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# A STUDY ON GENE REPAIR POLYMORPHISM AND OXIDATIVE STRESS AMONG VARIOUS OCCUPATIONAL WORKERS EXPOSED TO ENVIRONMENTAL POLLUTANTS IN GASHUA SENATORIAL ZONE, YOBE STATE, NIGERIA.

***MUSTAPHA ALHAJI SALEH; *ALI BOYI DAUDA; *RABIU SABO; ABUBAKAR *ABDULKARIM; & **BABA SALEH AHMED**

*Department of science Laboratory Technology, Mai Idriss Alooma Polytechnic Geidam, Yobe State. **Department of Computer sciences, Mai Idriss Alooma Polytechnic Geidam, Yobe State.

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## Abstract

Single nucleotide polymorphisms in OGG1 gene modulates DNA repair capacity and functions as one of the first lines of protective mechanisms against 8-OXOdG mutagenicity. OGG1-Cys326 gene polymorphism may decrease DNA repair function, causing oxidative stress due to higher oxidative DNA damage. The main purpose of this study was to examine the link of oxidative and genotoxic DNA damage with DNA repair OGG1 gene polymorphism, in Various occupational worker such as metal workers exposed to polyaromatic hydrocarbons. Methods: Urinary 8-OXOdG excretion (occupational workers of oxidative



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DNA damage) was determined in both exposed and control populations. Genotyping of OGG1 DNA repair gene in the blood samples of subjects was carried out by PCR-RFLP method. Results: The 8-OXOdG urinary concentration was significantly higher ($p < 0.05$) in the exposed (geometric mean 12.33 ± 3.78) than in the unexposed (geometric mean 7.36 ± 2.29) population. DNA damage, as measured by 8-OHdG and TM content, was found to be significantly higher in OGG1 homozygous mutants (mt/mt; 18.81 ± 3.34; 6.04 ± 0.52) as compared to wild-type genotypes (wt/wt; 10.34 ± 2.25; 5.19 ± 2.50) and heterozygous (wt/mt) mutants (12.82 ± 2.81; 6.04 ± 0.93) in the exposed group.

## INTRODUCTION

The extent and complexity of health hazards inherent to workers who live with injuries caused by some types of work activities have highlighted the need to develop a model study of the future of occupational health (Higashi, 2006). Exposure to PAH in metalworkers can be due to inhalation or by dermal contact. PAH is known to exert toxic effects even at relatively low concentrations. Such toxicity could be modulated by mutant genotypes of DNA repair gene, causing an increase in the susceptibility to various diseases [18]. Thus, the main goal of our study was to assess the modulatory effect of gene polymorphism of DNA repair gene on oxidative DNA damage in metal workers. These workers had the higher levels of urinary 1-OHP as compared to reference values, indicating high exposure to PAH. Urinary 1-OHP levels in occupational workers were higher than those of control population as suggested by Wenjuan *et al.,* [19]. These observations are also in accordance with those reported previously by Fu *et al.,* [20] and Nguyen *et al.,* [21] who described the toxicity of PAH as a consequence of imbalance between pro-oxidant and antioxidant homeostasis, the so called oxidative stress, which ultimately leads to oxidative damage. Therefore, to assess the differential impact of such toxicants on the exposed individuals, we



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investigated the association of genetic polymorphism of OGG1 gene with oxidative and genotoxic damage of occupational workers in the exposed and control groups. Our study found that OGG1 polymorphisms are significantly related to oxidative and genotoxic damage, and mutant type genotype presented higher levels of DNA damage. These results are similar to findings of Chen *et al.*,[22] who showed higher repair activity of OGG1 Ser/Ser for 8-OHdG than the OGG1 Cys/Cys. Also, Aka *et al.*[23] and Pawlowska *et al.*[24] observed that Cys/Cys and Ser/Cys OGG1 genotypes had less DNA repair capacity compared to the Ser/Ser OGG1 genotype. There are a number of clinical studies in agreement with our results, indicating that the mutant genotypes of OGG1 have higher DNA damage and lower repair capacity of 8-OHdG than wild-type genotype [25,26], which is associated with the risk of lung cancer [26], type 2 diabetes [27], breast cancer[28] and nasopharyngeal carcinoma[29]. Some previous researchers have deduced that the reduced DNA repair capacity of mutant OGG1 genotypes may result from either the loss of a putative regulatory serine phosphorylation site or the introduction of a redox-sensitive cysteine amino acid at position 326[30]. The process of recognition and repair of 8-oxoguanine by OGG1 is well understood and reviewed [10], and it represents one of the oldest DNA base excision repair pathways, which constitutes a major area in studies of DNA repair mechanisms and led to the 2015 Nobel Prize in Chemistry. Metals workers with mutant OGG1 genotypes have higher urinary 8-OHdG content and TM as compared with wild-type genotypes.

Heavy metals, pesticides, diesel exhaust, tobacco smoke, polycyclic hydrocarbons, hormones, radioactivity, viruses, bacteria, and nutrients are known or suspected to influence epigenetic processes (Weinhold, 2006). Chemicals, drugs, ionising radiation, solar light, cigarette smoking, and air pollution can increase the production of reactive oxygen species (ROS). ROS is a product of metabolism and causes damage to DNA and other

macromolecules such as lipids and proteins. Oxidative DNA damage can lead to cancer, aging, and degenerative diseases (Halliwell, 2000).

Therefore, the concern is metals workers and petrol vendors who do not use any protective equipment, and there is high probability that they are exposed to PAHs during the activities. PAHs are considered as carcinogenic, mutagenic and immunosuppressive agents due to their interference with the normal functioning of cellular membrane and certain membrane-associated enzymes

- Exposure to PAHs causes the interaction of this genotoxicant with nuclear DNA, leading to the oxidative and genotoxic DNA damage.
- An oxidized form of guanosine, 8-OHdG, is a biomarker of oxidative DNA damage and is involved in the pathology of many diseases such as cancer, atherosclerosis, and diabetes
- Base excision DNA repair pathway recognizes 8-OXOdG as a threatening lesion, which results in its excretion in human urine without further metabolism
- Accordingly, estimation of urinary 8-OXOdG is increasingly used as a noninvasive biomonitoring approach to assess the oxidative DNA damage produced in the body
- Comet assay is applied to assess DNA damage/genotoxicity

Genetic variations in enzyme-encoding genes are involved in xenobiotic metabolism, and DNA repair may influence genotoxicity. The early effects of occupational workers exposure to distinct pollutant around the environment. Susceptibility to exogenous and endogenous genotoxicants may be modulated by genetic polymorphisms of DNA repair genes.

There are multiple DNA repair pathways for repairing various types of DNA damage. These repair pathways involve a number of proteins that play a role in protecting the genome from mutagenic effects. Single nucleotide polymorphisms in DNA repair genes have been identified and shown to

contribute to alteration in their DNA repair capacity. Therefore, genetic variations in DNA repair genes have been known to modulate the levels of DNA damage in peripheral blood lymphocytes of the workers exposed to PAH at workplace. OGG1 is a DNA repair polymorphic gene involved in the base excision repair pathway. Human OGG1 is a base excision DNA repair enzyme that participates in the removal of both 8-oxo-dG and 8-oxo-G.

## MATERIALS AND METHOD

The study included 77 individuals working in metal work and Petrol vendors from 24 work stations located in Gashua Senatorial zone.(Gashua, Nguru, Jakusko, Yusufari, Machina and Karasuwa), were defined as those involved in metal work and Petrol vendors with working hours more than seven hours per day. The control group was comprised of 79 individuals who were not exposed to PAH generated during metal and Petrol work, but the environmental exposure and socioeconomic status of the control population were matched with the exposed group. Control samples were collected from healthy volunteers of the same area of the exposed population but far from the metal and Petrol work. All the subjects enrolled in our study were of Gashua, Nguru, Machina, karasuwa, Jakusko and Yusufari, living in the rural and suburban area of the above area. A questionnaire was designed to assess the participants' demographic characteristics such as age, consumption habits (smoking, tobacco and drinking habits), and years of work exposure.

## SAMPLE COLLECTION

Sample collection Blood (approximately 5 ml) and urine samples were collected from metal workers and petrol vendors were exposed to smoke and control subjects with the help of a trained technician. All the exposed individuals had minimum of two years of work exposure. Blood and urine

samples of the subjects were gathered after the metal workers and petrol vendors finished their work shift.

**ESTIMATION OF URINARY**

1-OHP to assess PAH exposure in the exposed population, the level of 1-OHP in the urine samples of exposed and control subjects were analyzed by a former method, through GC/MS with minor modifications. 2 ml of the urine sample, 10 µl of β-glucuronidase/aryl sulfatase was added, and the sample was incubated at 37 °C for 3 h. The sample was extracted with 5.0 ml of ethyl acetate by mechanical shaking for 20 min. The solution was evaporated in a vacuum rotor to about 0.1 ml. The dry residue was dissolved in 50 µl of N Methyl-N-(trimethylsilyl) trifluoroacetamide, and the tubes were heated at 60 °C for 30 min. Subsequently, a 1-µl sample of the solution was injected into the GC system. The gas chromatograph used was an Agilent (U.S.) 7890 A with a splitless injector. The analytical column was a 30-m HP-5MS column (cross-linked 5% phenylmethyl silicon, 0.2 mm of column internal diameter × 0.25 µm column film thickness. Oven temperature program was set starting at 80 °C, held for 1 min, raised to 320 °C at 20 °C/min and held for 5 min. All mass spectra were obtained with an Agilent 5975 B instrument, and the ion source was operated in the selected ionization mode. Confirmation of the compound was completed by MS characteristic ions, and the ratio of MS characteristic ions and GC retention time was matched with the known standard compound. Estimation of urinary 8-OHdG Urinary 8-OHdG was evaluated with a competitive enzymatic immunoassay kit (Caymen chemical Company, Michigan, USA). The concentration of creatinine was measured spectrophotometerically as described before. Level of 8-OHdG was expressed relative to creatinine (ng/mg creatinine).

**RESULTS AND DISCUSSION**

Demographic characteristics This study was comprised of 77 PAH-exposed metal and petrol workers and 79 controls, and their demographic characteristics are shown in Table 1. The number of subjects and the mean

age in each age group in both control and exposed workers matched demographically. metal workers included in this study had demographically matched mean age i.e. smoking, alcohol consumption, and tobacco chewing, the distribution was found to be non-significant between the charcoal workers and control subjects. The metals and petrol workers under study were occupationally exposed to smoke for an average time of 7.99 ± 4.54 years. **Results of Estimation of urinary** 1-OHP in this study, the mean concentrations of urinary 1-OHP levels in both control and exposed subjects were found to be 25.59 ± 16.55 ng/mg this result has been reported in one of our published research article.

The 8-OHdG urinary concentration, a workers of oxidative DNA damage, was significantly higher ($p < 0.05$) in the exposed (geometric mean 12.33 ± 3.78; 6.33-24.25) than in the unexposed (geometric mean 7.36 ± 2.29; 3.46-15.20) populations (Table 2). Association between oxidative stress repair gene and DNA damage. The effects of genetic polymorphism of OGG1 gene on 8-OHdG content in both exposed and non-exposed population were investigated. Genetic variants of OGG1 in all the subjects were analyzed by PCR-RFLP. The subjects with the mutant OGG1 Ser326Cys genotype had significantly higher oxidative DNA.

**Table 1. Demographic characteristics of the control and exposed groups**

| Variables | Control (n = 79) | | Metal/Petrol vendor's workers (n =77) | |
|---|---|---|---|---|
| Age(y) | Sample of individual by age | Mean ± SD | Sample of individual by age | Mean ± SD |
| 10 – 20 | 5 (6.32) | 18.8 ± 0.84 | 7 (9.09) | 17.7 ± 1.25 |
| 21-30 | 21 (26.5) | 25.57 ± 2.75 | 23 (29.8) | 25.2 ± 2.84 |



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| | | | | |
|---|---|---|---|---|
| **31 -40** | 27 (34.1) | 32.63 ± 2.51 | 24 (31.6) | 36.3 ± 2.61 |
| **41 – 50** | 18 (22.7) | 45.22 ± 2.92 | 16 (20.7) | 45.5 ± 3.39 |
| **51 – 60** | 8 (3.41) | 54.38 ± 2.39 | 7 (9.09) | 54.3 ± 3.20 |
| Total | N - **79** Control | | N-**77** workers | |
| Smoking<br>**smoker**<br>**Non. Smoker** | <br>46 (58.2)<br>33 (48.1) | | <br>48 (62.3)<br>29 (37.7) | |
| Alcohol intake<br>**Alcoholic**<br>**Non – Alcoholic** | <br>41(51.9)<br>38 (48.1) | | <br>34 (44.2)<br>43 (55.8) | |
| Tobacco chewing<br>**Tobacco chewer**<br>**Non- Tobacco chewer** | <br>9 (11.4)<br>70 (88.6) | | <br>5(6.49)<br>72(93.5) | |
| Work exposure (y) | <br><10<br>10 -20<br>>20 | | <br>50(64.9)<br>25 (32.7)<br>2 (2.60) | 7.99 ± 4.54 5.25 ± 2.24 12.40 ± 1.96 21.50 ± 0.71 |

T- test was applied to compare the mean values of age between the control and exposed populations. Chi- square test was applied to test differences in age, consumption habits and work exposure experiences

damage as measured by urinary 8-OHdG levels, compared to the wild type of OGG1Ser326Ser genotype in exposed as well as non-exposed population ($p < 0.05$). The values of 8-OHdG were significantly higher in the exposed



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population compared to the controls (Table 2). The result for the effect of OGG1 polymorphism on TM content has been summarized in Table 3. The 8-OHdG and TM content were significantly higher in Cys/Cys OGG1 genotypes (mt/mt; 18.81 ± 3.34; 6.04 ± 0.52) as compared to Ser/Cys (wt/mt 12.82 ± 2.81; 5.40 ± 0.84) and Ser/Ser genotypes (wt/wt; 10.34 ± 2.25; 5.19 ± 0.78) in metal workers and petrol vendor's. The levels of both oxidative and genotoxic damage were significantly higher in metals workers than in the control population.

**Results** of linear regression analysis adjusted for demographic characteristics such as age, consumption habits, and exposure duration are shown in Table 4.

**Table 2. Levels of urinary 8-OHdG in the control and exposed populations**

| Metal worker of effect | Group | Mean ± SD | Minimum | Maximum |
|---|---|---|---|---|
| **Urinary 8-OHdG** | control | 7.36 ± 2.29 | 3.46 | 15.20 |
| **(ng/mg creatinine)** | Exposed | 12.33 ± 3.78* | 6.33 | 24.25 |

Mann-Whitney U test was applied to compare the mean values between the control and exposed groups. *Significant at $p < 0.05$

**Table 3. Effect of OGG1 genotype on urinary 8-OHdG content (ng/mg creatinine) and TM (µm)**

| Genotype | Control population (n = 79) | | | Metals workers (n = 77) | | |
|---|---|---|---|---|---|---|
| | N | Urinary 8-OHdG Content (mean ± SD) | TM content (mean ± SD) | N | Urinary 8-OHdG content (mean ± SD) | TM content content (mean ± SD) |
| wt/wt | 50 | 8.15 ± 2.50 | 1.40 ± 0.33 | 39 | 10.34 ± 2.25 | 5.19 ± 0.78 |
| wt/mt | 25 | 6.04 ± 0.93* | 1.33 ± 0.32 | 28 | 12.82 ± 2.81 | 5.40 ± 0.84 |
| mt/mt | 4 | 5.67 ± 0.62* | 1.01 ± 0.07* | 10 | 18.81 ± 3.34* | 6.04 ± 0.52* |

Multivariate analysis of covariance test was used to test the differences in the urinary 8-OHdG content; TM was adjusted for age, consumption habits,



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and exposure duration among the control and exposed groups. *Significant at $p < 0.05$.

**Table 4. Association of OGG1 genotype with the urinary 8-OHdG content (ng/mg creatinine) and TM (μm)**

| | Urinary 8-OHdG | | | TM (μm) | | |
|---|---|---|---|---|---|---|
| OGG1 polymorphism | $\beta^a$ | $R^2$ | $p^b$ value | $\beta^a$ | $R^2$ | $p^b$ value |
| Control group (n = 79) | 0.073 | 0.236 | 0.025* | 0.022 | 0.149 | 0.000* |
| Exposed group (n = 77) | -0.162 | 0.603 | 0.000* | -0.168 | 0.576 | 0.000* |

Regression analysis was used to test differences in the urinary 8-OHdG content and TM adjusted for age, consumption habits, and exposure duration. *values are significant. Unstandardized coefficient; b model p value.

These results imply that metals workers with mutant OGG1 genotypes are more susceptible to oxidative and genotoxic DNA damage. Since oxidative and genotoxic damage are important considerations in events leading to cancer after carcinogenic/ genotoxicant exposure, precautions should be taken by occupational workers. Accordingly, there is a need to inform these workers about the potential hazards of occupational exposure and should always be provided with appropriate personal protective equipment. The outcomes of the present study show that there is a significant association of OGG1 heterozygous (wt/mt) and homozygous (mt/mt) variants with the oxidative and genetic damage as assessed by 8-OHdG level and TM content, respectively in the studied populations well as the environment. These findings suggest that metal workers with mutant OGG1 genotypes are more susceptible to the oxidative and genotoxic DNA damage. However, further studies should be conducted on the DNA repair gene expression and DNA repair capacity with a large sample size.


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## CONCLUSION:

We found a significant association of OGG1 heterozygous (wt/mt) and homozygous (mt/mt) variants with oxidative and genotoxic damage, suggesting that these polymorphisms may modulate the effects of PAH exposure in occupational workers which were exposes to environmental pollution within Gashua Senatorial Zone.

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