

# Effect of occupation on lipid peroxidation and antioxidant status in coal-fired thermal plant workers

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## ABSTRACT

**Background:** Air pollution from coal-fired power units is large and varied, and contributes to a significant number of negative environmental and health effects. Reactive oxygen species (ROS) have been implicated in the pathogenesis of coal dust-induced toxicity in coal-fired power plants. **Aim:** The aim of the study was to measure free radical damage and the antioxidant activity in workers exposed to varying levels of coal dust. **Material and Methods:** The study population consisted of workers in coal handling unit, turbine unit, and boiler unit ( $n = 50$  each), working in thermal power plant; and electricians ( $n = 50$ ) from same department were taken as controls. Lipid peroxidation was measured by malondialdehyde (MDA) levels and antioxidant activity was determined by superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels. Statistical analysis was carried out by Student's unpaired *t*-test. **Result:** MDA levels showed significant increase ( $P > 0.001$ ) in the thermal power plant workers than the electricians working in the city. The levels of SOD and GPx were significantly higher ( $P > 0.001$ ) in electricians as compared to subjects working in thermal plant. Among the thermal plant workers, the coal handling unit workers showed significant increase ( $P > 0.001$ ) in MDA and significant decrease in SOD and GPx than the workers of boiler and turbine unit workers. **Conclusion:** Oxidative stress due to increase in lipid peroxidation and decrease in antioxidant activity results from exposure to coal dust and coal combustion products during thermal plant activities.

**Key words:** Glutathione peroxidase, lipid peroxidation, malondialdehyde, superoxide dismutase

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## INTRODUCTION

In India, mostly the coal-fired thermal plants are used for generation of electricity which converts the energy available in the coal to electricity. Among power plants, the coal-fired facilities are reported to produce the most serious pollution.<sup>[1]</sup> The working of the thermal plant involves various procedures like preparation of coal in the coal handling unit, combustion

of coal, and utilization of its heat for boiling water in boiler unit; rotation of the turbines with high pressure steam in the turbine unit which makes the generator rotor to produce electricity. The working environment from coal-fired power plants contains high levels of various particulate matter (PM) and huge amount of gases derived from coal combustion, that are dependently on the coal sources and the combustion processes. The particulate matter includes nitrates, acids of sulfur, metal salts, and carbon particles.<sup>[2]</sup> Coal damages the respiratory, cardiovascular, and nervous systems through pollutants acting directly on the body.<sup>[3]</sup> European data reported by Markandaya and Wilkinson show that for each terrawatt hour of electricity generated, 24.5 deaths are expected in addition to 225 serious illnesses and 13,288 minor illnesses.<sup>[4]</sup> Similar increased health risk associated with their inhalation has been observed in Santa Catarina power station (Brazil) which uses coal enriched in ashes.<sup>[5]</sup> Inflammation of tissues is a critical element in the pathophysiology of illness caused by air pollution. Increased levels of serum proinflammatory cytokines interleukin (IL)-1 $\beta$ , IL-6, tumor necrosis factor (TNF)- $\alpha$

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have been found in subjects with occupational exposure to PM mixture from coal fly ash and coal dust in power plant environment.<sup>[6]</sup>

The permissible limit for the suspended PM (SPM) according to Punjab Pollution Control Board in ambient air is 60 µg/m<sup>3</sup> and limit for the 'at source air' is 150 mg/m<sup>3</sup> which is much higher than that for the ambient air. The thermal plant has digital meters to gauge the suspended particulate matter being emitted. Even the plant officials had been instructed to use ammonia gas in all units for conditioning of fuel gases to reduce the concentration of pollutants. In a study, carried out around a power plant in Mexico, it is reported that even if such units are complying with national standards, their emissions can still have significant impacts on the health of the surrounding population.<sup>[7]</sup> Reactive oxygen species (ROS), such as free radicals and oxygen ions, appear to be central to this process has been implicated in the pathogenesis of diseases caused or made worse by coal pollutants, such as cardiovascular and pulmonary diseases. The continuous production of ROS may damage antioxidant defenses and result in an oxidative stress leading to cellular injury in the lung.<sup>[8]</sup> This damage causes denaturation of proteins, destruction of carbohydrates, and peroxidation of lipids.<sup>[9]</sup> The detrimental effects on the exposed subjects depend both on the physical characteristics of PM, their chemical composition, the exposure time, and on the health state of the people exposed. The purpose of the present study is to better understand the effect of occupational coal dust exposure if any on thermal power plant workers by measuring oxidative stress although, and the local occupational hygienists considered these jobs to be unexposed to the contaminants. According to the literature, this study is the first investigation conducted on thermal plant workers on blood values mentioned above. That is why, in this study we compared our data with previous studies published on coal miners. We proposed that some people who are having physical contact with coal might have insufficient accoutrements to combat the ROS that occur after exposure to coal dust. We can adopt the hypothesis that antioxidant parameters in whole blood may reflect oxidative stress in the workers.

## MATERIALS AND METHODS

### Area of study

The study was done on workers of Guru Nanak Dev Thermal Plant (GNDTP, Punjab) on Bathinda-Malout Road about 10 km from the main city. Each unit of GNDTP, Bathinda, when operated at full capacity is capable of generating 26.4 lac units of electricity a day and the total daily coal requirement is about 6500 metric ton (MT) (about two rakes of 58 wagons each) when all the four units are in operation.

### Subjects

The study was conducted from December 2011 to February 2012 during winter months. The study population consisted of nonsmoking 200 healthy male workers of electricity department who were randomly selected from their attendance records and divided into four groups depending upon their nature of work viz. coal handling unit workers, turbine unit workers, boiler unit workers, and electricians working in the city.

The size of each group ( $n = 50$ ) was decided from the measurement of biomarkers of lipid peroxidation in aging studies done in normal healthy subjects in the age group of 30-40 years of northern India.<sup>[10]</sup> Only male subjects were taken for study as 80% of the workers in thermal plant were males. The mean age of the workers was  $35 \pm 5$  years. Subjects of same socioeconomic status with no history of smoking, alcohol, or drug intake and had completed 10 years in the same unit were included in the study. Those suffering from any chronic disease like hypertension, diabetes mellitus, rheumatoid arthritis, malignancy, collagen disorders, or any allergic diseases were excluded from the study. A written consent to participate in the study was obtained from the subjects after they were thoroughly informed about the research details. Ethical approval for study was obtained from local institutional ethical committee.

### Measurement

A detailed history including the history of diet and lifestyle was taken and general physical and systemic examination was done. Weight was taken on a weighing scale with standard minimum clothing to the nearest 0.5 kg. Hemoglobin was estimated by using Sahli's method.<sup>[11]</sup> For assessment of other parameters, 10 ml of fasting venous blood was taken from each worker with dry disposable syringe and needle under all aseptic conditions by venipuncture in the anticubital vein in a sterile, dry, acid washed vials; 2 ml of it was taken in tube with ethylenediaminetetraacetic acid (EDTA), and the rest in biochemical tube. Samples were kept in a cool box at  $-4^{\circ}\text{C}$  until they were transferred immediately to the laboratory, where they were centrifuged. Malondialdehyde (MDA) levels in serum were estimated by the method of Satoh.<sup>[12]</sup> Superoxide dismutase (SOD) activity in serum was assayed by using the method of Marklund and Marklund.<sup>[13]</sup> Glutathione peroxidase (GPx) activity in plasma was measured by using hydrogen peroxide as a substrate by applying the method of Rotruck.<sup>[14]</sup>

### Statistical analysis

Statistical analysis was carried out by unpaired *t*-test. The data were expressed as mean  $\pm$  standard deviation (SD) and the  $P < 0.001$  was taken as highly significant.

## RESULTS

The mean weight and mean hemoglobin did not show much variation in different workers. The mean MDA levels in turbine, boiler, and coal workers were more than the control group. The increase was maximum in coal workers as compared to control group, that is, electricians. Mean SOD and GPx were lower in all thermal plant workers as compared to electricians working outside the thermal plant [Table 1].

Table 2 shows percentage change in MDA, SOD, and GPx in boiler, turbine, and coal workers as compared to electricians. The mean SOD was significantly greater in electricians as compared to boiler, turbine, and coal workers. Similarly, glutathione peroxidase (GPx) levels were significantly lower in coal workers than the other workers of thermal plant. GPx levels were higher in electricians working in city as compared to thermal plant workers. On comparing, SOD and GPx levels in coal workers and electricians, a change of 37.08 and 31.57%, respectively was observed. The lipid peroxidation measured by MDA levels in contrast showed significant increase in all the thermal plant workers as compared to electricians. On comparison of MDA levels in coal workers and electricians, 31.92% change was found.

## DISCUSSION

Levels of MDA, measure of lipid peroxidation were significantly higher in the thermal plant workers as compared to electricians from same department in the city. It has been estimated that coal particles cause damage by their metal constituents.<sup>[15,16]</sup> The rising levels of MDA in thermal plant workers may be related to oxidation of lipid and protein by ROS in cells. Generally, it is thought that coal dust forms ROS in two different ways. It is either generated by intrinsic properties of particles and iron content of coal dust or by the oxidative burst

of macrophages and neutrophils activated during phagocytosis and persistent inflammation.<sup>[8]</sup> Lipid peroxidation, a chain reaction initiated by the attack on the membrane lipids by free radicals. The carbon radical so formed is stabilized by molecular rearrangement to produce conjugated diene which reacts with oxygen molecule to form peroxy radical. Peroxy radicals forms peroxides and endoperoxides the fragmentation of which results in formation of MDA.<sup>[17]</sup> Plasma MDA level has been used in many diseases as an indirect indicator of tissue lipid peroxidation and general oxidant stress. It has been established from various studies that parameters like age and sex account for the variations in MDA, SOD, and GPx.<sup>[10]</sup> These factors were taken into considerations and study was done in males of same age group only.

MDA levels were higher in coal handling unit workers as compared to workers in boiler and turbine unit. These biochemical parameters varied with magnitude of exposure to coal dust and combustion products. These findings may be considered as a result of exposition to respirable coal dust, which leading to production of ROS may be predictive of the inflammation and cell injury. As the coal has to be sized, processed, and handled; it leads to abrasion of coal material and generation of coal dust in coal handling unit. These workers are exposed to coal through both inhalation and skin contact in coal handling unit. Workers in this unit also have additional direct exposure to coal dust inhalation besides being exposed to coal combustion products present in thermal power plant. Similar increase of lipoperoxidation in plasma and change in prooxidant/antioxidant status in blood of subjects has been seen with both acute and long-term coal dust exposure.<sup>[18,19]</sup> These results match with animal studies done earlier, which showed increase of MDA levels in blood after exposition to inhalation of coal dust.<sup>[20]</sup> Oxidative stress which results from alteration in oxidant-antioxidant ratio has been observed in coal miners and other workers in dusty jobs.<sup>[21]</sup> Even acute exposure to

**Table 1: Mean±standard deviation (SD) of hemoglobin, weight, serum lipid peroxidation levels (nmol/ml; i.e., malondialdehyde), superoxide dismutase levels (U/ml), and glutathione peroxidase levels (U/ml) of different groups**

Groups	Mean±SD of weight	Mean±SD of hemoglobin	Serum lipid peroxidation (nmol/ml)	SOD levels (U/ml)	GPx levels (u/ml)
Electricians	66.90±8.22	12.06±1.53	3.39±0.38	2.44±0.20	0.5±0.10
Turbine workers	68.92±9.18	12.90±1.10	4.07±0.32	2.34±0.19	0.46±0.10
Boiler workers	71.96±6.42	12.62±0.94	4.63±0.36	1.98±0.17	0.4±0.10
Coal workers	68.68±13.50	12.70±1.66	4.98±0.38	1.78±0.16	0.38±0.10

**Table 2: Comparison of MDA, SOD, and GPx levels of different thermal plant workers with electricians working outside thermal plant**

Group	Electricians vs. turbine workers		Electricians vs. boiler workers		Electricians vs. coal workers	
	Percent change	P value	Percent change	P value	Percent change	P value
MDA	16.70	<0.001*	26.78	<0.001*	31.92	<0.001*
SOD	4.27	<0.01-0.001†	23.23	<0.001*	37.08	<0.001*
GPx	8.69	0.0532	25	<0.001*	31.57	<0.001*

\*Highly significant; †Significant; ‡Not significant; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase

coal dust induce oxidative stress in some human subjects.<sup>[22]</sup>

However, the ambient exposure conditions did not significantly alter hemoglobin levels of the exposed groups compared to control. We did not estimate serum iron levels in our study which is thought to reflect the exposure to transition metal ion and which with the coal dust appears to be important mediator of oxidative damage *in vivo/in vitro* together with coal dust.<sup>[23]</sup>

As a response to these potentially direct actions of ROS, the human body possesses antioxidant mechanisms; they include enzymes that have antioxidant activity and many actual and putative physiological antioxidants (such as a tocopherol). The main enzymes that provide cellular protection against damage due to ROS in human cells are three SODs, catalase, and a selenoenzyme GPx.<sup>[8]</sup> Selenium dependent GPx removes both H<sub>2</sub>O<sub>2</sub> and lipid peroxides by catalyzing the conversion of lipid hydroperoxides to hydroxy acids in the presence of reduced glutathione.

A decrease in the activity of GPx in our study in thermal plant workers as compared to electricians was observed. Among the thermal plant workers GPx levels were significantly lower in coal handling unit workers as compared to boiler and turbine workers. The continuous production of ROS damage antioxidant defenses and result in an oxidative stress.<sup>[24]</sup> The workers in boiler unit had lower GPx levels than turbine workers probably because of their close proximity to coal combustion processes where large number of gases and particles are liberated. Similar decrease in erythrocyte glutathione s-transferase activity and glutathione concentration was seen in the early stages of pneumoconiosis in coal workers.<sup>[25]</sup>

SOD is a copper containing enzyme occurring widely in cells tissues and erythrocytes, liver, and brain. It is a free radical metabolizing enzyme, catalyzing dismutation of superoxide anion hydrogen peroxide. This protects the cell membrane from damage by highly ROS. The low activity of SOD in workers of coal handling unit could be due to inactivation of the enzyme by crosslinking or damage to DNA lipid peroxidation and decreased expression of the enzyme.<sup>[26]</sup> Coal dust concentration was not measured in this study although there was variable amount of dust in different units. Occupational exposure to coal showed a decrease in all the red blood cell antioxidants in some stages of coal workers' pneumoconiosis.<sup>[27,28]</sup>

In spite of our small samples, our findings indicate that SOD activity may be considered as a marker of the effect of respirable coal dust in exposed workers, and plasma GPx activity may be considered as a biological marker for

various health effects in coal workers. The changes in blood antioxidants that we have measured are in agreement with the hypothesis that production of ROS is an important event in the exposure to coal dust particles.

The test tool assaying blood parameters we have used in our study is a noninvasive, rapid methodology and can be useful for identifying early occupational health hazard before it may come clinically apparent and for monitoring of individuals occupationally exposed to particulate coal pollutants in power plants.

The workers in thermal power plants are more susceptible to health problems. Ambient air monitoring and more specific tests to confirm the effect of coal pollutants can be carried out in the area in further studies. Follow-up studies which closely monitors dose of exposure to the effects of the plant and smoking can also be recommended. Even individual evaluation of the metal concentrations in relation to clinical findings can be done. With prolonged exposure, the situation can become more urgent not only because of chronic poisoning; but also because of the cancerogenic effects of these elements on the human organisms. So, precise exposure estimates based on specific exposures and work in thermal plant are to be developed in next phase of the study to further investigate the findings based on the nature of work.

The data may provide information for the development of preventive measures to minimize the adverse effects of the exposures and offer the opportunity to improve worker health and reduces health-related costs. Use of personal protective equipment and good hygiene practices can be encouraged while at work. Washing hands before eating and drinking at work place should be advised. Antioxidant supplements should be strongly recommended in thermal plant workers and change in shift of duties in various units must be done frequently.

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