EXECUTIVE SUMMARY OF THE RESEARCH PROJECT

STUDIES ON GENETIC VARIANTS OF DNA REPAIR AND ANTIOXIDANT GENES AS MODULATOR OF DNA DAMAGE AND OXIDATIVE STRESS IN OCCUPATIONAL WORKERS.

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BAHADUR SHAH ZAFAR MARG

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PRINCIPAL INVESTIGATOR

Dr Ranjan Gupta,

Associate Professor,

Department of Biochemistry,

Kurukshetra University, Kurukshetra

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INTRODUCTION

Several workers while conducting studies on wood burning have found a variety of chemicals being emitted, including benzene, toluene, naphthalenes, substituted naphthalenes and oxygenated monoaromatics and Polyaromatic hydrocarbons (PAH). Many of these PAHs which are present in small amounts during wood burning are suspected to be carcinogenic/mutagenic to humans. Usually, PAHs are formed in the course of all processes that involve incomplete combustion, pyrolysis, or pyrosynthesis of organic materials. PAH exposure is inherent to many industrial workplaces, i.e. coke plants, coal gasification facilities, aluminum factories, iron and steel foundries, as well as to the rubber industry. Various biomarkers, including PAH-DNA adducts, PAH-protein adducts, and urinary metabolites of PAHs, have been used to assess human uptake and/or metabolic activation of these carcinogens. Among these, urinary 1-hydroxypyrene (1-OHP) is a firmly established useful biomarker of PAH uptake, and is found to be clearly elevated in occupational settings, within a few hours or days of exposure to PAHs. 1-OHP is a urinary metabolite of the non-carcinogen pyrene, which always occurs in PAH mixtures that include carcinogens, such as benzo[a]pyrene.

PAHs can be metabolized by cytochrome P450 (CYP) enzymes to generate reactive oxygen species (ROS). The ROS can then cause oxidative modification of DNA and lipids in the body. 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). Comet assay is another rapid and sensitive genotoxicity biomarker for detection of chemically induced DNA damage, in occupationally exposed workers. 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 by detoxifying free radicals.

These ROS produced during exposure, are also responsible for oxidative changes in polyunsaturated fatty acids contained in the membranes of mammalian cells. This oxidation of membrane lipid causes increased permeability of the cell membrane. MDA is the most abundant aldehyde formed during lipid peroxidation which is considered a biomarker of the lipid peroxidation. Increased concentrations of MDA are indicative of high oxidative stress and aging.

Both occupational and environmental sources of PAHs contribute to individual exposure thus increasing cancer risk. However, inter-individual variability in the capacity to activate or inactivate potential genotoxic and carcinogenic PAHs might influence susceptibility to cancer or other chronic diseases. Increased attention has been recently focused on genetic polymorphisms that could modulate human response to genotoxic insult. Therefore, genetic polymorphisms in antioxidants genes have been often used as a biomarker of susceptibility. They provide an indication of the extent to which an individual may be at risk of developing an adverse health effect as a result of an exposure to the genotoxicant. Several enzymes, which play key roles in antioxidant defense mechanism are polymorphic in humans. Oxidative stress in vivo might be modulated by the enzymes such as Catechol-O-methyl transferase (COMT), Glutathione peroxidase (GPX), Manganese superoxide dismutase (MnSOD) and NAD(P)H:quinone oxidoreductase (NQO1). The genetic variants of these enzymes may play a key role in modulating oxidative and genetic DNA damage in both occupationally exposed and control population. The human 8-oxo-guanine glycosylase 1 (OGG1) gene encodes a protein responsible for the cleavage of 8-oxoGuanine, a major form of base lesion produced by oxidative damage to DNA. If this lesion is not excised, it can be matched with adenine leading to a GC to TA transversion. Therefore, decreased function of OGG1 could lead to mutations that activate oncogenes or inactive tumour suppressor genes. The DNA repair protein XRCC1 as a part of the BER pathway forms complexes with DNA polymerase beta, DNA ligase III and poly-ADP-ribose polymerase (PARP) in the repair of DNA single strand breaks. The genetic variants of these enzymes may play a key role in modulating oxidative and genetic DNA damage in both occupationally exposed and control population. The role of genetic polymorphism in susceptibility to specific genotoxic exposure can be more easily revealed by examining different biomarkers in exposed and control subjects. There is no information on the levels of oxidative DNA damage and genotoxic damage among tandoor workers and charcoal workers exposed to PAH emitted from wood smoke. To the best of our knowledge no such studies are available for tandoor and charcoal workers exposed to PAH from wood smoke.

In our study, the population under study included 76 tandoor workers, 77 charcoal workers and 79 unexposed subjects. We studied the demographic characteristics viz. age, consumption habits and exposure duration of control and exposed subjects using a specially designed questionnaire. We found no statistically significant difference in distribution of age and consumption habits among control and exposed subjects ($p>0.05$). The tandoor workers assessed were occupationally exposed to wood smoke for an average time of 12.94 ± 7.92 years whereas charcoal workers were exposed to 7.99±4.54 years.

Biomarkers of exposure

Urinary level of 1-OHP found in tandoor workers and charcoal workers are 0.31 µg/ml and 0.25 µg/ml respectively while urinary level of 1-OHP in control population is 0.065 µg/ml. 1-OHP is a biomarker of PAH exposure which is significantly higher in exposed workers as compared to control population. So it is quite evident that tandoor and charcoal workers were exposed to PAH emitted from wood combustion.

Biomarkers of oxidative stress

We evaluated the effect of exposure to PAH on level of the oxidative stress biomarkers. MDA and 8-oxodG used in our study are biomarkers indicating lipid peroxidation and oxidative DNA damage, respectively. In the present study, concentration of 8-oxodG in PAH exposed population i.e. tandoor and charcoal workers is 15.25 ± 4.52 ng/mg creatinine and 12.34 ± 3.79 ng/mg creatinine respectively whereas in control population it has been found to be 7.36 ± 2.30 ng/mg creatinine. Oxidative stress was also measured by MDA content which was found to be 4.93 ± 0.93 nmole/ml in tandoor worker and 4.55 ± 1.56 in charcoal workers as compared to the values of 2.01 ± 0.55 nmole/ml in unexposed population. MDA content was found to be significantly higher in exposed population as compared to control population. So, tandoor and charcoal workers exposed to polyaromatic hydrocarbons are more susceptible to oxidative stress as compared to control population.

Biomarkers of genotoxicity

In the current investigation, the genotoxic damage was evaluated in peripheral blood lymphocytes of control and exposed subjects with the help of comet assay. In comet assay, the extent of DNA damage was measured quantitatively as tail moment (TM) value using Lucia Comet Assay IV Software. TM is defined as the percentage of DNA in the tail multiplied by the length between the center of the head and tail. Tandoor and charcoal workers manifested a significant higher amount of DNA damage as compared to control as assessed by observing TM values. Mean TM value observed in tandoor workers was 5.63 ± 0.90 μM (range 4.11 to 7.88) and in charcoal workers the value was 5.38 ± 0.82 (range 3.99 to 6.95). Significantly lower values were found in control subjects with mean TM value as 1.36 ± 0.31 (range 0.75 to 1.98). So, tandoor and charcoal workers exposed to PAH undergo more genotoxic damage as compared to control population.

Antioxidant enzymes levels

Superoxide dismutase activity in exposed subjects i.e. tandoor workers and charcoal workers was 7.47 ± 2.67 U/ml and 6.23 ± 1.43 U/ml respectively which was significantly high than that observed in control subjects (3.29 ± 0.68 U/ml).

Catalase activity was evaluated individually in exposed and control subjects. Mean catalase activity in tandoor workers have been found to be 20.13 ± 9.50 U/ml and in charcoal workers the

mean value observed was 25.85 ± 4.31 U/ml whereas in control population it was 12.50 ± 3.08 U/ml. Catalase activity was found to be significantly increased in the tandoor and charcoal workers as compared to control population.

Gpx activity was found to be 4.56 ± 0.98 U/mg protein in tandoor workers and 4.10 ± 0.76 U/mg protein in charcoal workers as compared to control population (8.1 ± 0.57 U/mg protein). The decrease in Gpx activity was found to be significant ($p<0.05$). These observations clearly suggest that PAH exposure causes an imbalance between ROS production and antioxidant defense system leading to the alteration in antioxidant enzyme levels.

Effects of confounding factors on biomarkers

The effect of various confounding factors viz. age, consumption habits (smoking, alcohol intake and tobacco chewing) and exposure duration on various biomarkers was analyzed. Higher oxidative and genetic damage was found in individuals of age group greater than 45 years. Among consumption habits, smoking showed significant association with studied biomarkers whereas alcohol intake and tobacco chewing didn't show any regular pattern or significant association with studied biomarkers. Individuals in age group greater than 45 years were found to have higher 8-oxodG Content, higher TM value, higher lipid peroxidation, higher Gpx activity and lower SOD and CAT activities as compared to < 25 years and 25-45 years. Mean value of 8-oxodG, TM, and MDA content were found to be significantly higher in smokers as compared to non-smokers in both exposed and control subjects. The exposed individuals having >20 years of work exposure experience had higher mean value of 8-oxodG, MDA and TM as compared to individuals having < 20 years of work exposure experience. Activity of antioxidant enzymes was found to be significantly higher in individuals having 10-20 year work exposure experience as compared to <10 years exposure duration but significantly lower antioxidant enzymes activity was observed in individuals having >20 years work exposure duration. These observations imply that with the increase of work exposure duration antioxidant enzyme activity increases because antioxidant system is induced during oxidative stress but greater than 20 year experience antioxidant enzyme activity decreases. This may be due to a decrease in the antioxidant defenses or due to an increase in the processes that produces oxidants

Biomarkers of susceptibility

The role of genetic polymorphisms of antioxidant genes (MnSOD, Gpx-1, COMT and NQO1) as biomarkers of susceptibility was investigated. There was a significant difference in SOD activity between mutants and wild type genotype of MnSOD gene. SOD activity was found to be higher in MnSOD homozygous mutants (mt/mt) (3.47 ± 0.66; 8.59 ± 2.46) as compared to heterozygous mutants (wt/mt) (2.99 ± 0.68; 6.38 ± 2.48) and wild type genotypes (wt/wt) (3.27 ± 0.57; 7.56 ± 2.63) in control and tandoor population respectively. The difference was found to be significant. Similar results were obtained in charcoal workers but difference was not significant. Homozygous mutants of Gpx gene showed significantly lower Gpx activity as compared to wild type in both control and exposed subjects. Gpx-1 polymorphism was negatively and significantly associated with Gpx activity in tandoor and charcoal workers which was further confirmed by linear regression model. Higher mean value of TM, high 8-oxodG content and MDA content was observed in individuals having homozygous mutants of MnSOD, Gpx-1, COMT and NQO1 genotype as compared to individuals having wild genotypes. The association of the above mentioned biomarkers of effect and homozygous mutant genotype of MnSOD and COMT difference was found to be significant in both control and exposed population. However, the association of the biomarkers of effect and homozygous mutant genotype of NQO1 was non-significant in control group but significant in exposed group. For GPX-1 gene, the association of the biomarkers of effect and homozygous mutant genotype was found to be non-significant.

Based on the results of the present investigation, important findings are enlisted below

1. Results of our study are consistent with the hypothesis that continuous exposure to wood smoke increases oxidative and genetic damage. Exposure to PAH increases the plasma concentration of MDA content and urinary concentration of 8-oxodG and TM suggesting that it might be a useful biomarker of oxidative and genotoxic damge resulting from wood smoke.

2. Age and years of work exposure duration were found to be significantly associated ($P<0.05$) with oxidative and genetic damage in studied population. Smoking has significant effect on oxidative and genotoxic damage. However, consumption habits (alcohol intake and tobacco chewing) did not significantly affect the oxidative and genetic damage ($P>0.05$).

3. Polymorphisms of MnSOD, CAT, COMT, NQO1, *OGG1* and *XRCC1* genes were found to play a significant role in human susceptibility to oxidative stress induced by wood smoke in tandoor and charcoal workers. However, more studies need to be carried out before establishing the role of antioxidant genes as biomarkers of susceptibility and for better understanding of gene-environmental exposure interactions. .

This study shows the effect of exposure from wood smoke on tandoor and charcoal workers with regards to oxidative and genetic damage. Genetic polymorphisms of antioxidant genes (MnSOD and Gpx-1) were found to be associated with oxidative stress. The impact of genetic polymorphism as a biomarker of susceptibility is of key significance to the understanding of the process of genetic damage involved in mutagenesis and carcinogenesis. The relationship between genotypes and biomarkers of effects could be important in risk assessment of human exposure to mutagens and carcinogens. It may be helpful in early detection of risk to cancer or other chronic diseases, if any. Mostly raised health concerns by tandoor and charcoal workers were skin and eye infections, nose and throat irritations, cough, asthma and nausea. None of the exposed personnel used facemasks or any other protective equipment. The results of the present study emphasize that ignorance about using protective measures while working on tandoor and during charcoal production, could be the potential reason for high oxidative and genotoxic damage in the tandoor and charcoal workers. So, there is a need to inform these workers about the potential hazard of occupational exposure and hence should always be provided with appropriate personal protective equipment. Since oxidative and genotoxic damage are important considerations in events leading to cancer after carcinogen/genotoxicant exposure, precautions should be taken by occupational workers to minimize direct exposure to PAH arising from incomplete combustion of wood. On the other hand, controversial results obtained from different studies are always difficult to interpret because each population under study has different socioeconomic status and work in different areas under different climatic conditions. It is therefore suggested that bio-monitoring of oxidative and genotoxic effects in tandoor and charcoal workers with furthermore comprehensive controlled studies are needed to support our observations.