**	**≥56**
Control 10	10.47±7.32 n=19	21.26±4.73 n=34	22.55±7.43 n=45	15.96±6.37 n=51
≤1	84.16±6.93 n=29	99.33±14.28 n=31	93.53±16.35 n=39	81.58±09.97 n=41
>1-≤2	60.86±9.31 n=37	66.66±13.12 n=40	63.83±12.86 n=51	59.08±14.82 n=57
>2-≤3	51.65±9.63 n=35	60.14±11.63 n=48	58.41±11.41 n=60	53.96±9.39 n=45
>3-≤4	46.63±11.96 n=33	61.38±17.43 n=47	61,16±12.68 n=46	44.07±8.93 n=72
>4-≤5	31.68±5.71 n=36	39.19±16.46 n=39	40.91±17.68 n=45	34,86±10.36 n=53

Data:-Mean±SD

About 35%, 73% , 81% and 56% of males of age group 6-14, 15-35, 36-55 and ≥56 years show higher PbU level than RML, but 13% , 46% ,51% and 21% of age group 6-14, 15-35 ,36-55 & ≥56 years in males shows higher value then MAL, in villagers living in between the radius of 1km. In female about 32%, 69%, 64% and 43% of age group 6-14, 15-35, 36-55 and ≥56 years show females living in between 4-5 km radius show least PbU level in exposed study area than others. About 20%,32%, 27% and 29% of age group 6-14, 15-35, 36-55, and ≥56 years in males living in between 4-5 km radius shows greater value than RML, but 8% and 6% of males show greater value than MAL in age group 15-35 years and 36-55 years, no individuals in the age group 6-14 and >56 years show higher value than MAL .About 14%,23%, 25% and 17% of females in between 4-5km radius of age group 6-14,15-35,36-55 and ≥56 years shows higher values than RML but only 6% and 7% of female of age group 15-35 and 36-55 years shows greater values than MAL, may due to low exposure.

In all age groups the mean PbU level is less in females than males except age group 6-14, for example the females living in between 1km radius of age groups 15 -35, 36-55 and ≥56 years show 99.33 µg/lit.,93.53 µg/lit. and 81.58 µg/lit. respectively, where as males living in between 1km radius of age groups 15-35, 36-55 and ≥56 years shows PbU level 103.06µg/lit., 104.39µg/lit. and 94.02 µg/lit. respectively. This phenomenon is observed in all age group in villagers living in between 5 km radius. The females show less PbU level than males may due to less exposure because they are mainly engaged in house hold works, but the females of the age group 15-35 and 36-55 shows higher PbU than other groups may due to some of them engaged in outfield works.

Even normal PbB level that is < 20µg/100ml, affect the human biochemistry that is inhibit the action of ALA dehydrase (Environmental chemistry, 1998). Inhibition of ALAD activity inhibit formation of porphobilinogen (PBG) from δ-aminolevulinic acid (δ-ALA) in cell cytoplasm, which increases ALA level in the body, ultimately increase ALA excretion in urine and ALAU level increases.

ALA is regarded as being the earliest and most sensitive biological indicator of lead exposure in human population at very low PbB level (Telisman. et al., 1986).

During chronic occupational exposure to lead for prolonged period, there is fall in haematocrit value, haemoglobin concentration along with RBC and WBC number (Khatua et al. 2015). Increase in PbB level increases erythrocyte protoporphyrin level which shows lead interferes significantly with the formation of haem from protoporphyrin thus clearly indicates its toxic effect (Khatua et al. 2015).

Nakao et al.(1968) suggested that estimation of ALA in urine should give an early indication of lead poisoning. Estimation of ALA is reliable and rather more specific for lead, the result co-relate well with metabolically active lead in the body and have a linear relationship with body lead burden (Selander et al., 1970).

Table-3 Urinary δ-Amino levulinic acid (µg/100ml) in human male around Sargipali lead mine area.

Distance in KM	**Age in year**			
	6-14	**15-35**	**36-55**	**≥56**
Control 10	103.39±23.81 n=23	131.32±26.36 n=47	116.29±31.11 n=59	141.21±18.37 n=53
≤1	466.30±66.45 n=39	938.16±75.52 n=44	635.74±81.73 n=53	652.31±71.21 n=42
>1-≤2	387.74±73.57 n=43	685.06±66.39 n=56	559.13±89.86 n=71	532.57±43.56 n=63
>2-≤3	295.71±53.02 n=37	625.49±83.76 n=66	427.14±37.21 n=49	502.33±32.44 n=61
>3-≤4	352.85±69.27 n=40	671.42±76.81 n=62	497.99±46.32 n=79	440.53±51.12 n=81
>4-≤5	207.14±49.62 n=31	444.99±32.53 n=45	340.32±37.43 n=51	310.71±53.11 n=60

Data:-Mean±SD

Table-4 Urinary δ-Amino levulinic acid (µg/100ml) in human female around Sargipali lead mine area.

Distance in KM	Age in year			
	6-14	15-35	36-55	≥56
Control 10	107.94±23.43 n=19	111.42±21.46 n=34	126.71±19.56 n=45	128.10±27,98 n=51
≤1	435.73±53.01 n=29	798.03±61.87 n=31	663.28±51.67 n=39	636.64±68.13 n=41
>1-≤2	380.13±61.49 n=37	687.16±56.79 n=40	531.96±46.74 n=51	551.42±46.32 n=57
>2-≤3	272.14±52.52 n=35	555.53±59.31 n=48	401.17±54.04 n=60	435.28±69.72 n=45
>3-≤4	298.31±48.22 n=33	539.64±55.27 n=47	533.74±66.95 n=56	505.53±69.43 n=72
>4-≤5	207.85±40.57 n=36	335.27±43.60 n=39	349.64±48.71 n=45	279.10±29.36 n=53

Data:-Mean±SD

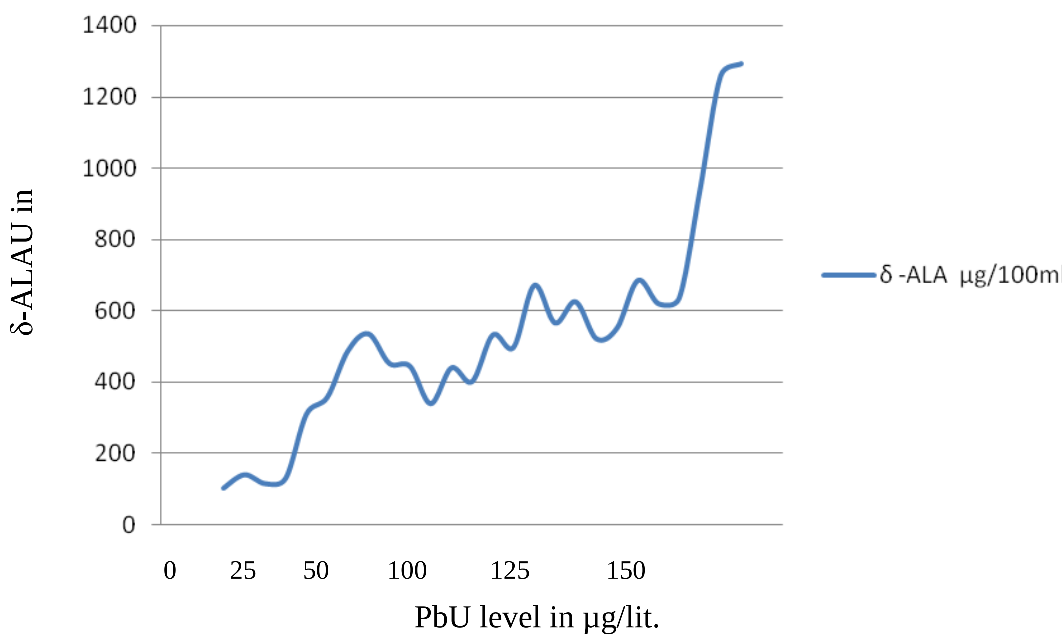

Fig.-2 Comparison between δ-Aminolevulinic acid (δ-ALA) with relation to Urine lead level (PbU) in people living around Sargipalli Lead Mine Area.

The δ-Aminolevulinic acid in urine is reflected in table-3 and 4. The observation in both the sexes shows that level of δ-ALA in control region is far below the acceptable limit but the mean δ-ALAU level in all the age groups living in 5 Km radius around mining site is above RML. Though in low blood lead level, the level of δ-ALA increases detectibly, indicates that lead interfere in porphobilinogen biosynthesis even in lower exposure.

In males the people living in between the radius of 1 Km shows higher mean values due to higher exposure, this may due to one of the village Bharatpur is placed nearby to ore concentrating plant, even 9% of the males shows δ-ALAU value greater than 1000µg/100ml. Among the males the people in the age group 15-35 years shows highest mean value (938.16 µg/100ml) as most of them are agriculture workers working in the crop field irrigated by pumped underground mine water. The mean δ- ALAU level increases sharply upto the age of 35 years and then decreases towards older age but still it is above the RML (table-3). In males the δ-ALAU level decreases with increase in distance from mining site except in people living in 4-5 Km radius , this may because one of the village Jhimirmoul is placed nearby to tailing dam & people are highly affected by the dusts in summer. The people of all age groups show higher δ-ALAU values than the children aged 6-14 years living in same distance except those are living in the radius of 4-5 Km from the mining site.

Like male females also shows higher δ-ALAU level in exposed study area (table-4). In most of the cases females shows lower δ-ALAU level than males except the mean value of age group 15-35 years living in 1-2 Km radius, 36-55 years living in 1-2 Km and 3-5 Km distance and age group ≥56 years living in 3-4 Km distance. In females the mean δ-ALAU value is highest in those living in 1 Km radius of 15-35 years age group and lowest in the people of age group 6-14 years living in 4-5 Km radius. Most of the females living in 4-5 Km distance show low δ-ALAU level may due to living in high distance and lower exposure.

Fig. 2 shows relation of PbU with δ-ALAU, which indicates that the level of δ-ALAU increases from very low level of PbU in people living around sargipali lead mine area. The δ-ALAU level increases considerably high from PbU level 150 µg\lit. The increase in δ-ALAU level indicates that lead inhibit haem biosynthesis from very low level of PbU and may create serious health problems as anaemic condition at very high level.

Table-5 Correlation co-efficient 'r' in between PbU and δ-ALAU of human male around Sargipali lead mine area.

Distance in KM	**Age in year**			
	6-14	**15-35**	**36-55**	**≥56**
Control 10	0.164	0.326	0.293	0.159
1≤	0.683*	0.725*	0.776*	0.652↑
>1-≤2	0.421↑	0.656↑	0.588 Ϯ	0.449 Ω
>2-≤3	0.425	0.526	0.638	0.572 Ϯ
>3-≤4	0.589 Ϯ	0.592 Ϯ	0.546 Ϯ	0.339
>4-≤5	0.365	0.485 Ϯ	0.442	0.206

*-P<0.001 ↑-P<0.01 Ω- P<0.05 Ϯ<0.1

Table-6 Correlation co-efficient 'r' in between PbU and Delta ALAU of human female around Sargipali lead mine area.

Distance in KM	**Age in year**			
	6-14.	**15-35.**	**36-55.**	**≥56.**
Control 10	0.226	0.325	0.319	0.191
1≤	0.591↑	0.689*	0.621*	0.626
>1-≤2	0.428 Ϯ	0.616*	0.524*	0.522 Ϯ
>2-≤3	0.425 Ϯ	0.475	0.527 Ω	0.519
>3-≤4	0.495 Ω	0.528 Ω	0.556 Ϯ	0.325 Ϯ
>4-≤5	0.225	0.322	0.395	0.262

*-P<0.001 ↑-P<0.01 Ω- P<0.05 Ϯ<0.1

The table-5 & 6 shows correlation co-efficient in between PbU and δ- ALAU in both the sexes. The males of 6-55 years living in 1Km radius shows r > 0.6 & P<0.001.,where as males living beyond 3Km radius shows 'r' value < 0.6. The females of age group 15-55 years living in between 2 Km radius shows higher significant values with $r > 0.6$ & $P<0.001$. The females living in control region and those are living in 4-5 Km radius shows $r < 0.4$ & $P>0.1$.

The above discussion concludes that significance correlation exists in between PbU and δ-ALAU, which clearly indicates the lead interference in δ-ALA metabolism, haem biosynthesis and creates disturbance in body metabolism, causes serious health hazards.

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