Coal Dust-Induced Systematic Hypoxia and Redox Imbalance among Coal Mine Workers

Aima Iram Batool, Naima Huma Naveed, Mehwish Aslam, Juliana da Silva, and Muhammad Fayyaz ur Rehman*

ABSTRACT: Continuous inhalation of coal dust among coal workers leads to a variety of disorders. The present study aims to evaluate the potential oxidative stress associated with coal dust generated from coal mining activities among exposed workers through the antioxidant enzyme system, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). In this study cohort, intensive coal mine workers were assessed for antioxidant variations. Blood samples were collected from dust-exposed workers (engaged in different activities at coal mines; $n = 311$) and residents of the same city (nonexposed, control group; $n = 50$). The workers’ exposure to coal dust was categorized based on working area (administrative group, surface workers, underground workers), working hours (up to 8 h and more than 8 h), and time of service. The results showed significantly altered activities of SOD, CAT, and GSH among the whole exposed group and its categories compared to the control group. A significant difference was also observed between high- and low-exposure groups. Statistical analysis revealed a negative correlation between antioxidant activity (catalase and SOD) and coal dust levels. Besides, coal exposure was associated with the time of service, smoking status, and dietary habits. The findings of this study reveal higher oxidative stress among highly exposed coal mine workers (underground workers > surface workers > administrative group > nonexposed group), and longer working hours have more pronounced adverse effects on workers’ health.

1. INTRODUCTION

Coal dust, the most detrimental chemical hazard for coal miners, is generated from all coal mining activities, from excavation till processing. Almost 60 different compounds (organic and inorganic) and their oxides constitute coal dust.1–3 Upon inhalation, this heterogeneous mixture (coal dust particles) gets deposited on the lungs of exposed workers, interacts with cellular machinery involved in balancing the reactive oxygen species (ROS),4,5 and damages the essential macromolecules (DNA, protein, and lipids), thus stimulating a cascade of adverse effects, ranging from mild respiratory symptoms to life-threatening diseases (coughing, phlegm, wheezing, chronic bronchitis, astringent asthma, pneumoconiosis, coal workers pneumoconiosis (CWP), chronic obstructive pulmonary diseases (COPD), emphysema, and silicosis).5,6–8

Continuous production of these ROS alters antioxidant defense, resulting in oxidative stress.9–14 The intensity and manifestation of coal dust-linked diseases are dependent upon the length of exposure to coal and coal dust. Thus, continuous inhalation of coal dust increases the number of activated macrophages (containing ingested carbon particles),7,15 which
upon activation release a battery of immune, proinflammatory, and profibrotic primary mediators (oxidants, bioactive lipids, cytokines, IL-1 and TNF, factors, proteases, and antiproteases) within the alveolar structures\textsuperscript{16} that target fibroblast and the epithelial and endothelial cells capable of releasing secondary mediators.\textsuperscript{17}

Under normal conditions, human body cells have an extremely sophisticated defensive antioxidant armor to take the helm and combat ROS assault. Such active antioxidant mechanisms boost and scavenge ROS for aerobic survival.\textsuperscript{18,19} Exposure to various coal toxicants and their components triggers the overstocking of signaling methods of oxidative stress. This intricate system of natural defense is incapacitated due to prolonged exposure to coal contaminants and their derivatives. Lifestyle factors play a significant mediating role in the development, progression, and treatment of diseases related to redox imbalance, for example, diet\textsuperscript{20−22} and physical activity.\textsuperscript{23−25}

The cell is endowed with an antioxidant defense mechanism against the damaging effect of ROS. A multiplex system of enzymes and nonenzymatic antioxidants works synergistically to maintain oxidant–antioxidant balance within cells,\textsuperscript{26} as well as to protect biomolecules from oxidative damage.\textsuperscript{27} Coal dust exposure also involves an increased number of alveolar inflammatory cells, which in turn are responsible for the change in superoxide dismutase (SOD) levels.\textsuperscript{28,29} Superoxide anion, which is the most important ROS, is dismutated by SOD. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide to water and of hydroperoxides to alcohol. Catalase (CAT), an antioxidant enzyme, specifically phagocytoses H\textsubscript{2}O\textsubscript{2} and shows higher phagocytic activity compared to glutathione peroxidase.\textsuperscript{30} Oxidative stress can be directly measured by quantification of oxidative parameters like SOD, peroxidases, glutathione reductase (GPx), and CAT, while indirectly measured by quantification of byproducts (such as MDA), produced by lipid peroxidation, in exhaled breath condensate, blood, bronchoalveolar lavage (BAL), and urine.\textsuperscript{31−33} Although a few studies have addressed the relationship of coal dust toxicity with biochemical changes in the body of exposed persons, no such study has been still performed in the labor-intensive coal mining areas of Pakistan with vast coal deposits. The present research is based on the evaluation of coal-induced cytotoxic changes in terms of antioxidant activity.

2. RESULTS

2.1. Characteristic of Study Population. The characteristics of the study population are shown in Table 1. Most of the study subjects (62.38\%) belong to the prime reproductive age group (20−40 years). Among them, 5.02\% were performing their tasks for more than 8 h per day and sometimes without any rest period, while 46.30\% had been a part of this profession for 5−10 years and 38.59\% had spent up to 20 years in this job. A proportion of 10.61\% of workers have mining experience of up to 30 years, while 4.50\% were those who almost sacrificed their whole life for this profession as they were performing different mining activities for more than 30 years. Regarding smoking, 61\% of exposed workers and 42\% of nonexposed workers were active smokers. Coal workers have extremely poor dietary habits as only lesser percentages of exposed workers were having fruits, meat, and milk frequently. Lower income was the main reason they explained for not having fruits, milk, and meat.

| characteristics | nonexposed group (control) (n = 50) | exposed group (n = 311) |
|-----------------|-------------------------------------|------------------------|
| age (mean years ± SD) | 33.6 ± 12.30 | 33.92 ± 11.54 |
| time of service (mean years ± SD) | 11.95 ± 7.67 | |
| smokers (%) | 42\% | 61\% |
| dietary habits | fruit | |
| sometimes | 7 (14\%) | 278 (89\%) |
| frequently | 43 (86\%) | 33 (11\%) |
| cow milk | never | |
| rarely | 12 (24\%) | 211 (68\%) |
| frequently | 38 (76\%) | 100 (32\%) |
| meat | rarely | |
| frequently | 5 (10\%) | 244 (78\%) |
| | frequently | 45 (90\%) | 67 (22\%) |

2.2. Antioxidant Enzyme Activities. Table 2 reveals the results of antioxidant enzymatic activities. One-way analysis of variance (ANOVA) was conducted to compare the effect of coal exposure on the activity of catalase, SOD, and glutathione peroxidase enzymes among underground miners, surface miners, administrative, and nonexposed groups. A comparison was also made between the whole exposed and nonexposed groups as well. There was a significant change in enzyme activities at P < 0.01 of exposed groups, contrary to the nonexposed group. Another comparison was made to check the exposed group with more altered enzyme activities. Comparisons indicated that the mean score for the CAT activity was significantly lower for underground miners, compared to the administrative group, surface workers, and nonexposed group. Taken together, these results suggest a significant decrease in catalase activity due to greater exposure to coal dust. A considerable reduction was observed in the SOD enzyme activity among the whole exposed group and nonexposed group. There was a 37\% reduction in underground group compared to the nonexposed group on equating the enzyme activities. A comparison of glutathione peroxidase activity between coal dust-exposed and nonexposed groups showed significantly lower enzyme activity among the whole exposed group. Underground miners have significantly lower levels of glutathione compared to the rest of the groups at P < 0.001.

2.3. Correlation Studies. Antioxidant activity was in an antagonistic relationship with age and smoking habits among both groups (exposed and nonexposed). Along with age and smoking habit, service time also appeared as a depressive element for the activity of catalase, SOD, and glutathione. A prolonged mining experience has a strongly negative interaction with catalase, SOD, and glutathione. The consumption of fruits has a positive relationship with the activity of catalase, SOD, and glutathione. Milk intake was positively and significantly related to exposed CAT, exposed SOD, exposed GSH, as well as to nonexposed CAT, nonexposed SOD, and nonexposed GSH. The relation of dietary meat was positive and significant for catalase and glutathione among both groups, while SOD relation was positively nonsignificant for the exposed group as well as for the nonexposed group (Table 3).

The interrelation of antioxidant enzymes was also analyzed among exposed and nonexposed subjects. Catalase was in a strongly positive relation with SOD and GTS, as well as CAT.
SOD activity, which was found as \( \text{SOD} = (56.713) + (\text{coal dust}) \) (Figure 2). The analysis revealed antagonistic interaction between the coal dust levels and antioxidant activity for SOD, CAT, and GSH.

A simple linear regression analysis was carried out to predict the changes in the activity of SOD based on the changes in coal dust levels. Coal dust levels significantly predicted a depression in \( \beta = -0.887, t (359) = 69.01, P < 0.001 \). Coal dust levels also explained a significant proportion of variance in SOD activity, \( R^2 = 0.787, F (1, 359) = 1326.928, P < 0.001 \). The linear regression equation for relating the activity of SOD was found as \( \text{SOD} = (56.713) + (-6.613) \times (\text{coal dust}) \).

The linear regression equation for relating the activity of glutathione and the level of dust is \( \text{GSH} = (61.282) + (-5.394) \times (\text{coal dust}) \). Regression analysis reveals that a 1 mg/m^3 increase in the coal dust level was responsible for the estimated decrease of 5.394 U/mL in glutathione activity \( \beta = -0.712, t (359) = 48.249, P < 0.001 \).

\[ \text{CAT activity was decreased by 25.811 U/mL with an increase of 1 mg/m}^3 \text{ in coal dust level, } \beta = -0.885, t (359) = 73.685, P < 0.001. \]

\[ \text{The linear regression equation for catalase remained as catalase} = (239.525) + (-25.811) \times (\text{coal dust}). \]

Coal dust exposure explained a significant proportion of variance in catalase activity, \( R^2 = 0.782, F (1, 359) = 1291.909, P < 0.001. \)

3. DISCUSSION

Although Pakistan produces and processes 3.34 million tons of coal annually, knowledge about the toxic effects of coal dust produced from mining activities and awareness about safety measures is still scarce. This study was carried out to assess and generate information about the hazardous impact of coal dust on the workers’ health. The coal dust level in the mines of Punjab remained at 4.81 mg/m^3, which is higher than the recommended levels by both NIOSH (National Institute for Occupational Safety and Health; 1 mg/m^3 for 10 h/day)\(^34\) and Indian mines (maximum exposure limit per day is 3 mg/m^3).\(^35\)

Similar exposure levels have been reported by Azad and colleagues,\(^36\) who reported an exposure range of coal dust in Pakistani mines as 4–5 mg/m^3. Such a higher level of coal dust could result from poor ventilation, inadequate haulage system, use of donkeys for transportation, and low coal seam height. Pauwels et al.\(^37\) have also considered unpaved haul roads as a source of coal dust generation in coal mines as this dust is coated in air with transportation. Dust generated from mining activities is believed to elicit hyper-sensitiveness and inflammation of the pulmonary system as it triggers oxidative stress. Higher and prolonged coal dust exposure is responsible for cellular and noncellular ROS sources.\(^28\) Lipid peroxidation, oxidation of proteins, and damage to nuclear material are some cellular effects of ROS.\(^28\)

Our study demonstrated that SOD, CAT, and GSH levels showed a decrease in the enzyme activity among the highly exposed group of miners to coal dust (underground workers < surface workers < administrative group). A decrease in antioxidant reserves reveals a higher level of oxidative stress.

### Table 2. Antioxidant Activity Parameters (U/mL) in Nonexposed and Exposed Groups (Mean ± Standard Deviation)\(^a\)

| Parameter levels | Nonexposed group | Exposed group | Administrative group | Surface mining | Underground mining |
|------------------|------------------|---------------|----------------------|----------------|--------------------|
| n                | 50               | 311           | 51                   | 84             | 176                |
| SOD              | 55.70 ± 6.26     | 25.13 ± 9.12  | 38.59 ± 8.60         | 26.56 ± 7.07  | 20.55 ± 5.24       |
| Catalase         | 255.31 ± 43.15   | 113.08 ± 15.86| 127.82 ± 11.49      | 116.58 ± 6.45 | 107.13 ± 16.83     |
| Glutathione      | 55.25 ± 41.16    | 35.38 ± 10.79 | 47.40 ± 16.16       | 36.76 ± 9.61  | 31.23 ± 5.25       |

\(^a\)Normal values for SOD, catalase, and glutathione peroxidase were obtained from nonexposed group and considered as 50–60, 240–280, and 50–70 U/mL, respectively. \(^b\)Significantly different at \( P < 0.001 \) in relation to the control group. \(^c\)Significantly different at \( P < 0.001 \) in relation to other groups using Dunn’s post hoc comparisons.

### Table 3. Correlation between the Biomarkers of Antioxidant Activity and General Characteristics for Control and Exposed Groups

| Variable         | CAT          | SOD          | GSH          |
|------------------|--------------|--------------|--------------|
|                  | \( r_s \)    | \( p \)      | \( r_s \)    | \( p \)      | \( r_s \)    | \( p \)      |
| Nonexposed group |              |              |              |
| Age              | -0.718       | <0.001       | -0.664       | <0.001       | -0.994       | <0.001       |
| Smoke status     | -0.445       | <0.001       | -0.368       | 0.040        | -0.557       | <0.001       |
| Dietary habits   |              |              |              |
| Fruits           | 0.322        | <0.05        | 0.321        | <0.05        | 0.527        | <0.001       |
| Milk             | 0.468        | <0.001       | 0.506        | <0.001       | 0.577        | <0.001       |
| Meat             | 0.297        | 0.036        | 0.156        | 0.279        | 0.536        | <0.001       |
| Exposed group    |              |              |              |
| Age              | -0.207       | <0.001       | -0.232       | <0.001       | -0.238       | <0.001       |
| Time of service  | -0.143       | <0.05        | -0.285       | <0.001       | -0.261       | <0.001       |
| Smoke status     | -0.094       | 0.097        | -0.049       | 0.393        | -0.251       | <0.001       |
| Dietary habits   |              |              |              |
| Fruits           | 0.284        | <0.001       | 0.415        | <0.001       | 0.339        | <0.001       |
| Milk             | 0.233        | <0.001       | 0.514        | <0.001       | 0.300        | <0.001       |
| Meat             | 0.288        | <0.05        | 0.214        | 0.136        | 0.570        | <0.001       |
This also reflects a perfectly functioned framework of the antioxidant enzyme defense system. Exceedingly, higher antioxidant enzyme levels reflect a compensatory response to elevated oxidative damage.\textsuperscript{38,39} This could be due to the maximum exposure to coal dust while working in underground mines for prolonged duration and confined space with a danger of explosion and collapse. In addition, coal mining environments usually have depleted levels of oxygen because of the oxidation of coal as well by the displacement of oxygen by gases liberated from coal strata. Reduction in ambient oxygen availability is related to prolonged systemic hypoxia and oxidative stress, which can cause redox imbalance among humans.\textsuperscript{40} The level of physical activity is also related to the modulation of redox balance. Strenuous physical efforts including repetitive heavy and frequent lifting; full force exertion such as shoveling, carrying, stooping, and climbing; pushing and pulling of high and heavy loads; vibration exposure; forceful gripping, jolting, and jarring; and handling awkward materials such as cabling or ventilation materials are part of a coal worker’s daily work routine.\textsuperscript{23} Working muscles require increased oxygen delivery that can intensify the production of superoxide anion and other oxygen-derived intermediates in mitochondria that promote oxidative damage within cells.\textsuperscript{24,25} We observed a decrease in SOD activity that may cause the overgeneration of intrinsic reactive oxygen species (ROS). Exhaustion in the antioxidant enzymes may be correlated with the consumption of activated enzymes to counter oxidative stress. The impeding activity of antioxidant enzymes disrupts phospholipid mobility that is essential for the proper functioning of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase. Coal miners may experience a drop in the antioxidant levels that may be a contributory factor for reduction in Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity.\textsuperscript{41} A significantly reduced expression level of SOD can be a predictor of coal dust-induced antioxidant coping strategy among coal miners, as demonstrated by different authors.\textsuperscript{42−44} Celli et al.\textsuperscript{28} described that the decreased levels of SOD explained an increase in ROS exposure, while SOD is inadequate to detoxify the high level of ROS. Exposure to coal dust enhances the inflammatory reactions of lungs and oxidative damage among exposed ones.\textsuperscript{45} Yan et al. reported that the CAT level significantly decreased among the exposed group. These results are in accordance with our findings of the lower CAT level among the high-exposed group. The present

Figure 1. Nonparametric Spearman’s correlation analysis among antioxidants enzyme levels for the exposed group (a, c, e) and the control group (b, d, f).
findings of CAT are also in agreement with the results of other studies.9,46−48

Nutrition plays an important role in keeping the body healthier and in fighting against undesirable changes. We observed a significant difference in dietary habits between the exposed and nonexposed groups. The consumption of fruits, meat, and milk was in lower percentages among the exposed group. Workers reported multiple reasons for this, such as lower income, unavailability of resources in the mining vicinity, as well as high price of these dietary items. Many of them reported that they consume meat only during Eid and family festivals. This poor consumption could be a reason for a decline in the level of antioxidant enzymes’ activity as fruits are a natural and rich source of antioxidants. We have also observed a positive relation of fruits, milk, and meat consumption with antioxidant levels among both groups (nonexposed and exposed) that depicts that increased consumption of fruits, milk, and meat may cause an increase in antioxidant activity and protect the cell from the dangers of oxidative stress. Vitamins are important in the prevention of free-radical-induced cytotoxicity directly (reactive dioxygen species scavenging and upregulation of antioxidant enzymatic activity).20 Vitamins E and C are considered most important in the inhibition of reactive oxygen species-induced release of lipid peroxyl radicals and free-radical-induced oxidative stress via the vitamin E redox cycle.49 Fruits and vegetables are a rich source of nutrients, vitamins (C, folate, and provitamin A), phytochemicals (flavonoids, carotenoids, and phenolics), and minerals (potassium, calcium, magnesium) that enhance antioxidant enzymatic activity and trigger multiple signal transduction pathways.21,22 Hypo- and hyperintake of protein, carbohydrates, fats, vitamins, and minerals can cause impairment in a body’s anti- and pro-oxidant balance.50 Direct scavenging of reactive oxygen species takes place through amino acids (arginine, citrullines, glycine, histidine, taurine), smaller peptides, and nitrogenous metabolites. Hypo-protein diets are associated with a decrease in the synthesis of antioxidant enzymes as well as an increment in superoxide anion generation.

Depleted levels of GSH were observed among the high-exposed group who worked in underground places and more subjected to coal dust. Linear regression also showed a negative effect (β = −0.712) of coal dust on the GSH level among coal workers. In accordance with our findings, Júnior et al.51 reported a decrease in GSH level due to coal dust exposure, and Altin et al.9 found a negative correlation of GSH level with exposure to coal dust. One possible reason for the decline in GSH reserves is that the exposure to coal dust leads to oxidative conditions, under which GSH is reversibly oxidized to GSSG.28 Redox imbalance could be associated with the chemical composition of coal dust. Coal from Pakistan has been ranked as lignite and presents silica in the form of quartz, calcium carbonate, and dolomite, as well as some proportions of elements such as aluminum, potassium, and sulfur.52 As observed, coal is a mixture of a variety of chemicals and include hydrocarbons.5

It should be noted that the International Agency for Research on Cancer (IARC) classified quartz as Group 1 because it presents sufficient evidence for carcinogenicity in experimental animals and humans.53 However, coal dust is classified as a noncarcinogen for humans (group 3) by the International Agency for Research on Cancer.53 The generation of free radicals in living systems is closely linked with the participation of redox-active metals such as iron, copper, chromium, and aluminum.54,55 Metals participate in the transfer of electrons between metals and substrates and therefore may play an important role in the maintenance of redox homeostasis; disruption of metal homeostasis may lead to the uncontrolled formation of harmful free radicals participating in the modifications to DNA bases, and be related to a different disease, including cancer. Thus, knowing the importance of coal as an energy source and the association of inorganic and organic elements with fine particulates makes its characterization and estimation of risks extremely important.

In conclusion, the findings of this study reveal higher oxidative stress among highly exposed coal mine workers.
(underground workers > surface workers > administrative group > nonexposed group) and its relationship with age, time of work, and lifestyle. Our results showed that chronic coal dust exposure leads to a redox imbalance and indicates that the use of antioxidants could have a prophylactic value, but further studies will address this issue.

4. MATERIALS AND METHODS

4.1. Individuals and Sampling. The study participants were workers employed at the labor-intensive coal mining area of Punjab, Pakistan. Ethical as well as study approval was obtained from Department of Zoology, University of Sargodha, Sargodha, via letter no. 467. A total of 311 workers performing different tasks at different mine sites (underground and surface) were recruited for the current study after informed consent. Exclusion criteria include miners who have currently joined the mining profession or have a job experience of less than 5 years, who were having any comorbidity, and who were performing tasks like electricians and truck drivers.

The nonexposed reference cluster consisted of 50 individuals who currently live in the same area not involved in any sort of coal mining activities. The coal mine worker group was considered as a whole exposed group, which was further divided into three groups based on the duties performed by them at mining sites: (i) surface mine workers (n = 84); (ii) underground mine workers (n = 176); and (iii) workers encompassed those involved in administration (n = 51). The administrative group consisted of mine supervisors locally known as Munshis, who were involved in keeping the record of miner’s duty hours, pay, as well as maintenance of mines.

All subjects answered an investigator’s administrated questionnaire about general health, lifestyle, diet, smoking habits, occupational information (job experience, working hours, working position, working conditions), living environment, and socioeconomic factors. Blood samples were collected at the end of the job shift on the last working day, and the samples were divided into two tubes (with/without ethylenediaminetetraacetic acid (EDTA)). The serum was collected from different mine sites (underground and surface) were recruited for the current study after informed consent. Exclusion criteria include miners who have currently joined the mining profession or have a job experience of less than 5 years, who were having any comorbidity, and who were performing tasks like electricians and truck drivers.

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4.2. Determination of Oxidative Stress Markers. 4.2.1. Determination of Superoxide Dismutase (SOD) Activity. For the assessment of SOD activity in blood samples, 160 μL of the reaction mixture was prepared to consist of diethylenetriamine penta acetic acid, Sigma (DETPAC-CAS Number: 67-43-6), with a final concentration of 1 mM in 0.05 M Na phosphate buffer (pH 7.8), MTT (thiazolyl blue tetrazolium bromide, Sigma; Case Number: 298-93-1, 1 mg/mL in phosphate buffer), xanthine (Sigma CAS Number: 69-89-6), and 1.8 mM in Na phosphate buffer. In microplate wells, 20 μL of serum sample was added to the prepared reaction mixture. Xanthine oxidase (20 μL; final concentration, 0.01 U/mL of phosphate buffer) was used as an initiator of reaction. After incubation for 60 min at 25 °C, the absorbance of samples and blank was obtained at 570 nm using a microplate reader. Values of absorbance (U of SOD/mL of serum) were compared to a standard curve prepared by diluting standard SOD in a range of 0–0.935 U/mL in Na phosphate buffer.56

4.2.2. Computation of Catalase (CAT) Activity. The method developed by Szuster-Ciesielska et al.55 was used for the evaluation of catalase in blood samples. The reaction mixture was composed of 500 μL of phosphate buffer (0.05 M, pH 7.0), 300 μL of double-distilled water, and 50 mL of 1.1 mM hydrogen peroxide (H2O2) in distilled water. The serum sample (50 μL) was added to the reaction mixture, followed by incubation for 5 min at 25 °C. Each serum sample tube was subjected to 5 min centrifugation (5000 g) after adding 50% trichloroacetic acid (TCA, Sigma; Product Number: T6399). Centrifugation was preceded by the addition of 10 μL of titanium(IV) reagent. A measured volume of 100 μL of supernatant was shifted to a 96-well microplate, and absorbance was measured at 405 nm using a microwell plate reader. Absorbance values (CAT activity U/mL) were obtained and compared to a standard curve generated from samples with known catalase activity.

4.2.3. Determination of Glutathione (GSH) Activity. GSH activity was measured according to the method of Bolzán et al.58 The reaction mixture was prepared by adding 0.05 M potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN3 (to block CAT activity in the sample), 1 mM GSH, 1 U/mL yeast GSSG-RD, 0.2 mM NADPH, and 0.25 mM hydrogen peroxide in a total volume of 1 mL.

4.3. Determination of Coal Dust Levels. A Type 113A Gravimetric Dust Sampler was used for the measurement of dust in coal mines. It is a self-powered portable instrument used for gravimetric sampling of respirable airborne dust. Coal dust measurement was carried out with the assistance of a coal mining inspector, who recorded coal dust levels at different points of coal mines. Respirable airborne dust particles were collected from different locations of the mines. They were hung at a height two-thirds of the total height of the roadway for a whole shift period. A gravimetric sampler was used that operates on the principle of collecting harmful particles on the filter. The differences in the weight of the filter give a measurement of airborne dust particles per cubic meter of air.

4.4. Statistical Analysis. Demographic, lifestyle, and occupational characteristics were summarized by calculating means and percentages. Antioxidant enzyme activities were analyzed using ANOVA and descriptive statistics. Correlations between different variables were determined by Spearman correlation test. At the same time, regression analysis was carried out to find out changes in antioxidant enzyme activities with respect to changes in the level of coal dust. Statistical analysis was carried out using SPSS ver. 24.059 and NCSS ver. 1220 software.

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Notes
The authors declare no competing financial interest.

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