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RESEARCH ARTICLE

EVALUATION OF OXIDATIVE DAMAGE AND ANTIOXIDANT DEFENCE POTENTIAL IN CHARCOAL WORKERS EXPOSED TO POLYAROMATIC HYDROCARBONS

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ABSTRACT

Occupational exposure to Polyaromatic Hydrocarbon (PAH) constitutes an important health hazard. PAH may lead to the increased production of reactive oxygen species (ROS) causing oxidative stress. In the present study, the effect of PAH was investigated on 77 charcoal workers and 79 control population by quantifying antioxidant enzymes such as Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) after recording the demographic characteristics (age of the subject), lifestyle (smoking habit, alcohol and tobacco consumption), and occupational features (lifetime exposure to wood smoke, use of personal protective equipment). Level of urinary 1-hydroxypyrene which is a marker of PAH and 8-OHdG which is a marker of oxidative stress were significantly higher in charcoal workers (0.25µg/ml) as compared with control workers (0.065µg/ml). Mean of SOD and CAT activity in charcoal workers was significantly higher, while GPX activity was lower than control workers. These results suggest that occupational exposure to PAH increase oxidative stress levels as a response to elevated ROS generation. Elevated levels of ROS has been known to causes health hazards as they possess high reactivity with biomolecules i.e. DNA, RNA and proteins. So, it is evident that preventive changes in work conditions and lifestyle are necessary to improve the quality of life of charcoal workers who are exposed to PAH.

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INTRODUCTION

Approximately 40,000 million tons of charcoal is produced annually in the world (FAO, 2003). In many countries, the charcoal is destined mainly for domestic use (Plas, 1995). In India, Brazil, Malaysia, Zambia, and the Philippines, charcoal is used mainly for industrial purposes (Rosillo-Calle *et al*., 1996; FAO, 2003). Numerous iron and steel industries depend on charcoal for their production (ABRACAVE, 2001). It is a poorly mechanized process. This process depends on workers practical knowledge. It consists of two main actions: tree cutting and carbonization of wood in kilns. The process occurs constantly, and the workers working under conditions are exposed to wood smoke and charcoal dust. Charcoal production process in detail has been described by Kato (2003). Various studies on wood burning have found a variety of chemicals being emitted, including benzene, toluene, naphthalenes, substituted naphthalenes, and oxygenated monoaromatics and PAH (Larson and Koenig, 1994; Smith *et al*., 1999; Lemieux *et al*., 1999; Simoneit *et al*., 2000; Re´-Poppi *et al*.,2002). PAHs which are present in small amounts during wood burning are suspected to be carcinogenic/ mutagenic to humans (IARC, 2010). PAHs can be metabolized by cytochrome P450 (CYP) enzymes to generate active semiquinones, (Penning *et al*., 2007) which are known free radical intermediates and can go through redox cycling and generate reactive oxygen species (ROS) (Palackal *et al*., 2002). The ROS can then cause oxidative modification of DNA and lipids in the body (Palackal *et al*., 2002). 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is one of the most abundant forms of oxidative lesions in DNA, which is a critical biomarker of oxidative DNA damage (Cheng *et al*., 1992). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX-1) are the primary endogenous antioxidant defense enzymes. They provide cellular protection against damage due to ROS and detoxify free radicals: SOD converts highly reactive superoxide radicals to hydrogen peroxide whereas CAT and GPX-1 converts hydrogen peroxide into water and oxygen (Yu 1994). However, there are only a few

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studies reported on the charcoal production workers (Ellegard, 1996; Tzanakis *et al*., 2001; Dias *et al*., 2002) and none have evaluated biological effects among charcoal workers. To evaluate the effect of wood smoke on workers engaged in charcoal production, we examined urinary metabolite i.e. 1-hydroxypyrene (1-OHP) which indicates exposure to PAHs, urinary 8-oxodG which is a biomarker of oxidative stress and biological effect which includes antioxidant defense enzymes i.e. Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPX).

MATERIALS AND METHODS

Subjects

The population under study included 77 individuals working on charcoal and 79 healthy control subjects. All the subjects were informed about the study before obtaining the consent. The research protocol was approved by the Institutional Human Ethics Committee of Kurukshetra University, Kurukshetra (Haryana). A standard questionnaire was used to assess standard demographic characteristics such as age, sex, consumption habits (smoking, tobacco, and drinking habits, etc.) and years of work exposure.

Sampling

Blood (approximately 5 ml) and urine samples were collected from charcoal workers exposed to wood smoke and control subjects in presterilized K_2EDTAcoated vaccutainer tubes and urine container respectively with the help of a trained technician. The blood and urine samples were brought to the laboratory in a well insulated ice box taking care to avoid freezing of samples. Assays were performed under low-temperature conditions immediately after samples were brought to laboratory, and the remaining samples were stored at 4ºC. All exposed individuals had minimum 2 years of working exposure. Blood samples of subjects were collected after finishing of their work shift.

Analysis of urinary 1-OHP

To assess the PAH exposure in exposed population, the level of 1-OHP in randomly selected urine samples of exposed and control subjects were analyzed by standard method (Jongeneelen *et al*., 1987) using gas chromatography with flame ionizing detector. In 2.0 ml urine sample, 10 µl of β-glucuronidase/aryl sulfatase was added and the sample was incubated for 3 h at 37ºC. Sample was extracted with 5.0 ml of ethyl acetate by mechanical shaking for 20 min. The solution was evaporated in vacuum rotor to about 0.1 ml. The dry residue was dissolved with 50 µl of MSTFA and the tubes were heated for 30 min at 60 C. 1µl sample of the solution was injected into the GC system. The gas chromatograph used was an Agilent 7890 A with a splitless injector. The analytical column was a 30m HP-5MS column (cross-linked 5% phenylmethylsilicon, 0.2 mm I.D × 0.25 µm F.T). Oven temperature program was set starting at 80ºC, held for 1min, raised to 320ºC at 20ºC/min and held for 5 min. All mass spectra were obtained with an Agilent 5975 B instruments. The ion source was operated in the selected ionization mode. Confirmation of the compound was completed by MS characteristic ions, the ratio of MS characteristic ions and GC-retention time matched to the known standard compound.

Analysis of urinary 8-OHdG

Different assay methods including HPLC/ECD, GC/MS and ELISA are available for urinary 8-OHdG measurement. ELISA is a more sensitive method and generally provides higher 8- OHdG levels than the other methods (Yin *et al*., 1995; Kasai *et al*., 2001). Urinary 8-OHdG was measured with a competitive enzymatic immunoassay (EIA) kit (Caymen chemical company). First, void urine samples were centrifuged at 300 x *g* for 10 min to remove any particulate material. All reagents and urine samples were brought to room temperature before analysis and to insure the accuracy and reproducibility of results, each sample was assayed in duplicates. To each antibody coated well of the ELISA kit, a 50 µl urine sample, 50 µl of tracer and 50 µl of reconstituted primary antibody were added. The plate was covered with plastic film and incubated for 18 h at 4°C. The amount of antibody bound to the plate was determined color metrically after the addition of a Ellman's reagent and read at 405 nm. Quantification of the 8-OHdG was achieved by comparing the optical densities of the chromogenic signal of each sample with that of an internal standard of known 8-OHdG concentrations. The greater the amount of 8-OHdG in the sample, the lower was the light absorption. The 8-OHdG excretion was expressed relative to creatinine (ng/mg creatinine). Creatinine concentration was measured by spectrophotometry, as described by Jaffe *et al*. (1886).

Estimation of Malondialdehyde (MDA)

Blood malondialdehyde (MDA) level, as an end product of lipid peroxidation was measured by the method of Stocks and Dormandy (1971). Thiobarbituric acid (TBA) reacts with MDA to produce a stable chromogen that is quantified spectrophotometrically. To 0.5 ml plasma added an equal volume of MDA reagent (20% TCA in 5% TBA) and kept in water bath at 95ºC for 40 min and immediately chilled on ice for 15 min. The mixture was centrifuged at 10,000 x g for 30 min and the absorbance of supernatant was measured at 532 nm. MDA content was calculated using the extinction coefficient of 156 mM^{-1} cm^{-1}. The results were expressed as nmol/ml blood.

Estimation of Catalase (CAT) Activity

Catalase (EC 1.11.1.6) activity was determined according to the modified method of Abei (1984). The assay mixture for determining CAT activity contained 50 mM potassium phosphate buffer (pH 7.0), 30% H_2O_2 diluted with distilled water in 1:1 ratio and serum in a total volume of 3 ml. A blank was run without addition of the enzyme sample. The decrease in H_2O_2 concentration was followed by recording the decrease in absorbance at 240 nm for 3 min at 15 second interval using UV-VIS spectrophotometer. One unit (U) of CAT activity was defined as the amount of enzyme catalyzing decomposition of 1 µmol H_2O_2 per min at 240 nm calculated from the extinction coefficient of absorbance for H_2O_2 at 240 nm of 0.036 cm^2 μmol^{-1}.

Assay of Superoxide Dismutase (SOD) Activity

In serum, activity of superoxide dismutase (SOD) was measured by the method of Marklund and Marklund (1988). Superoxide anion is involved in the auto oxidation of pyrogallol at alkaline pH 8.5. Superoxide dismutase inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance at 420 nm for 3 minutes at 15 seconds interval. The SOD activity was measured as unit/ml. One unit of superoxide dismutase is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation.

Estimation of Glutathione Peroxidase (GPx) Activity

GPx activity in plasma was measured by the method of Rotruck *et al*. (1973) using H_2O_2 as substrate in the presence of GSH. This method is based on the fact that GSH is oxidized during reduction of H_2O_2. The GPx enzyme preparation allowed the splitting of H_2O_2 utilizing the GSH. Reaction was stopped at a particular time by addition of TCA and remaining GSH was measured by developing the color complex with DTNB. GPx activity was expressed in µmol GSH oxidized/min/g. Assay mixture contained 0.4 ml Tris-HCl buffer, 0.2 ml of GSH, 0.1 ml of sodium azide, 0.1 ml of H_2O_2, approximately diluted enzyme preparation and distilled water in a total volume of 1.0 ml. Reaction was started by the addition of H_2O_2. After incubation at 37 ºC for 10 min, reaction was stopped by addition of 0.5 ml TCA. Reaction mixture was centrifuged at 3000 rpm for 10 min. Then 0.5 ml of supernatant was added to 2.0 ml of dibasic solution and 0.5 ml DTNB. The color was read at 420 nm against a suitable blank.

Statistical Analysis

Two independent groups were compared using the Student's *t*-test. Chi-square ($\chi 2$) test was applied for difference in consumption habits and exposure history among studied population. The results were expressed as mean ± standard deviation (SD). Comparisons between groups were achieved by the Kruskal Wallis H test. Correlation tests were performed according to Pearson's rank following the variables distribution. Values of $p<0.05$ was considered to be statistically significant. Multiple regression analysis was used to evaluate the influence of age, gender, years of work exposure and smoking status on oxidative stress parameters of study groups.

RESULTS

Characteristics of the study population

The demographic characteristics of the study subjects are presented in Table 1. In this study, 77 Charcoal workers exposed to wood smoke and 79 control individuals participated. No difference between the age of the exposed (35.97±10.44 years) and the control group (34.83±11.05 years) was observed. The exposed group had been working for 2 to 21 years in charcoal producing area. None of the workers reported the wearing of complete personal protective equipment — PPE (mask, gloves, protective clothes and glasses). No significant differences were observed in relation to smoking, drinking and tobacco chewing between the exposed and non-exposed groups.

Biomarker of Exposure

Urinary 1 - Hydroxy pyrene (1-OHP) assessment

The GC–MS analysis was performed with the derivatized extract. The derivatization reduces the polarity of the analytes and produces a number of benefits including better detection, sensitivity and no carry over between runs. Figure 1 shows chromatogram of standard 1-OHP whereas chromatogram of charcoal workers and control population is shown in figure 2 and figure 3 respectively. The retention time was same in all the peaks which were 12 minutes. The mean concentration of 1-OHP in random urine samples of control and exposed subjects were found to be 0.065 µg/ml and 0.25 µg/ml respectively. The level of 1-OHP in urine of exposed subjects was found to be significantly higher than that of control subjects ($p<0.05$).

Biological Effects

Antioxidant enzyme levels

It is known that increased ROS production is related to the increase of antioxidant enzymes. Activities of antioxidant enzymes, SOD and CAT were significantly increased in exposed subjects when compared to controls whereas GPX levels were found to be lower in exposed subjects when compared to controls (4.10 ±0.76 versus 8.11 ± 0.57; P< 0.05) as shown in Figure 4. Based on the measurement, Tables 2, 3 and 4 show the effect of age, consumption habits, and work year exposure on antioxidant enzymes activities i.e. SOD, CAT and GPX activities respectively. The effect of smoking on antioxidant enzyme levels revealed that the exposed subjects who were smokers showed significantly increased levels of antioxidant enzymes activity in comparison to their control counter parts. Studies revealed that aging, alcohol consumption and tobacco chewing had no effect on antioxidant enzyme levels such as SOD, CAT and GPX. As seen in tables 2 and 3, with the increase of work year exposure, SOD and CAT activity increases but in workers with greater than 20 year exposure, antioxidant enzyme activity decreases.

8-oxodG and MDA content

Table 5 shows the distribution of urinary 8-OHdG levels and plasma MDA content in occupationally exposed workers and controls. The data reveals a significant difference of the 8-OHdG and MDA levels between these two groups with respect to control.

Effect of age, consumption habits and Work year Exposure on oxidative stress biomarker

Distribution of oxidative DNA damage in terms of 8-oxodG and MDA content in charcoal workers and control population in different age groups, smoking and alcohol status and occupational work year exposure to wood smoke has been depicted in table 6 and 7 respectively.

Table 1. Demographic Characterization of Control and Exposed (Charcoal) Population

Variables	Control Population		Charcoal Workers		95% CI		*p*-value	OR
					Lower	Upper		
Total	79 (100 %)	Mean ± S.D	77	Mean ± S.D	-2.26	4.54	0.507	
Age		35.97±10.44	17 (22.1 %)	34.83±11.05				
<25	13 (16.5 %)	21.88±3.16	45 (58.4 %)	20.53±2.67				
25-45	51 (64.6 %)	35.26±5.96	15 (19.5 %)	34.80±5.82				
>45	15 (18.9 %)	51.53±3.64		51.13±3.81				
Smoking					0.44	1.60	0.99	0.84
Smoker	46 (58.2 %)		29 (37.7 %)					
Non-Smoker	33 (41.7 %)		48 (62.3 %)					
Drinking					0.73	2.56	0.172	1.35
Drinker	41 (51.9 %)		34 (44.2 %)					
Non-Drinker	38 (48.1 %)		43 (55.8 %)					
Tobacco					0.59	5.80	0.590	1.85
Tobacco	9 (11.4 %)		5 (6.49 %)					
Non-Tobacco	70 (88.6 %)		72 (93.5 %)					
Work Exposure		12.94±7.92		7.99±4.54			<.05	
<10 Year								
10-20 Year			50 (64.9 %)	5.25±2.24				
>20 Year			25 (32.7 %)	12.40±1.96				
			2 (2.60 %)	21.50±0.71				

Student t-test was applied for comparing mean value of age among control and exposed group. SD- Standard deviation, $\chi 2$ test was applied for difference in sex and consumption habits exposure history among studied population. OR- Odd ratio, CI- Confidence Interval.

Table 2. SOD enzyme activity by Age, Consumption Habits and Work Year Experience

Variables	Control Population		Charcoal Workers	
	N	SOD Concentration (U/ml) (Mean ± SD)	N	SOD Concentration (U/ml) (Mean ± SD)
Age				
<25	13	3.18±0.73	17	5.74±1.47
25-45	51	3.37±0.69	45	6.48±1.44
>45	15	3.15±0.87	15	6.06±1.23
Smoking				
Non-smoking	33	3.05±0.62	29	6.44±1.37
Smoking	46	3.45±0.66	48	6.11±1.46
Alcohol				
Non-alcoholic	41	3.10±0.656	54	6.09±1.41
Alcoholic	38	3.46±0.638	23	6.56±1.44
Tobacco				
Nontobacco	70	3.28±0.644	72	6.28±1.41
Tobacco	9	3.32±0.873	5	5.52±1.61
Year of Work Exposure				
<10 years	-	-	50	5.70±1.32
10-20 years	-	-	25	7.04±1.36
>20 years	-	-	2	6.18±0.95

Significant at $p< 0.05$; multivariate ANOVA test was used with post hoc analysis for the comparison in SOD activity in multiple subgroups among studied population.¥Significant at $p < 0.05$ + highest mean rank; ¥highest mean rank (Kruskal-Wallis *H*test).

Table 3. CAT enzyme activity by Age, Consumption Habits and Work Year Experience

Variables	Control Population		Charcoal Workers	
	N	Catalase activity (U/ml) (Mean ± SD)	N	Catalase activity (U/ml) (Mean ± SD)
Age				
<25	13	11.64±2.85	17	25.03±3.21
25-45	51	12.62±3.11	45	26.16±4.91
>45	15	12.78±3.12	15	25.8±0.822
Smoking				
Non-smoking	33	11.45±2.76	29	25.11±4.09
Smoking	46	13.25±3.07*¥	48	27.06±4.46*¥
Alcohol				
Non-alcoholic	41	12.60±3.30	54	25.99±4.25
Alcoholic	38	12.39±2.82	23	25.49±4.55
Tobacco				
Nontobacco	70	12.59±3.09	72	25.76±4.39
Tobacco	9	12.50±2.96	5	27.16±2.97
Year of Work Exposure				
<10 years	-	-	50	23.36±3.75
10-20 years	-	-	25	28.68±4.41*¥
>20 years	-	-	2	23.95±3.47

Significant at $p < 0.05$; multivariate ANOVA test was used with post hoc analysis for the comparison in CAT activity in multiple subgroups among studied population.*¥Significant at $p < 0.05$ + highest mean rank; ¥ highest mean rank (Kruskal-Wallis *H*test).

Table 4. GPX enzyme activity by Age, Consumption Habits and Work Year Experience

Variables	Control		Charcoal Workers	
	N	GPX Concentration (IU/mg protein) (Mean ± SD)	N	GPX Concentration (IU/mg protein) (Mean ± SD)
Age				
<25	13	7.923±0.704	17	4.28±0.661
25-45	51	8.21±0.522	45	3.93±0.674
>45	15	7.96±0.478	15	4.39±0.822
Smoking				
Non-smoking	33	8.22±0.62	29	3.87±0.711
Smoking	46	8.06±0.515	48	4.24±0.763*¥
Alcohol				
Non-alcoholic	41	8.16±0.602	54	4.11±0.747
Alcoholic	38	8.05±0.532	23	4.07±0.807
Tobacco				
Nontobacco	70	8.12±0.563	72	4.07±0.776
Tobacco	9	7.97±0.602	5	4.49±0.300
Year of Work Exposure				
<10 years	-	-	50	4.02±0.713
10-20 years	-	-	25	3.65±0.657
>20 years	-	-	2	3.33±0.170

Significant at $p < 0.05$; multivariate ANOVA test was used with post hoc analysis for the comparison in GPX activity in multiple subgroups among studied population.¥Significant at $p < 0.05$ + highest mean rank; ¥ highest mean rank (Kruskal-Wallis *H*test).

Table 5. 8-OHdG and MDA content in charcoal workers and control population

Variables	Control	Charcoal Workers
8-OHdG Concentration (ng/mg creatinine) (Mean ± SD)	7.36 ± 2.30*	12.34 ± 3.78*
MDA content (nmole/ml)	2.01 ± 0.55	2.86 ± 0.74

*Significant at $p < 0.05$, student t-test was used

Table 6. MDA content by Age, Consumption Habits and Work Year Experience

Variables	Control		Exposed	
	N	MDA Content (nmole/ml) (Mean ± SD)	N	MDA Content (nmole/ml) (Mean ± SD) (Charcoal Workers)
Age				
<25	13	3.19±0.73	17	4.28±0.72
25-45	51	3.36±0.68*¥	45	4.91±0.86*¥
>45	15	4.13±0.58*¥	15	5.68±0.82*¥
Smoking				
Non-smoking	33	1.19±0.30	29	4.07±0.60
Smoking	46	1.48±0.30*¥	48	5.44±0.68*¥
Alcohol				
Non-alcoholic	38	1.84±0.57	54	4.94±0.96
Alcoholic	41	1.98±0.53¥	23	4.88±0.86¥
Tobacco				
Nontobacco	70	1.97±0.55	72	4.91±0.95
Tobacco	9	2.34±0.4*¥	5	5.12±0.54¥
Year of Work Exposure				
<10 years	-	-	50	4.55±0.81
10-20 years	-	-	25	5.51±0.62*¥
>20 years	-	-	2	6.94±0.26*¥

Table 7. 8-oxodG content by Age, Consumption Habits and Work Year Experience

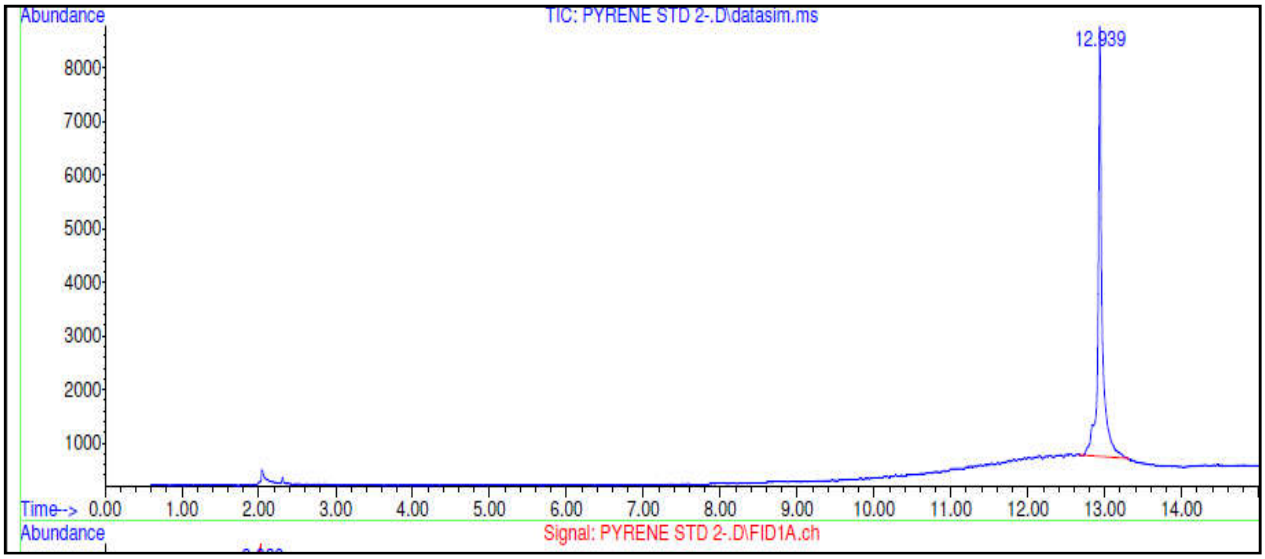
Variables	Control		Charcoal Workers	
	N	8-oxodG level (ng/mg protein) (Mean ± SD)	N	8-oxodG level (ng/mg protein) (Mean ± SD)
Age				
<25	13	5.55 ± 1.65	17	8.95±1.47
25-45	51	7.03 ± 1.43*	45	12.57±3.34*
>45	15	10.07 ± 2.92*	15	15.48±3.94*
Smoking				
Non-smoking	33	6.54 ± 1.27	29	11.58±3.98
Smoking	46	7.95 ± 2.68*¥	48	12.80±3.62*¥
Alcohol				
Non-alcoholic	41	8.16±0.602	54	12.23±3.72
Alcoholic	38	8.05±0.532	23	12.57±4.02¥
Tobacco				
Nontobacco	70	7.09 ± 2.11	72	12.34±3.83
Tobacco	9	9.48 ± 2.76*¥	5	12.24±3.49
Year of Work Exposure				
<10 years	-	-	50	10.63±2.39
10-20 years	-	-	25	15.45±3.99*
>20 years	-	-	2	16.18±3.24*

Significant at $p < 0.05$. Multivariate analysis of covariance tests with post hoc analysis was used for the comparison of MDA content in multiple subgroups among exposed and control groups separately. *¥Significant at $p < 0.05$ + highest mean rank; ¥highest mean rank (Kruskal-Wallis *H*test).

Table 8. Linear and logistic regression model for Interaction of age, consumption habits, exposure, 8-oxodG and MDA content

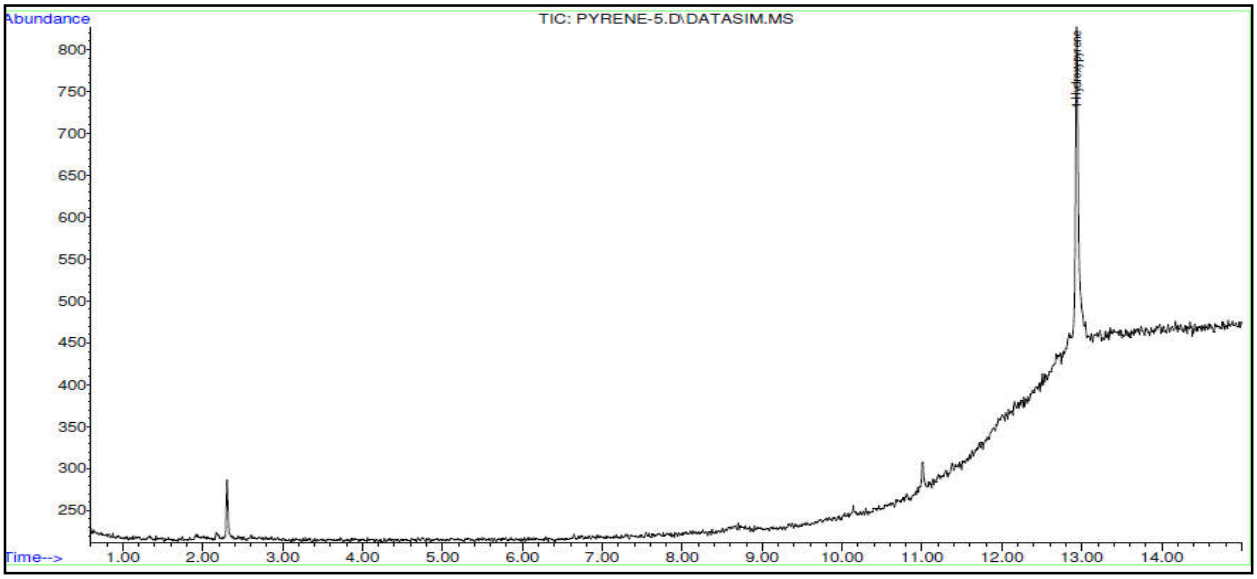
Dependent Variable	Independent Variable	R-square	Regression coefficient	Odd Ratio	P-Value
MDA Content					
	Intercept	0.405			
	Age		-0.002	0.023	0.846
	Drinking		-0.108	0.898	0.107
	Smoking		3.21	24.68	0.000
	Tobacco		0.373	1.452	0.490
	Work Experience		0.135	0.651	0.000
8-oxodG					
	Intercept	0.369			
	Age		0.123	0.358	0.005
	Drinking		0.023	1.023	0.726
	Smoking		0.098	1.098	0.175
	Tobacco		-0.007	0.993	0.952
	Work Experience		0.259	0.308	0.015

Models using stepwise method and adjusting by 8-oxodG and MDA Content. CI: confidence interval, R^2: correlation coefficient, Models excluding control individuals. Significant at $p < 0.05$



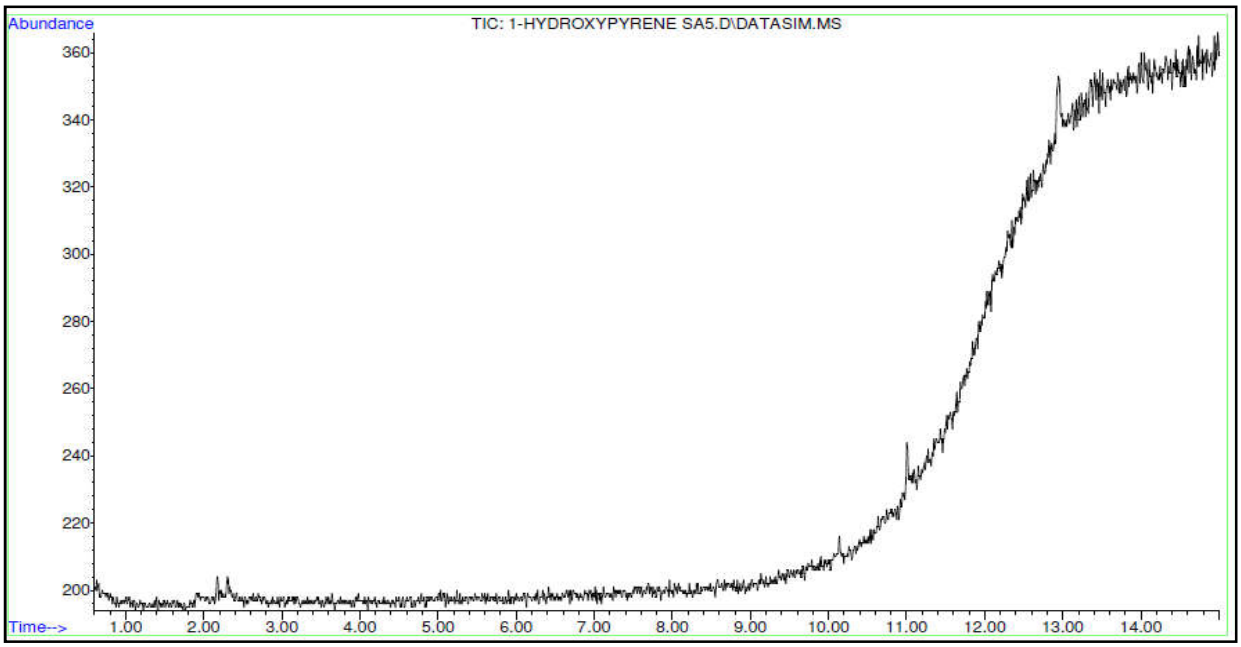
Retention time (minutes)

Figure 1. Chromatogram of standard. The standard solution was used having concentration of 1.0 µg/ml for 1-OHP



Retention time (minutes)

Figure 2. Chromatogram of Exposed Population



Retention time (minutes)

Figure 3. Chromatogram of control Population

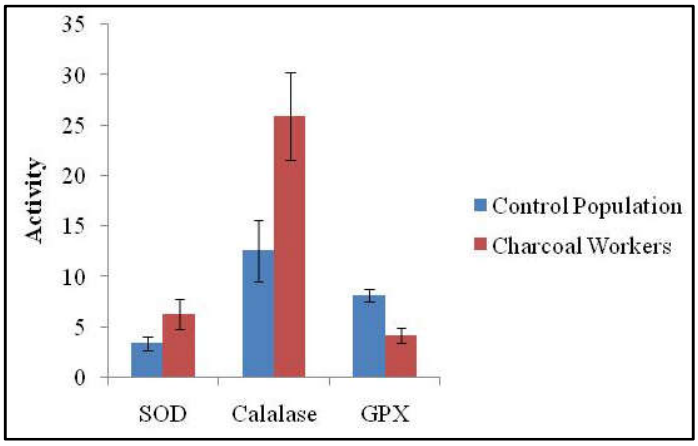


Figure 4. Antioxidant enzymes activity in control and exposed population

As can be seen in Tables 6 and 7, both urinary 8-oxodG level and MDA content increases with age in both the control group and in charcoal workers respectively. With regard to smoking (Tables 6 and 7), significant variations in 8-oxodG and MDA levels were detected between control population and charcoal workers but not with respect to alcohol drinking and tobacco chewing. The level of 8-oxodG and MDA content also significantly increases with the increased of work year exposure.

Linear and multivariate logistic regression analysis between MDA levels, DNA strand breakage and interference factors including age, consuming habits and work experience

Table 8 shows the results of multiple regression analysis applied to investigate the influence of age, smoking status, alcohol drinking, tobacco chewing and years of exposure on oxidative stress parameters of the studied populations. A strong association was found between smoking and MDA levels in the exposed group (r = 3.21, $p<0.05$). A significant relation between years of employment and MDA level was observed in the charcoal workers (r = 0.351, $p<0.05$). MDA level showed significant positive association for smoking (r= 0.568, $p=0.018$) but drinking ($p = 0.846$) and aging ($p = 0.107$) didn't show any significant alteration in MDA content. A statistically significant association was also detected between age and 8-oxodG in the charcoal workers (r = 0.123, p = 0.005). Smoking status did not affect 8-oxodG content in exposed group, but a significant association was detected with extended period of work exposure and 8-oxodG content (r = 0. 259, p = 0.015).

DISCUSSION

Wood smoke is known to be a human carcinogen with several health hazards associated with its exposure. Biological

monitoring of exposure to deleterious chemicals is important in the evaluation of risks to human health and it is considered as a strategy to improve conditions of occupational safety. In this study, we assessed the biological effects in charcoal workers exposed to wood smoke and other substances generated in the course of manufacturing of charcoal. The investigation was conducted by detecting antioxidant enzymes activity and 8-oxodG level in charcoal workers with respect to control population. Oxidative stress causes various types of damage followed by cell death or the activation of repair systems. ROS generated during oxidative stress not only triggers various types of tissue damage, but also act as inducers of anti-oxidative and other preventive systems. It is known that PAHs can generate oxidative stress (Chuang *et al*., 2003; Marczynski *et al*., 2005; Pan *et al*., 2008) which could be partially reduced by antioxidant enzymes (Cassini *et al*., 2011). It has been observed that low stress, which may not damage cells seriously, also induces preventive systems in cultured cells (Guo *et al*., 2003). In the present study, we observed that significantly higher SOD and CAT activity and lower GPX activity in the exposed group with respect to the control group. Literature on biochemical studies in wood smoke-exposed workers are scarce but similar results were obtained in other type of occupational workers i.e. metal-shelf factory workers (Polat *et al*,. 2013) Whereas Rekhadevi *et al*. (2009) determined that antioxidant enzyme levels significantly decreased in workers working in carpentry shops exposed to wood dust containing PAH. In our study, confounding factors i.e. age, alcohol drinking, and tobacco chewing didn't significantly influence the antioxidant enzymes level. Effect of smoking habit was apparent in univariate comparison between smokers and nonsmokers but it could not be confirmed by a regression analysis model. The present results indicated that with the increase of the period work exposure there was a significant increase of CAT and SOD activities in charcoal workers but in workers with greater than 20 years work experience, the enzyme activity decreases because more PAHs exposure inhibit the activities of antioxidant enzymes. This may be due to a decrease in the antioxidant defenses or due to an increase in the processes that produce oxidants (Hussain *et al*., 1999; Whaley-Connell *et al*., 2011). When the rate of formation of ROS exceeds the cellular antioxidant levels, the intracellular redox status is disturbed and depletion of antioxidant enzyme levels occurs. Free radicals generating during PAH metabolism causes alterations in membrane integrity which increases MDA content by lipid peroxidation. Pan *et al*. (2008) found that urinary MDA level was significantly associated with urinary 1-OHP levels ($p<0.001$) and working hours per day in male workers of Chinese restaurant which are exposed to PAH from cooking oil fumes, and similar results were obtained among workers occupationally exposed to diesel engine exhaust, a byproduct of the fuel composed by PAH and element carbon (Bin *et al*., 2016).

Oxygen radicals generated during PAH metabolism causes extensive damage to DNA, and the repair product 8-OHdG can be detected in both leukocytes and potential target tissues such as lung (Asami *et al*., 1996) and in the urine (Loft *et al*., 1992). So 8-OHdG is the most represented biomarker of oxidative stress. Comparisons between groups (by smoking habits) were performed on data adjusted by creatinine levels. The level of 8-OHdG was higher in charcoal workers than in control population. These results are consistent with the result of other studies which aimed to determine oxidative damage due to PAH exposure in occupational workers (Al Zabadi *et al*., 2011; Huang *et al*., 2013). The average level of 8-OHdG for smokers in this study (12.80±3.62 ng/mg creatinine) was significantly higher than that of non-smokers in charcoal workers but it could not be confirmed by a regression analysis model on smokers. Cigarette smoking is a factor contributing to PAHs exposure and DNA damage (Autrup *et al*., 1999). The main limitations of this study were that we used randomly 2-3 samples for detection of 1-OHP (Biomarker of PAH exposure) and large portion of the charcoal population were having less than 20 years experience, only two samples had more than 20 years period of exposure. So we can't evaluate of oxidative damage and antioxidant defense potential in charcoal workers having > 20 years experience.

Conclusion

Results of our study are consistent with the hypothesis that exposure to wood smoke air pollution increases oxidative stress. Exposure to PAH increases the plasma concentration of MDA content and urinary concentration of 8-OHdG suggesting that it might be a useful biomarker of oxidative stress resulting from wood smoke. Exposure to PAHs also increases antioxidant enzymes level. This is indicative of the damage caused by oxidant and ROS generating nature of wood smoke. Workers working in such area encounter various health hazards. Further, the workers are not advised on safety and hygiene. This data help to estimate of exposure risk assessment and emphasizes the necessity of surveillance in exposed workers. So there is a need to inform the charcoal workers about the potential hazard of occupational exposure and to improve conditions in workplace environment. Hence these workers should always be provided with appropriate personal protective equipment.

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