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Original Research Article

Identification of Heme Oxygenase-1 (HO-1) for Risk Assessment and Disease Progression in Bauxite Mine Workers

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ABSTRACT

Background- Bauxite ore is mined all over the world because of industrial importance and presence of abundant aluminium. The exposure to Bauxite dust concern that its toxic effects may be due to dust overload rather than direct effect of aluminium in lung tissue. Heme Oxygenase-1 is known marker of stressful condition induced in the lung. Present study may try to give answers to the questions, whether HO-1 is helpful as a predictable biomarker covering Al exposure and its toxic effects in bauxite mine workers.
Objective- Find out relationship between years of Bauxite dust exposure with levels of Heme Oxygenase-1 with confounder factors and biochemical parameters.
Materials and Methods-The study was conducted in three different opencast Bauxite mines in India. In this exploratory and stratified randomized study, blood samples from 273 subjects were collected from Bauxite miners. Subjects were divided into three study groups as experimental (n=150), experimental control (n= 73) and control (n=50).
Results- Descriptive statistics of baseline characteristics of subjects in three study groups were analyzed according to mines as well as after pooling over the three mines. However, considering that age, BMI and the behavioural habits i.e. smoking, tobacco and alcohol consumption have no effect of these confounding factors on Heme Oxygenase-1. Bar plots for the mean duration of exposure shows positive correlation with Heme Oxygenase-1. It was also observed that increased level of aluminium is statistically significant but lies within normal range.
Discussion- On the basis of findings it was observed that exposure to Bauxite dust (even at low levels of aluminium) changes biochemical profile leading to high levels of Heme Oxygenase-1 (HO-1). These changes show positive correlation with duration of exposure with bauxite dust.
Conclusion- The rationale of the study is the Heme Oxygenase-1 may be used as a biomarker for early detection of health risks in Bauxite dust exposed miners.

Keyword: Aluminium, Bauxite dust, Biomarker, ELISA, Heme Oxygenase-1.

INTRODUCTION

India is endowed with rich Bauxite reserve of 2,300 million tonnes (approx. 6.76% of the world total) and ranks 5th in the world Bauxite reserve base. [1] Bauxite is mined all over the world because of its industrial importance and abundant availability throughout the world, therefore a lot of industries got engaged with its extraction and production. Bauxite is refined to produce alumina, which is then reduced to Aluminium (Al), a major mineral present in the Bauxite ore. [2]

Long term exposure to Bauxite dust may lead to adverse health condition, which may be identified by the expression of biomarkers on progression of disease. [3] Therefore, scientists are trying to investigate the biomarkers which might contribute to early detection of occupational diseases among workers as well as helps towards understanding the mechanism of progression of the disease. Heme Oxygenase-1 is the probable potential biomarkers used in this study of bauxite exposed workers.

Heme Oxygenase (HO-1): an inducible stress response enzyme

Heme Oxygenase-1 (HO-1) is a stress responsive protein that is highly induced by many agents, including cytokines, endotoxin, heavy metals, Nitric Oxide, Reactive Oxygen Species (ROS), and its own substrate heme. [4,5] Lung is the primary organ for vulnerable attack of the hazardous dust, which is the major site for the induction of HO-1. Lungs have potent defense mechanism of HO-1 which can protect from increase oxidant burden under more stressful circumstances. The mechanism by which HO-1 confers protection against oxidative stress posed by the environmental dust has not yet been fully understood.

HO-1 is a rate-limiting enzyme in heme catabolism. Al competes with iron of porphyrin ring due to similar valency, which disturbs catabolism of heme and thus influences the whole pathway. It has been noted that exposure to high level of Al may increase the level of HO-1 in the serum and thus affects the catalytic activity of HO-1, which in turn imbalances the synthesis of bilirubin and thus increases Oxidative Stress (OS). [6]

Scientist embarks on toxic inducers in asthma on expression of isoenzymes of HO-1 in the sub mucosal macrophages and airway epithelium as a cyto-protective molecule. The high endogenous expression of HO-1 present in the airways, particularly in the epithelium may be resulted due to daily environmental pollutants. [7,8] Clinical relevance of up regulation of HO-1 was also reported in case of pulmonary diseases, such as Acute Respiratory Disease Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD), which helps to defend against the insults in lungs. [9,10] Actual risk may be substantially underreported regarding Bauxite exposed mine workers. HO-1 induction can give more decisive results as biomarker in Bauxite dust exposed workers.

MATERIALS AND METHODS

The current study is based on Exploratory and Stratified Randomized design which was carried out in three different major Bauxite producing mines. Total 273 mine workers and control subjects were selected. Workers, who were directly exposed to bauxite dust were categorized into experimental group (n=150). Workers, who were age and sex matched and residing at same geographical region but not directly exposed to Bauxite dust were selected as experimental control group (n=73). Healthy individuals from same geographical area were considered as a control group (n=50). All subjects of control group had no history

of Al containing Bauxite dust exposure. Workers having exposure period of more than 1 year were included and those who were occupationally exposed to any known chemical agents, history of chronic diseases and female workers were excluded from the study. A standard questionnaire was used to record information on base line characteristics. Informed consent was obtained from all study subjects. The study was approved by Institutional Ethics Committee (IEC) of National Institute of Miners' Health (NIMH), Nagpur.

Blood collection:

Blood samples were collected from mine workers in dust free environment. Collected blood samples were allowed to clot and centrifuged at 1000 rpm for 5min. Separated serum samples were allowed to freeze immediately and stored at -40ºC in accordance with accepted procedures. Whole blood samples (2ml) were used for Aluminium metal analysis by Inductive Coupled Plasma Atomic Emission Spectrometry method and hemoglobin while serum samples were used for Heme Oxygenase-1 (HO-1), Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetate transaminase (SGOT) and Bilirubin analysis.

a) Determination of Heme Oxygenase-1 (HO-1) by ELISA:

HO-1 was evaluated by Sandwich ELISA in the collected serum samples for the study (Kit-Uscn Life Science Inclusion Cat log No.E90584Hu). A mouse Monoclonal Antibody (MAb) specific for human HO-1 is pre-coated on the wells of the microtiter plate. Add 100ul of standards and samples (after preparation in sample diluent) to the wells of Anti-HO-1 Immunoassay microtiter Plate (precoated plate) and incubated at 37°C for 30 min. Wells were then washed six times with wash buffer followed by addition of 100µl diluted Anti-Human HO-1 antibody. After 1 hour of incubation, the wells were washed six times with the wash buffer and 100µl secondary antibody, affinity purified anti rabbit IgG conjugated to Horse Radish Peroxidase (HRP) was added to wells and incubated at 37°C for 30 min. After incubation the wells were washed six times extensively with wash buffer followed by addition of 100µl of TMB/H_2O_2 substrate and incubated at 37°C for 15 min. The reaction was stopped with addition of 100µl stop solution and the absorbance of colour in each well was read at 450 nm. Each sample was tested in Duplicate.

b) Determination of Hemoglobin, Bilirubin concentration and enzyme activity:

Hemoglobin (Hb) level was estimated in the blood by using commercially available Drabkin's reagent by BEACON. Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetate transaminase (SGOT) was measured in the sera by commercially available kit from BEACON. Bilirubin was measured by DMSO (Dimethyl sulfoxide) Method. Hb and Bilirubin were read at 530nm while kinetic assay was done at 340nm by Semi autoanalyser. Each sample was tested in duplicate.

c) Determination of Aluminium by Inductive Coupled Plasma Atomic Emission Spectrometry ICP-AES method:

A set of experiment was prepared by using 2 ml blood sample with 1 ml triton x-100, 2ml of concentrated Perchloric acid, 5ml ultrapure concentrated HCl and 8.5 to 10ml ultrapure concentrated Nitric acid. This mixture was digested on hot plate at 150^0C for 30 minutes and filtered through whatman filter paper no. 40 and filtrate was diluted with nitric acid (5%) and made up volume up to 10 ml. Instead of blood

sample, ultrapure water was used in another set of experiment done with same procedure. This set of experiment was used as blank. Then final solution was aspirated in ICP-AES for further analysis. Each sample was tested in duplicate. [11]

Statistical analysis

All the statistical analysis was performed using R-2.15.1 programming language with pre-validated programs. Descriptive statistics of basic characteristics of subjects in three study groups were done by using *one-way analysis of variance*, *t-test of independent sample*, *and Chi-square test* followed by *Tukey's post-hoc* for comparison. Graphs of respective data were prepared using Prism (version 5) software (Graph Pad Software, Inc. San Diego, CA). *p* value of <0.05 was considered statistically significant for all the analysis.

RESULTS

Table 1: Descriptive statistics for demographic and behavioral parameters according to study groups

Parameter	Study groups (n=273)											
	Control (*n*=50)				Experimental control (*n*=73)			Overall	Experimental (*n*=150)			
Mines	Phase I (*n*=10)	Phase II (*n*=20)	Phase III (*n*=20)	Overall	Mine I (*n*=17)	Mine II (*n*=20)	Mine III (*n*=36)		Mine I (*n*=51)	Mine II (n=49)	Mine III (n=50)	Overall
Age (yrs.) [M ± SD]	28.2 ± 4.44	42.5 ± 5.58	38.15 ± 8.89	37.9 ± 8.61	48.24 ± 9.30	41.25 ± 12.15	48.31 ± 7.79	46.36 ± 9.88	45.88 ± 9.90	41.92 ± 10.12	45.98 ± 9.99	44.62 ± 10.11
Exposure (yrs.)[M±SD]	-	-	-	-	22.65 ± 10.58	15.35 ± 10.46	22.78 ± 6.87	20.71 ± 9.36	21.06 ± 9.97	15.67 ± 9.66	22.06 ± 7.38	19.63 ± 9.45
BMI (kg/m2) [M ± SD]	20.95 ± 2.59	26.78 ± 4.43	24.40 ± 3.84	24.66 ± 4.38	26.65 ± 5.04	22.80 ± 3.92	27.70 ± 3.27	26.11 ± 4.38	23.90 ± 3.46	23.09 ± 3.37	25.14 ± 3.65	24.05 ± 3.58
Diet												
Vegetarian	4 (40)	5 (25)	6 (30)	15 (30)	7 (41.18)	2 (10)	8 (22.22)	17 (23)	2 (3.92)	12 (24.49)	12 (24)	26 (17)
Both	6 (60)	15 (75)	14 (70)	35 (70)	10 (58.82)	18 (90)	28 (77.78)	56 (77)	49 (96.08)	37 (75.51)	38 (76)	124 (83)
Smoking (*Yes*)	3 (30)	4 (20)	0	7 (14)	3 (17.65)	8 (40)	7 (19.44)	18 (25)	20 (39.22)	23 (46.94)	18 (36)	61 (41)
Tobacco (*Yes*)	0	10 (50)	9 (45)	19 (38)	5 (29.41)	12 (60)	10 (27.78)	27 (37)	23 (45.10)	29 (59.18)	24 (48)	76 (51)
Alcohol (*Yes*)	4 (40)	11 (55)	8 (40)	23 (46)	10 (58.82)	10 (50)	6 (16.68)	26 (36)	33 (64.71)	33 (67.35)	20 (40)	86 (57)

Note: n= Data are presented as n=number of cases, ( )= Percentage for categorical data, Abbreviations: BMI;body mass index
During the course of the study, 273 Participants were enrolled. The study subjects were divided into three groups; (a) mine workers exposed to Bauxite dust as experimental group (n=150); (b) subjects from same geographical region working in mines but not exposed to dust as experimental control (n=73); and (c) healthy individuals from Nagpur region categorized under control group (n=50).

Table 1 provides the descriptive statistics of basic characteristics of subjects in three study groups. As regards age, the difference in the mean age across study groups was statistically significant with *P*-value < 0.0001 using *one-way analysis of variance*. The mean age of subjects in Control group (37.9 ± 8.61 yrs) was significantly lower than the other two groups. The mean duration of exposure for subjects in Experimental control group (20.71 ± 9.36 yrs) was insignificantly different than that of Experimental group (19.63 ± 9.45 yrs) as indicated by a *P*-value of 0.422 as per *t-test of independent samples*. Further, the mean body mass index (BMI) of subjects across study groups differed significantly as revealed by a *P*-value of 0.001 ($P < 0.05$) using *one-way analysis of variance*. The mean BMI in Experimental control group (26.11 ± 4.38 kg/m2) was significantly higher than the Control (24.66 ± 4.38 kg/m2) and Experimental groups (24.05 ± 3.58 kg/m2).

The dietary habits of subjects showed insignificant association with the study groups as indicated by a *P*-value of 0.1463 using *Chi-square test*. The proportion of subjects with smoking habit in Experimental group (40.6%) was significantly higher than that of Experimental control (24.6%) and Control (14%) group as revealed by *P*-value of 0.0007 ($P < 0.05$) using *Chi-square test*. As regards tobacco consumption, the proportion of subjects in Control (38%) and Experimental control (36.9%) groups was nearly same and differed insignificantly with that of Experimental group (50.6%) as indicated by *P*-value of 0.089 ($P > 0.05$) using *Chi-square test*. The proportion of subjects consuming alcohol in Experimental group (57.3%) was significantly higher than that of Experimental control (35.6%) and Control (46%) groups as revealed by *P*-value of 0.008 ($P < 0.05$).

Table 2 provides the mean and standard deviation of different biochemical parameters and biomarkers according to study groups. The estimates were obtained according to mines as well as after pooling over the three mines. However, considering that *age*, *BMI* and the behavioral habits i.e. *smoking*, *tobacco* and *alcohol* consumption could have a possible confounding effect on the levels of these parameters; *analysis of covariance* (ANCOVA) was carried out for each parameter independently to adjust for these confounders and to determine the true effect of exposure. As a result, the adjusted parametric levels were obtained for each subject and were summarized in terms of adjusted mean and standard deviation as shown in Table 3. The statistical significance of difference in the overall mean adjusted values of parameters across study groups was evaluated using *one-way analysis of variance* (ANOVA). The parameters violating the assumption of normality were log-transformed and then the significance testing was carried out. One-way ANOVA revealed that all the parameters differed significantly across three groups. For majority of the parameters, the significance was contributed by the mean levels in the Control group as confirmed through *Tukey's post-hoc* comparison. The Experimental control and Experimental groups showed statistically insignificant difference of mean in all the parameters. It was also observed that there was slight increased in the level of aluminium in experimental group as compared to both the groups but it was not statistically significant and lies within normal range.

Table 2: Unadjusted mean and standard deviation for different biochemical parameters , Heme Oxygenase-1 and Aluminium according to study groups and mines

Parameter	Study groups / Mine											
	Control (*n*=50)			Overall	Experimental control (*n*=73)			Overall	Experimental (*n*=150)			
Mines	Phase I (*n*=10)	Phase II (*n*=20)	Phase III (*n*=20)		Mine I (*n*=17)	Mine II (*n*=20)	Mine III (*n*=36)		Mine I (*n*=51)	Mine II (n=49)	Mine III (n=50)	Overall
Hb% (12-15 g/dl)	13.52 ± 0.84	13.90 ± 1.03	14.28 ± 2.09	13.97 ± 1.52	13.58 ± 0.94	12.7 ± 1.38	13.56 ± 1.73	13.33 ± 1.52	13.27 ± 2.01	13.2 ± 1.47	12.86 ± 1.67	13.12 ± 1.73
Bilirubin (0.3-1.2 mg/dl)	0.99 ± 0.26	0.95 ± 0.50	1.06 ± 0.75	1.00 ± 0.58	0.97 ± 0.35	1.32 ± 0.62	1.21 ± 0.94	1.18 ± 0.76	1.10 ± 0.43	1.01 ± 0.43	0.98 ± 0.74	1.03 ± 0.55
SGPT (upto 42 U/L)	13.8 ± 3.71	17.1 ± 5.27	16.85 ± 3.76	16.34 ± 4.52	29 ± 12.28	26.95 ± 11.09	23.53 ± 11.31	25.74 ± 11.56	25.88 ± 11.75	21.53 ± 9.30	20.36 ± 9.10	22.62 ± 10.35
SGOT (upto 40 U/L)	15.6 ± 6.18	19.75 ± 9.78	22.05 ± 4.90	19.84 ± 7.69	24.29 ± 12.51	30.60 ± 15.59	26.42 ± 11.18	27.07 ± 15.85	27.16 ± 13.73	32.73 ± 20.63	22.04 ± 8.09	27.27 ± 15.52
HO-1 (less than 0.087 ng/ml)	1.14 ± 0.75	1.6 ± 0.39	1.56 ± 0.41	1.49 ± 0.51	1.67 ± 0.51	1.71 ± 0.25	1.69 ± 0.55	1.69 ± 0.47	4.59 ± 2.55	1.79 ± 1.44	2.76 ± 1.66	3.07 ± 2.11
Aluminium (upto 17 µg/dl)	0.71 ± 0.34	0.56 ± 0.33	0.44 ± 0.33	0.54 ± 0.34	0.72 ± 0.38	0.75 ± 0.32	0.94 ± 0.57	0.84 ± 0.49	0.82 ± 0.39	0.98 ± 0.53	0.90 ± 0.47	0.90 ± 0.47

The percent change in the overall mean levels of parameters in three groups after adjusting for the confounders is shown in Table 4. Considering a thumb rule of 10% change as noticeable, in Control group, SGPT and SGOT showed marked reduction in the mean levels of respective parameters after adjustment. Also, in Experimental group, SGOT levels reduced by ~12% after adjustment, implying the effect of one or more confounders on the parameter. The percent change was less than 10% suggesting that the confounders had a very negligible role on the parametric levels in statistical sense.

Table 3: Adjusted mean and standard deviation for different biochemical parameters, Heme Oxygenase-1 and Aluminium according to study groups and mines*

Parameter	Study groups / Mine											
	Control (*n*=50)			Overall	Experimental control (*n*=73)			Overall	Experimental (*n*=150)			Overall
Mines	Phase I (*n*=10)	Phase II (*n*=20)	Phase III (*n*=20)		Hindalco (*n*=17)	Balco (*n*=20)	Nalco (*n*=36)		Hindalco (*n*=51)	Balco (n=49)	Nalco (n=50)	
Hb% (12-15 g/dl)†	13.76 ± 0.32	13.65 ± 0.33	13.75 ± 0.28	13.71 ± 0.31	13.04 ± 0.36	13.22 ± 0.26	13.22 ± 0.21	13.17 ± 0.27	12.93 ± 0.29	12.92 ± 0.33	13.00 ± 0.34	12.95 ± 0.32
Bilirubin (0.3-1.2 mg/dl)†	0.88 ± 0.05	0.97 ± 0.08	0.93 ± 0.10	0.94 ± 0.09	1.17 ± 0.09	1.08 ± .12	1.22 ± 0.09	1.17 ± 0.11	0.96 ± 0.07	0.93 ± 0.09	0.99 ± 0.08	0.96 ± 0.08
SGPT (upto 42 U/L)†‼	15.07 ± 1.56	13.36 ± 2.88	13.73 ± 2.41	13.85 ± 2.51	23.70 ± 2.55	23.90 ± 1.91	24.66 ± 1.74	24.23 ± 2.02	20.88 ± 2.01	20.43 ± 2.48	21.14 ± 2.56	20.82 ± 2.36
SGOT (upto 40 U/L)†‼	16.65 ± 1.61	16.92 ± 2.66	17.09 ± 2.62	16.94 ± 2.43	25.18 ± 2.61	23.45 ± 2.86	26.33 ± 2.30	25.27 ± 2.78	24.19 ± 2.32	23.23 ± 2.30	24.86 ± 2.48	24.10 ± 2.45
HO-1 (less than 0.087 ng/ml)†‼	1.68 ± 0.14	1.55 ± 0.12	1.53 ± 0.06	1.57 ± 0.12	1.68 ± 0.16	1.83 ± 0.14	1.71 ± 0.13	1.73 ± 0.15	3.15 ± 0.12	3.21 ± 0.15	3.16 ± 0.15	3.17 ± 0.14
Aluminium (upto 17 µg/dl)†	0.48 ± 0.09	0.49 ± 0.08	0.53 ± 0.05	0.50 ± 0.07	0.81 ± 0.09	0.79 ± 0.08	0.80 ± 0.08	0.8 ± 0.08	0.85 ± 0.08	0.84 ± 0.09	0.85 ± 0.07	0.85 ± 0.08

*Adjusted for *age*, BMI, *smoking*, *alcohol* and *tobacco* using logistic regression model; ‡*P*-value < 0.05 (S) for overall data from each group; † *P*-value < 0.0001 (HS) for overall data from each group; ‼ Statistical significance evaluated using log-transformed data

Table 4: Percent change in the mean levels of each parameter after adjusting with the confounders

Parameter	Percentage change		
	Control (n=50)	Experimental control (n=73)	Experimental (n=150)
Hb% (12-15 g/dl)	1.86	1.21	1.3
Bilirubin (0.3-1.2 mg/dl)	6	0.85	6.8
SGPT (upto 42 U/L)	15.24	6.23	7.96
SGOT (upto 40 U/L)	14.62	7.12	11.62
HO-1 (less than 0.087 ng/ml)	5.37	2.31	3.26
Aluminium (upto 17 µg/dl)	7.41	5	5.56

Table 5: Comparative study on three mine wise distribution of data, adjusted mean and standard deviation for different biochemical parameters, Heme Oxygenase-1 and Aluminium according to study groups

Parameter	Mine I				Mine II				Mine III			
	Control (n=10)	Experimental control (n=17)	Experimental (n=51)	*P*-value	Control (n=20)	Experimental control (n=20)	Experimental (n=49)	*P*-value	Control (n=20)	Experimental control (n=36)	Experimental (n=50)	*P*-value
Hb% (12-15 g/dl)	13.76 ± 0.32	13.04 ± 0.36	12.93 ± 0.29	< 0.0001	13.65 ± 0.33	13.22 ± 0.26	12.92 ± 0.33	< 0.0001	13.75 ± 0.28	13.22 ± 0.21	13.00 ± 0.34	< 0.0001
Bilirubin (0.3-1.2 mg/dl)	0.88 ± 0.05	1.17 ± 0.09	0.96 ± 0.07	< 0.0001	0.97 ± 0.08	1.08 ± .12	0.93 ± 0.09	< 0.0001	0.93 ± 0.10	1.22 ± 0.09	0.99 ± 0.08	< 0.0001
SGPT (upto 42 U/L)	15.07 ± 1.56	23.70 ± 2.55	20.88 ± 2.01	< 0.0001	13.36 ± 2.88	23.90 ± 1.91	20.43 ± 2.48	< 0.0001	13.73 ± 2.41	24.66 ± 1.74	21.14 ± 2.56	< 0.0001
SGOT (upto 40 U/L)	16.65 ± 1.61	25.18 ± 2.61	24.19 ± 2.32	< 0.0001	16.92 ± 2.66	23.45 ± 2.86	23.23 ± 2.30	< 0.0001	17.09 ± 2.62	26.33 ± 2.30	24.86 ± 2.48	< 0.0001
HO-1 (less than 0.087 ng/ml)	1.68 ± 0.14	1.68 ± 0.16	3.15 ± 0.12	< 0.0001	1.55 ± 0.12	1.83 ± 0.14	3.21 ± 0.15	< 0.0001	1.53 ± 0.06	1.71 ± 0.13	3.16 ± 0.15	< 0.0001
Aluminium (upto 17 µg/dl)	0.48 ± 0.09	0.81 ± 0.09	0.85 ± 0.08	< 0.0001	0.49 ± 0.08	0.79 ± 0.08	0.84 ± 0.09	< 0.0001	0.53 ± 0.05	0.80 ± 0.08	0.85 ± 0.07	< 0.0001

*Adjusted for *age*, *bmi*, *smoking*, *alcohol* and *tobacco* using logistic regression model.

Table 5 shows the mean and standard deviation of each parameter according to mine and three groups. The significance of difference in the mean levels of each parameter across group was evaluated separately for each mine. Majority of the parameters showed statistically significant difference in the mean levels of groups. The significance was mainly contributed by the Control group for all the three mines.

Figure 1 shows the bar plot for mean HO-1 and Hb levels for the three groups. As regards HO-1, the mean level was significantly different across three groups as revealed by *P*-value < 0.0001 (Table 3). The mean showed increasing trend with the exposure. The increase in the mean HO-1 level of Experimental control group with respect to Control group was only 1.1 times, but for Experimental group, the increase was 1.83 times that of Experimental control group. The mean Hb level also showed statistically significant difference across three groups. However, the mean levels showed decreasing trend with the exposure. The mean reduction in Experimental control group was 0.96 times that of Control group, while in Experimental group it was 0.98 times that of Experimental control group.

Figure 2 shows the bar plot for mean duration of exposure and the mean HO-1 levels for Experimental control and Experimental groups. Although the mean duration of exposure was same for the two groups, the mean HO-1 levels in Experimental group was significantly higher than that of Experimental control group with *P*-value < 0.0001. The mean level in Experimental group was 1.83 times higher than the Experimental group. This again points towards some hidden factors playing role in raising HO-1 levels in Experimental group.

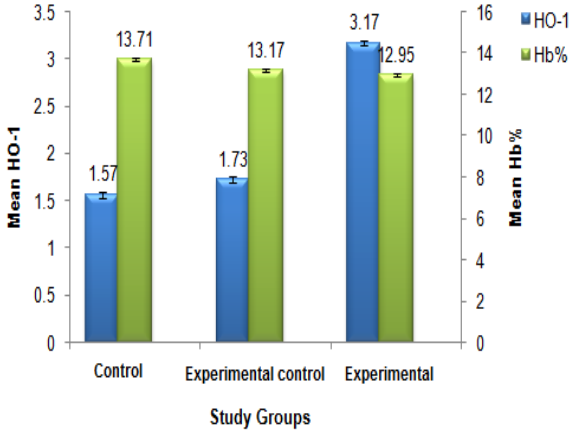

Figure 1: Bar chart with error bars showing mean Heme Oxygenase-1 and Hemoglobin %

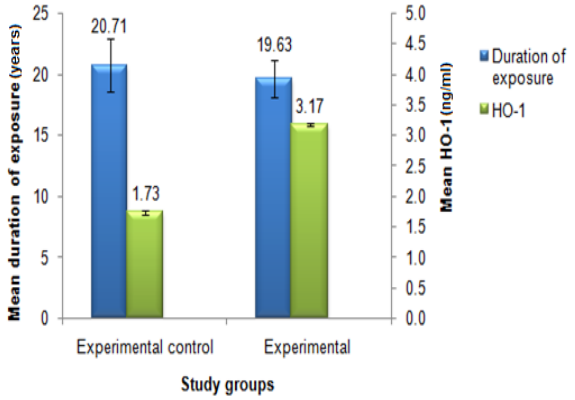

Figure 2: Bar chart with error bars showing mean duration of exposure and Heme Oxygenase-1

Table 6 provides the mean and standard deviation for the biomarker according to behavioral habits of subjects. As regards parameter HO-1, the difference in the mean levels for the three behavioral habits was statistically insignificant as indicated by *P*-value > 0.05.

Table 6: Comparison of Heme Oxygenase-1 according to behavioral habits

Parameter	Smokers (n=86)	Non-smokers (n=187)	Alcoholic (n=135)	Non-alcoholic (n=138)	Tobacco (n=122)	Non tobacco (n=151)
HO-1 (ng/ml)	2.48 ± 1.77	2.38 ± 1.75	2.51 ± 1.88	2.31 ± 1.62	2.48 ± 1.76	2.35 ± 1.76
P-value	0.653		0.357		0.552	

DISCUSSION

Toxicity of aluminium is reported to be diverse and not well documented; therefore a cohesive pattern of its cellular mechanism fails to emerge after reviewing the literature. [12] The present study focuses on evaluating certain biochemical parameters, selected enzymes and HO-1as a biomarker which may be affected by exposure to Bauxite dust at workplace.

The results of the current study, shows that overall adjusted mean of biochemical parameters in all three groups, control, experimental control and experimental were significantly different ($p<0.0001$) but within the normal range therefore has no clinical significance. (as shown in Table 3).

This study showed elevated levels of HO-1 which were found in all the groups. The reported normal range of the HO-1 is less than 0.087 ng/ml. It is significantly noted that the HO-1 levels in the control group was increased by 20 times as compared to the normal range. This needs to be studied further for setting the range of HO-1 in large group of common population. So the threshold limit values should be further investigated for reasons of elevation of stress responsive protein. There may be several reasons for elevation of HO-1 levels in common population, which needs to be further investigated from different point of inception of stress and associated responsible factors. This study represents HO-1 as a biomarker due to its highly elevated levels in experimental group as compared to other two groups and it reports as biomarkers for health related risk to Bauxite dust exposure.

Hemoglobin is known potent inducer of HO-1 induction. Workers having more conc. of Hb are less susceptible to oxidant mediated lung injury. It was reported that the increased HO-1 activity with subsequent increased destruction of heme was observed in aluminium exposed workers. Our study correlates with the above finding as all the subjects were having normal hemoglobin concentration but there was slight decrease in the level of hemoglobin in experimental group. [13,14] One of the possible mechanisms may be the stress response of HO-1 via generation of ROS and OS may lead to the destruction of Heme followed by enhanced level of HO-1

Citations are directly correlated with the HO-1 levels and confounder factors like smoking, tobacco chewing and alcohol consumption. [15-18] It was interesting to correlate induction of HO-1 with smokers and non smokers along with the other factors. Studies on HO-1 induction in smokers reported that oxidative stress due to cigarette smoking increase the number of alveolar macrophages in lungs and it shows that the alveoli spaces in smokers are more. HO-1 plays a critical role in counterbalance of oxidative stress. Oxidative stress could be involved in the predisposing effects of cigarette smoking because cigarette smoke contains a high oxidative burden. [19] Our results are not supporting previous findings. The line of evidences in our study, suggests that a potential induction of HO-1 is not due to smoking, consumption of alcohol and tobacco chewing, though different references are supporting for hypothesis of induction of HO-1 in same consumers.

Our findings showed that although the duration of working years to bauxite dust was same for both experimental and experimental control groups, exposure to bauxite dust correlates with HO-1. As far as the exposure increases the expression of HO-1 increases while this increase in the level was higher among experimental group as compared to experimental control. It is clear that the expression of HO-1 increases after 10 years of exposure to Bauxite dust.

On the basis of results, it is noted that exposure to Bauxite dust changes biochemical profile leading to high level of HO-1. HO-1 as a biomarker may be used for early detection of health risks in workers exposed to Bauxite dust in mines.

CONCLUSION

The present study may be the first study in which the work was carried out in three different Indian open cast mines located in three different geographical regions concerning detection of occupational diseases among bauxite mine workers. The effects of smoking, tobacco chewing and alcohol consumption had no effect on HO - 1. On the basis of findings it is concluded that exposure to Bauxite dust (even at low levels of aluminium) changes biochemical profile leading to high levels of HO-1. The study suggested for the setting of the normal range of the HO-1 on large reprehensive samples due to highly elevated values in control samples. The outcome of this study suggests that HO-1 may be used for early detection of health risks in workers exposed to Bauxite dust in mines.

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Conflict of interest: NIL

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