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A Bio-assessment of DNA damage by Alkaline Comet Assay in metal workers of Kano metropolis, Nigeria

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ABSTRACT

Metallic work is one of the widespread economic activities in urban Kano. Little or no attention is usually directed at occupational health risk by local or state authorities in Kano. The present work was aimed at the evaluation of DNA damage in metal workers by Alkaline Comet Assay in blood lymphocytes. The results showed that there was significant difference statistically between the level of DNA damage in blood lymphocytes of metal workers and control group ($p < 0.05$). In addition, the level of damage to DNA in blood of subjects with long term exposure and old age is of serious concern. There is the need to monitor occupational activities that can pose serious health risks. The relative ignorance of the metal workers about the health risks they are exposed to as well as the public should be addressed.

1. Introduction

Adverse health effects have resulted by the release of airborne particles into the environment through metal works. Higher incidence of pneumonitis, bronchitis and metal fume fever were reported by metal workers that were permanently exposed to high concentrations of metal fumes [1].

High risk of lung cancer has been associated with occupational exposures to metal fumes. However, the causal relationship is affected by the lack of a clear dose–response and exposure to other agents [2]. Effective biological survey of metal workers is imperative to determine the genotoxicity and carcinogenicity of exposure. Several studies have reported that contamination by heavy metals have induced chromosome/genome mutations and DNA damage using the comet and the cytokinesis-block micronucleus (CBMN) assays [3–7].

Metallic work is one of the widespread economic activities in urban Kano. Most of the workshops are situated either in open spaces or relatively enclosed setting so as to attract more customers. As such, the fumes and the dusts are released into the environment (atmosphere) while the workers are exposed directly to most of the metal dust/fume pollutants. The metal fumes produced in the process of welding composed of at least 13 kinds of metals, including manganese (Mn), beryllium (Be), cadmium (Cd), chromium (Cr) cobalt (Cu), iron (Fe), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), zinc (Zn), antimony (Sb), and vanadium (V) [8]. The chemicals contained in these fumes and gases depend on several factors including; type of welding being performed, material of the electrode, type of metal being welded, presence of coatings on the metal, duration and severity of exposure and ventilation [9]. In addition, the workers only wear glasses to prevent light sparkles from having contact with their eyes and often wore hand gloves as the only protective measure. It is very clear that these workers are excessively and persistently exposed to the contaminants through their skin or accumulate in crevices of the hands and perhaps also from food and drink contamination at the work premises. The aim of the study was to assess the extent of DNA damage in blood lymphocytes of metal workers in Kano metropolis through identifying the presence of DNA damage in blood lymphocytes of the metal workers and control groups.

2. Materials and methods

2.1. Study area

The study area was Kano state which was located at the northern part of Nigeria. Kano metropolitan encompasses eight local governments. The sampling sites where along both Jakara and Gabari road all located at Kano Municipal.

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2.2. Experimental design

Metal workers (n = 60) were sampled through random sampling from two major sites within Kano metropolis: Jakara and Gabari roads. The control group (n = 20) were selected randomly from the population whose occupations have no relation and history of exposure to metal fumes. However, they were exposed to traffic pollution just like the metal workers but not to metal fumes. Control groups do not differ from metal workers in gender, age and smoking habits [10].

All subjects were informed of the objective of the study and their consent was obtained. Ethical clearance from ethical committee of the state ministry of health was also obtained.

2.3. Study plan

- Selection of test subject, control group and relevant biographical information.
- Designing a questionnaire which provided valuable information on age, years of exposure, health history and working conditions of the metal workers.
- Laboratory analysis which involved DNA damage detection in blood lymphocytes by Alkaline Comet Assay and was conducted at Centre for Biotechnology Research and Training, Ahmadu Bello University, Zaria.

2.3.1. Questionnaire (Design and administration of structured questionnaire)

An interviewer questionnaire was used for general population studies [11]. Data on sex, age, and social habits (e.g. smoking and alcohol consumption) were collected. The questionnaire also included data on medical history and years of exposure, daily working hours.

2.3.2. Blood samples collection

Blood samples were collected from test subjects in the morning around 7 and 8 am. 5 mL of blood samples were collected through venipuncture. Samples were collected in a royal blue EDTA tube. The collection site on the subject body was washed with soap and water, followed by alcohol swab. Blood was collected by venipuncture using a phlebotomy needle. EDTA tube was inverted about 8–10 times to prevent clotting. Each specimen tube was attached with an identification label. Specimens were stored at 4 °C [12].

2.3.3. Alkaline comet assay

Comet assay was performed, according to [13,14]. 750 μL of whole blood was added to the same volume of freezing mixtures (10% DMSO and RPMI 1640) in a clear 2.0 mL microcentrifuge tube and inverted several times to ensure complete mixing. It was then transferred to a ziplock pack and then placed in a −80 °C freezer to be step frozen at the rate of −1 °C/min. 10 μL of single cells suspensions were mixed with 75 μL of 0.5% (w/v) low melting point agarose at 37 °C and layered on 0.5% (w/v) normal agarose precoated on frosted slides. The slides were covered with coverslips and placed at 4 °C for 10 min to allow the agarose to solidify. Coverslips were carefully removed and the slides were covered with a layer of low melting point agarose and placed at 4 °C for another 10 min. The slides were then placed in a freshly prepared alkaline lysis solution (100 mM EDTA, 2.5 M NaCl, 10 mM Tris, 1% v/v Triton X-100 and 10% DMSO, pH = 10) at 4 °C for 1 h. After lysing, slides were incubated for 20 min in a horizontal electrophoresis tank with alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA, pH 13) at 4 °C for DNA to unwind before electrophoresis. Samples were electrophoresed for 20 min at 300 mA, 25 V at the same temperature. After the removal of slides from the solution, slides were then dried with filter paper and neutralize using neutralization buffer (0.4 M HCl, pH 7.5) for 5mins 3 times. Subsequently, cells were stained with ethidium bromide (30 μg/mL) for at least 20 min and slides were covered with cover slips and stored in a dark humidified chamber until analysis. All procedures were performed under dimmed light to prevent additional DNA damage. Cells were observed at a magnification of 40 × by a fluorescence microscope with green light excitation and a 590 nm barrier filter. DNA damage was calculated by randomly counting tailing DNA in 50 cells/sample. Classification of comets was five damage levels according to Tail DNA%, including grade 0, 1, 2, 3 and 4. Grade 0 was defined as no DNA damage and grade 4 was the most serious DNA damage.

Table 1
Profile and health history of sampled population in Kano metropolis.

Characteristics	Metal workers (n = 60)	Control group (n = 20)
Age (Yrs)	32.7 ± 12.5	25.4 ± 5.40
Exposure (Yrs)	15.4 ± 11.9	0
Protective devices	Sun glasses (100%)	None

Table 2
Level and extent of DNA damage among various age groups of metal workers in Kano metropolis, 2015.

Age groups(Yrs)	DNA damage (abitrary units)
15–25	5.14*
26–35	5.91*
36–45	9.60*
>45	16.87*

* $p \leq 0.05$ = positive correlation with age.

Table 3
Level and extent of DNA damage at various exposure rates in metal workers of Kano metropolis, 2015.

Exposure(Yrs)	DNA damage (Arbitrary units)
0–10	4.12*
11–20	7.36*
21–30	13.40*
>30	15.25*

* $p \leq 0.05$ = positive correlation with exposure.

2.3.4. Data analysis

Data analysis was performed using Sigma Stat 3.5 statistical software for Windows as follows: The extent of DNA damage was compared between metal workers and control groups using *t*-test. Pearson product moment correlation analysis was carried out to check whether there is a relationship between different variables. Statistical significance was defined as $p \leq 0.05$.

3. Results

3.1. Discussion

The respondents did not provide any precautionary measures taken other than sun glasses as presented in Table 1. Perhaps their understanding is that sun glasses shield the eyes from the intensity of the light but do not serve adequate protection from fumes and dusts of metals going into the eyes.

The extent of DNA damage at various age groups was shown in Table 2 for the metal workers and in Table 4 for the control group. Table 3 showed the extent of DNA damage at various exposure rates among metal workers.

The difference in level and extent of DNA damage caused between the metal workers and control groups was also determined by *t*-test. There was a significant difference in level of DNA

Table 4
Level and extent of DNA damage among various age groups of control group in Kano metropolis, 2015.

Age groups (Yrs)	DNA damage** (Arbitrary units)
15–25	4.58**
26–35	4.20**
36–45	5.00**

** $p > 0.05$ = no correlation with age.

damage between test subjects and control groups ($p < 0.05$). The induction of DNA damage might be as a result of exposure to metal fumes which consist of several heavy metals. The metal workers were likely exposed to higher levels of traffic air pollution than the control subjects, so the Comet effects reported here could at least partly be attributable to traffic exposure in addition to metal fumes [15]. Aluminium and zinc might produce DNA strand breaks via the oxidative stress induced by metal-fume fever [16,17].

The fumes from industrial stainless steel welding processes also increased chromosomal aberrations in 11 welders [18]. As part of a large study on tannery workers, [19] reported that five welders had a higher frequency of DNA-protein crosslinks than the control group. However, probably due to the small number of welders involved in this study, the authors reported no increase in the frequency of micronuclei for these individuals. In contrast, [4] showed that 102 welders had an increased frequency of micronuclei in epithelial buccal cells. Furthermore, elevated levels of DNA damage were reported in nucleated peripheral cells using the Comet assay.

Oxidized forms of chromium and nickel, which also are present in welding fumes, are genotoxic [20,21]. Other metals that may be present in welding fumes, and that could induce DNA damage, include lead [22], manganese [23], cadmium [24] and cobalt [25].

Pearson product moment correlation was performed to determine significant relationship between variables.

However, There was a relationship between age and DNA damages ($p < 0.05$) as shown in Table 2. Similarly, there was a relationship between years of exposure and DNA damage ($p < 0.05$) as shown in Table 3. [3,4] reported that DNA damage was higher in subjects with longer duration of exposure ($P < 0.05$). A related study by [26] on genotoxic exposure among welders in Copenhagen found that chromosomal aberrations and sister chromatid exchanges had increased significantly with age. A recent study confirmed the effect of exposure to welding fumes on micronucleus frequency, although the target cells in this study were binucleated lymphocytes [6].

3.2. Conclusion

Based on the findings of the study, it was concluded that metal workers of urban Kano have some level of DNA damage. The age and years of exposure of the metal workers had affected the extent of the damage. Metal works as currently practiced in urban Kano should be closely monitored by relevant environmental protection agencies such as National Environmental Standards and Regulations Enforcement Agency (NESREA), Federal Environmental Protection Agency (FEPA) and Ministry of Environment etc. Further studies are required on genotoxicity of metals from a wider sampled population to generate the necessary data for better understanding of this phenomenon.

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