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# Association of Superoxide Dismutase (*SOD1*) Gene Polymorphism with Oxidative Stress in PAHs Exposed Brick Kiln Workers in Haryana

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**ABSTRACT**

In developing countries like India brick kiln in suburban areas pose a major threat to environment and health of brick kiln workers and those who lives in close vicinity. Due to long working hours and lack of safety measures workers were exposed to various harmful pollutant like polycyclic aromatic hydrocarbons (PAHs). To assess the health risk of brick kiln workers due to PAHs exposure the present study was carried out on group of 100 PAH exposed brick kiln workers and 100 healthy individuals of Haryana. We have examined SOD enzyme activity and *SOD1* A251G polymorphism against PAH induced oxidative stress in human population. From our result we have found significantly lower value of SOD enzyme in exposed workers and polymorphism *SOD1* A251G also contributes significantly to its phenotype in exposed workers as compared to healthy control. Current study suggested high level of oxidative stress in brick kiln workers of Haryana due to long exposure to heat and PAHs compounds. Protective measure should be taken to prevent health risk.

***Key words:*** *SOD, PAHs, Haryana, Oxidative stress, Brick kiln*

## Introduction

The brick manufacturing sector contributes 0.7% of India's GDP and employs one of the nation's largest workforces after agriculture. But along with economic benefit brick kilns are also known to be a leading cause of air pollution because they use a variety of fuels such as coal and wood. But to earn more profit low cost fuels like waste oil, plastic, oil based drilling cutting and even rubber are also used in unscientific way to fire kilns, resulting in the production of Polycyclic aromatic hydrocarbons (PAHs) and other toxic pollutants (Liu *et al*., 2022, Yusups, 2014).

PAHs are hydrocarbon compounds having fused benzene rings (Saldarriga-Norena *et al*., 2018). Many PAHs compounds viz. Benzo (a)pyrene [B (a) P], anthracene, naphthalene and chrysene are potent

carcinogens and mutagens (IARC, 2016). Along with carcinogenic properties PAH compounds also have much long time health effect like oxidative stress, cardiovascular diseases, anemia and neurological disorders (Kamal, 2014).

Oxidative stress is the stage when amount of reactive oxygen and nitrogen species increase as compared to antioxidants (Pisoschi *et al*., 2021). These reactive molecules can cause membrane lipid peroxidation and attack cellular genetic material and proteins (Volka *et al*., 2006). To combate the effect of oxidative stress various antioxidant enzymes like SOD, Catalase and Glutathione S transferase etc are present in human body as a safeguard. Out of all the antioxidant enzymes Superoxide dismutase are the most abundant class of metalloproteins which catalyze dismutation of superoxide radical in to molecular oxygen and hydrogen peroxide (Hayyan *et al*., 2016). Three form of superoxide dismutase enzyme are present in human these are SOD1 (Cu-Zn SOD), SOD2 (Mn-SOD) and SOD3 (EC-SOD). Out of these three forms SOD1 contributes 85% of total enzymatic activities. Gene coding for SOD1 enzyme is 9307bp long and contains five exon and four intermittent intron. It is present on 21q22.11 region of chromosome 21.

In this study we have examined the enzymatic activity of SOD as a biomarker of effect resulting from PAH induced oxidative stress in brick kiln workers. Present study was based on some previous finding of different researchers who reported association of PAHs induced oxidative stress and SOD enzyme level in exposed population (Gómez-Oliván *et al*., 2012; Kamal *el al.*, 2014).

Gene coding for SOD1 enzyme is polymorphism prone which leads to altered enzymatic activity. Various SNP has been found in *SOD1* gene out of which A251G (rs2070424) polymorphism is the most important one as various previous studies found association of A251G (rs2070424) polymorphism with oxidative stress (Bizon *et al*., 2022; Saremi *et al.*, 2021). Aim of present study was phenotypic and genotypic characterization of SOD enzyme in PAH exposed brick kiln workers of Haryana. To the best of our knowledge this is one of the first study of its type to be conducted in among the people of Haryana.

## Materials and Methods

The study population comprised of 100 PAH exposed brick kiln workers and 100 healthy person of Haryana with age in the range of 18-60 years. All the individuals were of Haryana origin (North Indian Ethnicity). Blood samples were collected from these individuals with informed written consent. The present study was approved by the Institutional Ethical Committee, Maharaja Agrasen University, Baddi (HP) India and all participating subjects signed a term of consent after being informed about the objectives of the study. A standardized questionnaire, including data on social habits such as life style, age, marital status, ethnic group, occupational history, smoking habits and health problems (family history of cancer any other related disease).

### Sample collection

With the help of trained technician 3ml blood sample was taken from each participant in $K_2$EDTA coated vacutainer tubes which were immediately transported to laboratory for further processing in well insulated ice box. 200 µl blood sample was separated for DNA isolation and from remaining blood total serum was separated using centrifuge and immediately proceeded for photometric assays. Genomic DNA was isolated from 200 µl of whole blood by Spin column kit (Promega, Cannada).

### Determination of SOD activity

The activity of Superoxide dismutase antioxidant enzyme was determined by using the approach of Marklund *et al.,* 1974 with minor modifications. Briefly, assay mixture contained 100 µl blood serum, 2.8 ml 0.1 M Tris HCl buffer (pH 8.2). After that, 0.1 ml of 2 mM pyrogallol was added to the test mixture to start the reaction. The SOD activity was measured by UV-visible spectrophotometer at 420 nm. One unit of SOD corresponds to the amount of SOD that will result in 50% inhibition of the oxidation of pyrogallol to pyrogallol-ortho-quinine for 1 minute. The enzyme activity was measured in units per mg of protein.

### SOD1 genotyping

The polymorphism analysis for the *SOD1* A251G region was done by PCR-RFLP method described by Silig *et al.,* 2017 with minor modifications. Reaction mixture (25 µL) consisted of 1 µL of genomic DNA template (~100 ng/ µL), 0.5 µl of each forward and reverse primers (25pmol), 0.5 µL of dNTPs (200 µM), 2.5 µL PCR buffer with 15 m M/L $MgCl_2$ (1x), 0.5 µL of Taq polymerase (3 U/µl) and 19.5 µL nuclease

free water (Bangalore Genei, Bangalore, India) and 5% dimethyl sulfoxide.

The PCR products (10 µl) were digested with 10U of specific restriction enzyme and then subjected to electrophoresis in 3.0 % agarose gel stained with ethidium bromide (0.5 µg/ml). The PCR conditions, primers sequences, restriction endonuclease (for RFLP methods) and length of the expected fragments (amplified and after digestion) to identify *SOD*1 alleles are given in Table 2. All PCR amplifications were performed in Eppendorf Gradient Thermal Cycler.

### Statistical Analysis

All the data were expressed as mean and standard error. Different variable were compared using student t-test and chi square test among exposed and control population. Genotype and allele frequency was calculated by counting the no. of each particular allele, Hardy–Weinberg law was used to compare observed and expected genotype frequencies of *SOD*1 gene. Influence of *SOD*1 A251G polymorphism on SOD enzyme activities among multiple subgroups was determined by post hoc analysis using multivariate ANOVA test. All the tests were performed using system software SPSS 21.0. Level of significance was set at 0.05

## Results and Discussion

Current study focused on assessment of oxidative stress and SOD enzyme activity in PAHs exposed brick kiln workers of Haryana. Brick kiln is the most unprivileged sector where workers perform under very unhygienic conditions. Workers were not using any safety gears and also due to long working hours they comes in contact with various harmful pollutant which further increase their health risk. To assess the health risk of brick kiln workers due to PAHs exposure present study was carried out on group of 100 PAHs exposed brick kiln workers and 100 healthy individuals of same socioeconomic status.

Demographic characteristics of both the studied population are given in Table 1. We found no significant (p value›0.05) difference in between demographic characteristics of both populations.

The mean value of SOD enzyme activity was found significantly (p value <0.05) lower in exposed workers (1.94±0.24 U/mg protein) as compared to

**Table 1.** Demographic characterstics of Exposed and Control population

| Variable | Exposed Population | Control Population | P value |
|---|---|---|---|
| Number (N/%) | 100 (100) | 100(100) | |
| Age in years(mean+SD) | 35.82±12.44 | 35.14±10.46 | 0.075 |
| Age Categories | | | |
| 18-30 | 39 | 33 | 0.152 |
| 31-45 | 46 | 53 | |
| 45-60 | 15 | 14 | |
| Gender (N/%) | | | |
| Male | 77 | 71 | 0.333 |
| Female | 23 | 29 | |
| Smoking Habits (N/%) | | | |
| Smoker | 46 | 45 | 0.887 |
| Non Smoker | 54 | 55 | |
| Alcohol intake (N/%) | | | |
| Alcoholic | 49 | 37 | 0.087 |
| Non Alcoholic | 51 | 63 | |
| Diet | | | |
| Vegetarian | 74 | 76 | 0.744 |
| Non Vegetarian | 26 | 24 | |
| Exposure duration (in years) | | | |
| 1-10 | 64 | - | <0.05 |
| 11-25 | 32 | - | |
| 25-50 | 4 | - | |

Student 't' test was applied for comparing mean value among control and exposed population. Chi-square was applied for difference in sex and consumption habits. Level of significance was set at 0.05

healthy control (2.85±0.77 U/mg protein). SOD is the most important antioxidant enzyme as it provide first line of defense against reactive oxygen species. Hence drop in the level of SOD enzyme could cause oxidative stress in brick kiln workers. Result of current study is consistent with some earlier findings which also suggested decrease level of SOD enzyme as a result of PAHs exposure (Jahan *et al.*, 2016; Kaushik *et al.*, 2012; David *et al*., 2022). Decrease in enzyme activity could be due to increase use of enzyme for detoxification of reactive oxygen and nitrogen species produced as a result of PAH exposure. While some other studies like (Gómez-Oliván *et al*., 2012) suggested higher level of antioxidant enzyme as a result of increase in reactive oxygen species. Inal *et al.*, 2001 also suggested age could be a factor for reduced antioxidant enzyme activities.

Amplified PCR products for *SOD1* gene which is 570 bp long are shown in Figure 1. Results of RFLP are shown in Table 3. There was no significant difference between observed and expected genotypic frequencies of both the studied population indicating that they were in Hardy-Weinberg equilibrium.

Frequency of mutant allele G of *SOD*1 gene was found more in exposed workers. Many previous studies found protective role of G allele of *SOD1* A251G Polymorphism in gastric cancer (Ebrahimpour *et al*., 2014), ulcerative colitis (El-Kheshen *et al*., 2016) and Alzheimer disease (Spisak, *et al*., 2014). Some other studies give contradictory results as higher G allele frequency was associated

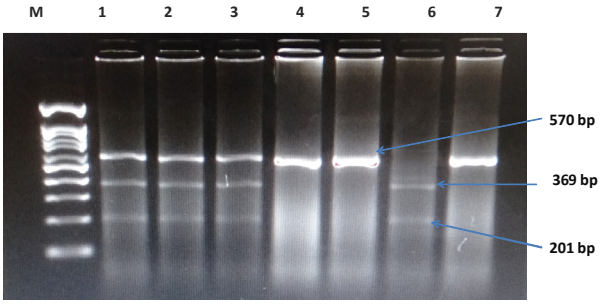


**Fig. 1.** 2.5% Agarose gel showing PCR-RFLP pattern of polymorphism of *SOD 1* A251G. M lane is 100 bp marker, Lane 1,2, and 3 are presenting Heterogygous genotype AG (570 bp + 369 bp + 201 bp), Lane 4,5 and 7 presenting Homozygous wild Genotype AA (570 bp), Lane 6 is presenting Homozygous mutant Genotype GG (369 bp + 201 bp)

**Table 2.** Reaction conditions for genotyping of *SOD1* gene

| Gene | Primer sequence (52-32) | PCR condition | Restriction enzyme and reaction temperature | PCR and RFLP products size | Reference |
|---|---|---|---|---|---|
| *Superoxide dismutase (SOD1)* rs2070424 | Forward: CACCAGCACTAGCAGCATGT Reverse: GGTGACGTTCAGGTTGTTCA | 94° C 4 min<br>94 °C 50 sec<br>58 °C 50 sec<br>72 °C 50 sec } 35<br>72 °C 7 min | *Msp* I 37°C for 15 min | **PCR Product 570bpAA**: 570bp **AG:** 570bp+ 369bp+201bp **GG**: 369bp+ 201bp | Silig Y.*et al.,* 2017 |

AA-Homozygous wild, AG-Heterozygous, GG-Homozygous mutant, PCR-RFLP-Polymerase Chain Reaction, Restriction fragment length polymorphism. Restriction enzymes are of New England Bio Labs, UK

**Table 3.** Genotypic frequency of *SOD1* gene polymorphism in studied population

| | Exposed Population (Brick Kiln worker) | | | Control Population | | |
|---|---|---|---|---|---|---|
| *SOD1* Genotype | Observed frequency (N) | Expected frequency By HWE (N) | Variant allele and frequency | Observed frequency (N) | Expected frequency By HWE (N) | Variant allele and frequency |
| AA | 58 | 61.6225 | G (0.215) | 78 | 79.21 | G(0.11) |
| AG | 41 | 33.755 | | 22 | 19.58 | |
| GG | 1 | 4.6225 | | 0 | 1.21 | |

AA-Homozygous wild, AG-Heterozygous, GG-Homozygous mutant, N-no.of individuals, HWE- Hardy Weinberg Equation

**Table 4**. Influence of *SOD1* gene polymorphism on SOD activity

| *SOD1* Genotype | Exposed Population | | Control Population | |
|---|---|---|---|---|
| | N | SOD activity | N | SOD activity |
| AA | 58 | 2.00±0.24 | 78 | 2.82±0.75 |
| AG | 41 | 1.85±0.23 | 22 | 2.97±0.81 |
| GG | 1 | 1.76 | 0 | 0 |

Influence of *SOD1* A251G polymorphism on SOD enzyme activities among multiple subgroups was determined by post hoc analysis using multivariate ANOVA test. Level of significance was set at 0.05

with cervical cancer (Datkhile *et al*., 2020), Parkinson diseases (Liu *et al*., 2019), Colorectal cancer (Jamhiri *et al*., 2017) etc.

Influence of SOD1 gene polymorphism on SOD activity in both the studied population is shown in Table 4. We found non-significant (p value 0.443) association of *SOD 1* genotype and SOD enzyme activity in control population while in exposed population we found significant (p value 0.010) association between *SOD1* genotype and SOD enzyme activity.

## Conclusion

Results of present study suggested high level of oxidative stress in brick kiln workers of Haryana due to long exposure to heat and PAHs compounds. Despite various health problems in brick kiln workers health care facility and occupational safety is negligible. To prevent the health hazards in brick kiln workers early detection of oxidative stress biomarker is very important. Mutant allele G frequency in *SOD1* gene could also be used as practical tool in early analysis and treatment of various disorders. Protective measure should be taken to prevent health risk.

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### Statement of Ethics

Participants were requested to sign a permission form (consent form), and each was questioned in person using a predetermined set of questions (standard questionnaire) which included information about their socioeconomic situation. Institutional ethical committee, Maharaja Agrasen University, Baddi (HP) India approved the study's research methodology (Ref. no. IEC/MAU/2021/03, Dated: 11/08/21).

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### Conflict of Interest statement

The authors declare no conflict of interest.

### Authors Contributions

Shiv Kumar Giri conceived, designed the study and review the original draft. Monika Rani collected blood samples, conducted experiments and writing original draft. Hemlata and Soniya jhangra helped to conduct analysis of data. Anil Kumar, Gulab Singh, Anita Saini, Anuradha Bhardwaj and Kanu Priya provided critical inputs during the data analysis and manuscript preparation.

### Data Availability Statement

The data support the findings of this study, which are not publicly available due to the organization's administrative policy and procedures. However, the data are available from the corresponding author upon request.

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