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# Research Article
# Changes in the Haematological Profile and Frequency of Nuclear Abnormalities in Exfoliated Buccal Cells of Automobile Welders in Enugu, Nigeria

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## Abstract
**Background and Objectives:** Welders are often occupationally exposed to potentially harmful substances. This study assessed the haematological profile and the frequency buccal cell nuclear abnormalities of automobile welders who use calcium carbide as a source of fuel in Enugu, Nigeria. **Materials and Methods:** A total of 50 participants (30 automobile welders and 20 controls) were recruited for the study. Questionnaires were used to obtain participant's bio data and exposure characteristics. Haematological parameters were measured and assessment of buccal cell nuclear abnormalities was done by cytological evaluation of participants' the buccal cells. **Results:** The welders were observed to had significantly lower total red blood cells and haemoglobin concentration. They also had significantly higher micronuclei frequency compared to the control group. Further analysis showed that number of years spent in the occupation, alcohol consumption and ongoing medications significantly affected the frequency of micronuclei among the welders. **Conclusion:** Automobile welders may be exposed to toxic substances which may cause increased frequency of micronuclei and reduced red cell count and haemoglobin concentration.

Key words: Calcium carbide welding, nuclear abnormalities, micronuclei, automobile welders, haematological changes

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Repeated chronic exposure to low levels of harmful factors at work has been found to be related to the incidence and occurrence of many diseases[1]. Welders are a group of semiskilled and skilled workers who join and cut metal parts using flame, electric arc or other heat sources with over five million people estimated to be either part time of full time welders[2,3]. Welders in the non-industrial settings are mostly involved in the automobile repairs and real-estate construction[4].

Welding exposes workers to a potentially harmful factors such as radiation, heat, high noise levels, gases and fumes via various routes[5]. The composition of the resultant gases and fumes depends on the welding type and the substrate being welded[6]. Welding fumes have been classified by The International Agency for Research on Cancer (IARC) as a possible human carcinogen[7]. Use of Personal Protective Equipment (PPE) including overalls, gloves, goggles and boots is known to minimize exposure and health risk. Adherence to such safety measures and practices such as adequate use of PPE among welders is vital in checkmating health hazards associated with these occupational exposures[8]. Several studies carried out in different parts of Nigeria however show that even though majority of welders in the area were aware of the welding hazards, only a third of them used some form of protection against these hazards[1,9,10]. A similar study carried out in Enugu also observed a wide gap between awareness rate and possession rate of PPE among welders and that electric welding was more significantly associated with use of PPE than carbide welding[11]. Due to the unstable nature of power supply, most automobile welders in Nigeria mainly use acetylene gas generated from the reaction of calcium carbide with water[12].

Pure calcium carbide is colorless though most of the industrially produced impure ones are black or grayish-white in colour[13]. Crude calcium carbide often contains arsenic, phosphorus and sulphur impurities and therefore its reaction with water yields, in addition to acetylene: arsine, phosphine, ammonia, hydrogen sulphide and related compounds[14,15]. Recent evidence suggested that arsenic may be a genotoxic carcinogen[16] and that chronic exposure to inorganic arsenic may lead to many cancer types including lung, skin, liver, kidney and urinary bladder cancers[17]. Arsenic has also been reported to increase the incidence of certain cancers in experimental animals[18]. Also, fruits ripened using calcium carbide have been shown to cause adverse haematological and biochemical effects in experimental animals[13,19].

Automobile welders in developing countries like Nigeria are constrained by paucity of funds to rely solely on acetylene generated *in situ* from the reaction of calcium carbide and water. Published evidence in a study that assessed the effects of chronic exposure to crude acetylene on some biochemical and haematological indices in New Zealand white rabbits shows that the treatment caused a significant elevation of aspartate and alanine transaminases in the plasma of the test rabbits relative to controls[14] suggesting that industrial acetylene produced from technical-grade calcium carbide may have deleterious effects on vital organs and blood constituents.

Since most welders in Nigeria who use calcium carbide as fuel source rarely use PPE and are thus potentially exposed to toxic compounds during the welding process, this study was designed to assess the haematological profile and frequency of nuclear abnormalities in the buccal cells of automobile welders who use calcium carbide as a source of fuel for welding.

## MATERIALS AND METHODS

**Ethical considerations:** This study was conducted after obtaining an ethical clearance from the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla with approval number NHREC/05/2008B-FWA00002458-1RB00002323. Prior to recruitment, the details of the procedures involved in the study were explained to the participants. Those who gave their approval by signing an informed consent form were recruited into the study.

**Study area and design:** The study was conducted in Enugu, the capital city of Enugu state, Nigeria and lasted for 5 months from March, 2017 to July, 2017. A total of 50 participants were involved in this study; 30 of them were automobile mechanic welders in Enugu metropolis who used calcium carbide as a source of fuel for welding and 20 control subjects who were not welders. The study adopted a case-controlled, cross-sectional approach.

**Study participants and data collection:** Only apparently healthy subjects aged between 18 and 55 years were recruited. The test group consisted only of automobile welders who used calcium carbide as a source of fuel for welding. The control group was selected from the general population and were individuals who were not welders. Individuals who reported having a chronic disease or being exposed to X ray 12 months before the sampling were excluded from the study.

Also, any individual with obvious oral lesion were not recruited. A structured questionnaire was administered for the purpose of obtaining relevant information on demographics and lifestyle (age, smoking and alcohol intake, medication) and occupational exposure characteristics (number of years in occupation, frequency of exposure and use of PPE).

**Specimen collection and analysis**

**Full blood count:** About 5 mL of blood was collected aseptically from the antecubital vein of each participant by venepuncture. The samples were then dispensed into commercially prepared ethylenediaminetetraacetic acid (EDTA) plastic containers and were dispatched to the laboratory where they were immediately used for the full blood count using an automated haematological analyzer (Mindray BC-5300, China).

**Buccal cell cytology:** Prior to collection of buccal smears, participants were advised to rinse their mouth thoroughly with water. Exfoliated buccal cells were obtained by gently scrapping the inside of both cheeks with a wooden spatula. Once collected the exfoliated cells were smeared immediately on clean grease free microscope slides. Smears were fixed in 95% ethanol, stained using the Giemsa staining technique and allowed to air dry prior to microscopy[20].

To minimize bias, the slides were evaluated independently under the microscope by two individuals who had experience with micronuclei counts and identification of nuclear abnormalities using the X 40 objective. A minimum of 1000 buccal cells per participant were scored. The counting of cells was done according to the criteria developed by Tolbert *et al.*[21]. Out of the 1000 cells counted the number of cells with micronuclei (CMN), total number of micronuclei (TMN) and other nuclear abnormalities such as nuclear buds (NB), bi-nucleation (BN) and karyorrhexis (KH) were scored.

**Statistical analysis:** Data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS). Results obtained were expressed as mean±standard deviation. The Students t-test was used to compare haematological parameters and nuclear abnormalities among test and control groups. Mann-Whitney U and Kruskal-Wallis tests were used to assess differences in nuclear abnormalities based on demographic and occupational characteristics. The level of significance was set at $p<0.05$.

## RESULTS

**Effect of exposure on haematological parameters:** The haematological parameters were compared between the control subjects and automobile welders (Table 1). Of all parameters measured, only the red blood cell count (RBC) and haemoglobin concentration (Hb) were observed to be significantly lower among the automobile welders than the controls ($p = 0.003$ and $p = 0.004$, respectively).

**Effect of exposure on buccal cell nuclei:** Indices of nuclear damage assessed in the buccal cells of participants in the study were CMN, TMN, BN, NB and KH. Table 2 compared the mean values of the nuclear abnormalities observed among the control and exposed automobile welders. The automobile welders were found to have significantly higher CMN ($p = 0.00$) and TMN ($p = 0.00$). They also had a higher number of bi-nucleated cells and cells with nuclear buds, though not statistically significant. The control subjects were however found to have a significantly higher number of karyorrhectic cells ($p = 0.01$) than the automobile welders.

**Variation of nuclear abnormalities based on demographic, lifestyle and exposure characteristics:** The automobile welders were further sub-divided into groups according to age, occupational exposure factors and lifestyle to assess the

Table 1: Comparison between the haematological parameters of the control subjects and automobile welders

| Groups | WBC total ($X10^9$) | Lymphocytes ($X10^9$) | Monocytes ($X10^9$) | Granulocytes ($X10^9$) | Hb ($g\ dL^{-1}$) | RBC ($X10^{12}$) | Haematocrit (%) | Platelet count ($X10^9$) |
|---|---|---|---|---|---|---|---|---|
| Controls | 5.89±1.22 | 2.74±0.65 | 0.56±0.28 | 2.60±0.78 | 14.48±1.47 | 5.67±0.77 | 45.39±4.54 | 240.44±59.77 |
| Welders | 5.96±1.60 | 2.78±0.89 | 0.54±0.36 | 2.64±0.72 | 13.05±1.30* | 5.00±5.62* | 45.12±4.10 | 213.00±67.07 |
| p-value | 0.46 | 0.25 | 0.10 | 0.24 | 0.003 | 0.004 | 1.41 | 20.58 |

Values are presented as Mean±SD, *Significantly different from control with significance set at $p<0.05$, WBC: White blood cells, Hb: Haemoglobin, RBC: Red blood cells

Table 2: A comparison of the frequency of buccal cell nuclear abnormalities between control subjects and automobile welders

| Groups | CMN | TMN | BN | NB | KH |
|---|---|---|---|---|---|
| Control | 4.10±3.74 | 5.15±4.53 | 15.45±5.87 | 0.75±0.79 | 4.65±4.72 |
| Welders | 15.90±5.62* | 26.37±12.70* | 32.80±9.65 | 1.27±0.91 | 2.27±1.11* |
| p-value | 0.00 | 0.00 | 2.20 | 0.24 | 0.01 |

Values are presented as Mean±SD, *Significantly different from control with significance set at $p<0.05$, CMN: Cells with micronuclei, TMN: Total number of micronuclei, BN: Binucleate cells, NB: Nuclear buds and KH: Karyorrhectic nuclei

Table 3: Frequency of buccal cell nuclear abnormalities in the automobile welders by demographic and exposure variables

| | | Nuclear abnormalities | | | | |
|---|---|---|---|---|---|---|
| Characteristics | N | CMN | TMN | BN | NB | KH |
| **Age** | | | | | | |
| 18-30 | 4 | 13.75±8.18 | 22.00±16.06 | 28.75±11.64 | 0.50±0.58 | 2.25±0.96 |
| 31-42 | 14 | 14.64±5.00 | 24.71±12.20 | 35.93±8.14 | 1.36±0.93 | 1.93±0.83 |
| 43-54 | 6 | 19.33±2.80 | 29.00±7.95 | 28.17±13.75 | 1.00±0.63 | 2.67±1.63 |
| Above 54 | 6 | 16.83±6.85 | 30.50±16.68 | 32.83±5.60 | 1.83±0.98 | 2.67±1.21 |
| p-value | | 0.204 | 0.607 | 0.529 | 0.097 | 0.545 |
| **Time in occupation** | | | | | | |
| 1-3 years | 2 | 5.50±2.12 | 7.00±0.00 | 18.50±9.19 | 1.00±0.00 | 2.00±0.00 |
| 4-9 years | 2 | 12.50±3.53 | 22.00±11.31 | 42.50±9.19 | 1.50±0.71 | 1.50±0.71 |
| Above 10 years | 26 | 16.96±5.02* | 28.19±12.12 | 33.15±8.79 | 1.27±0.96 | 2.35±1.16 |
| p-value | | 0.035 | 0.067 | 0.051 | 0.819 | 0.553 |
| **Frequency of exposure** | | | | | | |
| Weekly | 4 | 15.00±9.87 | 27.00±19.37 | 29.25±11.87 | 1.50±0.58 | 1.50±0.58 |
| Daily | 26 | 16.04±4.98 | 26.27±11.91 | 33.35±9.42 | 1.23±0.95 | 2.38±1.13 |
| p-value | | 0.746 | 0.930 | 0.837 | 0.536 | 0.157 |
| **Use of protection** | | | | | | |
| No | 8 | 13.00±5.37 | 22.00±14.84 | 35.25±7.07 | 1.13±0.64 | 2.13±0.83 |
| Yes | 22 | 16.95±5.45 | 27.95±11.80 | 31.91±10.43 | 1.32±0.99 | 2.32±1.21 |
| p-value | | 0.078 | 0.256 | 0.368 | 0.696 | 0.836 |
| **On-going medication** | | | | | | |
| No | 28 | 15.18±5.08 | 25.00±12.00 | 32.61±9.95 | 1.29±0.90 | 2.29±1.12 |
| Yes | 2 | 26.00±0.00# | 45.50±0.71# | 35.50±3.54 | 1.00±1.41 | 2.00±1.41 |
| p-value | | 0.005 | 0.005 | 0.556 | 0.777 | 0.777 |
| **Smoking habit** | | | | | | |
| Non-smoker | 27 | 15.30±5.61 | 24.59±12.12 | 32.70±10.18 | 1.30±0.95 | 2.11±1.01 |
| Occasional smoker | 2 | 21.50±0.71 | 41.50±2.12 | 33.00±1.41 | 1.00±0.00 | 4.00±1.41 |
| Moderate smoker | 1 | 21.00±0.00 | 44.00±0.00 | 35.00±0.00 | 1.00±0.00 | 3.00±0.00 |
| p-value | | 0.113 | 0.120 | 0.898 | 0.860 | 0.115 |
| **Alcohol consumption** | | | | | | |
| Non-consumer | 5 | 10.40±6.02 | 17.60±14.43 | 28.40±10.16 | 0.80±0.45 | 1.80±0.84 |
| Moderate consumer | 6 | 15.00±6.81 | 22.17±16.77 | 33.00±4.86 | 1.67±0.52 | 2.00±0.89 |
| Regular consumer | 19 | 17.63±4.26* | 30.00±9.70 | 33.89±10.67 | 1.26±1.05 | 2.47±1.22 |
| p-value | | 0.031 | 0.074 | 0.746 | 0.204 | 0.456 |

Values are presented as Mean±SD, *p<0.05 when compared to other groups using the Kruskal-Wallis test, #p<0.05 when compare to other group using the Mann Whitney U test

influence of these factors on observed nuclear abnormalities (Table 3). Analysis showed that age of the welders did not significantly affect the frequency of nuclear abnormalities among the welders. Welders who had spent above 10 years on the job were found to have a significantly higher CMN (p = 0.035). Frequency of exposure, use of PPE and smoking did not significantly affect the distribution of nuclear abnormalities. However those who reported being on medication had significantly higher CMN (p = 0.005) and TMN (p = 0.005). Regular consumers of alcohol also had a higher CMN than moderate consumers and non-consumers.

## DISCUSSION

In this study the haematological parameters and frequency of nuclear abnormalities in the buccal cells of automobile welders who use calcium carbide as fuel source were assessed. Both group of parameters were compared with control subjectswho were not welders.

The result of the haematological assay showed a significant decrease in the red blood cell count of welders compared to controls. Arsenic is a known impurity in commercial grade calcium carbide[14]. Anaemia is a known effect of chronic exposure to arsenic which may be due to direct cytotoxic effects on the red blood cells and a suppression of erythropoiesis. The production of toxic gases such as arsine, phosphine and carbon monoxide as part of the combustion process in the oxyacetylene welding process may have also played a role. The effects of arsine in humans have been shown to include rapid destruction of red blood cells by damage to their cell membranes with subsequent hemolysis[22,23].

The result of the buccal cell cytologyshowed that the frequency of micronuclei was higher in the buccal cells of automobile welders than in controls. The higher micronuclei count observed in the automobile welders may be attributed to several factors. Firstly, welding fumes-containing metallic nano-particles may be produced during the welding process[5]. These ultrafine particles usually constitute of metallic compounds such as iron oxide, lead, nickel and zinc oxide among others depending on the type of metal being welded. Some of these metals and their oxides are known carcinogens and can easily permeate the respiratory system rapidly due to their small size[10]. These fumes are known to induce oxidative stress which is one of the major indirect causes of genetic damage initially seen as micronuclei in certain cases[24-27]. Secondly, arsenic, when present as an impurity may interfere with the DNA repair processes thus either directly inducing DNA damage or amplifying mutagenic properties of other toxicants which may be present in welding fumes[17].

These results are consistent with the findings in a similar study on metal arc welders[28]. It is however contradictory to a study on welders in Mexico using the micronucleus test on buccal cells, in which the micronuclei count did not differ significantly between the welders and the control group even though they observed significantly higher number of other nuclear abnormalities such as binucleate cells and condensed-chromatin cells in the welders compared to controls[29].

The control participants were however observed to have a significantly higher number of karyorrhectic buccal cells when compared with the automobile welders. Karyorrhexis is one of the degenerative cellular manifestations seen in apoptosis-a protective mechanism against cancer. This process is often initiated to eliminate cells with irreparable genetic damage[30]. The reason for this pattern of result is not understood and it may be possible that some other factors unaccounted for in this study may have played a role.

Several occupational exposure and lifestyle characteristics were considered in the analysis of the findings. CMN was observed to increase significantly with the number of years in the occupation, on-going medication and also with alcohol consumption among the automobile welders. Most direct and indirect genotoxic substances often require a chronic exposure to induce their damaging effects hence the observed pattern[31]. Alcohol consumption is a known lifestyle factor which predisposes to DNA damage and possibly increasing the frequency of micronuclei in buccal cells[32].

An increase in frequency of micronuclei among automobile welders who were on medication was observed. It is known that certain medications like anti-neoplastic drugs may be genotoxic[33]. However, since we did not collect data on the specific medications which the participants were taking, it may be difficult to explain the observed increase in micronuclei frequency.

## CONCLUSION

It may be concluded that automobile welders who use carbide as a source of fuel for welding may be exposed to toxic substances which may have given rise to the observedlower red blood cell count, haemoglobin concentration and higher frequency of micronuclei among automobile welders when compared to controls. These findings highlights the need for such automobile welders to obtain and use relevant PPE to guard against undue exposure to potentially toxic substances at their work places.

## SIGNIFICANCE STATEMENT

This study presents data showing that automobile welders, especially those using calcium carbide as source of fuel may occupationally exposed to toxic substances which may have causedhaematotoxic and genotoxic changes seen as reduced haemoglobin concentration and red blood cell counts and increased frequency of micronuclei. The study also highlights relevant demographic and occupational exposure characteristics which play significant roles in the observed changes. The findings are relevant because it highlights the necessity of bio monitoring of unskilled, semi-skilled workers and artisans who are often not properly protected from toxic substances in the course of their work. This study will also serve as basis for further studies aimed at evaluating overall occupational health of welders and other artisans and driving advocacies for ensuring that welders use protective apparel during the course of their work.

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