Frequency of buccal cell micronuclei in abattoir workers exposed to smoke from singeing animal hide in Enugu, South East Nigeria

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ABSTRACT

Many abattoir workers are frequently exposed to smoke emanating from singeing animal hides which may give rise to genetic damage in such individuals. The study was designed to evaluate the frequency of micronuclei in buccal cells of abattoir workers exposed to smoke from singeing animal hides. A total of 60 consenting individuals participated in the study -- 30 of them were abattoir workers directly exposed to smoke, the other thirty were control subjects who are not directly exposed to smoke. Buccal samples were collected from all participants, stained using the Giemsa technique and evaluated for the presence of micronuclei to ascertain the total number of cells with micronuclei (CMN). Results obtained showed that abattoir workers directly exposed to smoke from singeing animal hides had significantly higher CMN (p < 0.001) and were up to 4 times more likely to have micronuclei in their buccal cells when compared to control subjects. It was also observed that those workers exposed to the smoke on a daily basis had a higher number of micronuclei in their buccal cells than others who were less frequently exposed. In conclusion exposure to smoke from singeing animal hides may be genotoxic.

Keywords: micronuclei, genotoxicity, occupational health

1. INTRODUCTION

Smoke is generally regarded as being potentially mutagenic and is thought to be responsible for inducing chromosomal changes which sometimes present as micronuclei [1]. It is generally known that there is an increase in the frequency of micronuclei during the very early stages of carcinogenesis especially in the oral mucosa [2,3]. The use of micronuclei in exfoliated buccal cells as a biomarker presents an easy and minimally invasive method for biomonitoring of human exposure to genotoxic environmental pollutants. Micronuclei are whole chromosomes or fragments of chromosomes lagging behind at the anaphase stage in mitosis [4]. An increased number of these micronuclei in buccal cells is indicative of genetic damage [5]. Abattoirs and slaughter houses in Nigeria are known to impact negatively on their surrounding environments. Factors such as poor location, unhygienic practices and improper waste management leading to environmental pollution are responsible for most of the health risks posed by these facilities [6]. Smoke emanating from singeing the slaughtered animals’ skin (a popular method of meat processing in Nigeria) is one of the major sources of health risk to the abattoir environment [7], including the abattoir workers themselves. Many of the abattoir workers do not use any form of protection when working and are often exposed to smoke from singeing the animal hide hence exposing themselves to various health risks known and unknown [8].

For this reason, this study was designed to evaluate the likelihood of genetic damage in abattoir workers who are directly exposed to smoke from singeing animal hides using buccal cell micronucleus frequency as a biomarker.
2. MATERIAL AND METHODS

2.1 Ethical considerations

The study was approved by the University of Nigeria Teaching Hospital Health Research Ethics Committee. Informed consent were obtained from all individuals who participated in the study.

2.2 Participants

Participants for the study were recruited from abattoirs in Enugu metropolis, South East Nigeria. A total of sixty individuals were randomly selected for the study. Thirty (30) of the participants were abattoir workers who are directly exposed to smoke from singeing animal skin. The other 30 participants neither work in the abattoir nor are directly exposed to smoke singeing animal hides. A structured questionnaire was used to collect demographic data, smoking habit and occupational exposure characteristics of the participants selected for the study.

2.2.1 Inclusion criteria

Consenting adult males aged 18 to 65 years who are apparently healthy with no oral lesions and who have worked in an abattoir for at least six months were included in the study.

2.2.2 Exclusion criteria

Individuals outside the age range of 18 to 65 years, individuals with oral lesions and those who had been exposed to X ray radiations in the past one year were not recruited. Also individuals who did not give consent were excluded from the study.

2.3 Sample collection, staining and evaluation

Prior to sample collection, the participants were asked to rinse their mouth with water. The inner part of both cheeks were scrapped gently with a wooden spatula and was used to make a smooth smear on appropriately labeled grease free slide. The slides were allowed to air dry and immediately fixed in 95 ethanol. The fixed slides were stained with Giemsa stain diluted with phosphate buffer in the ratio of 1:9 for 15min. The stained slides were allowed to air dry and viewed under microscope to identify and record the micronuclei (MN) count. At least 1000 intact buccal epithelial cells per individual were examined and the number of cells with micronuclei recorded. Cells with micronuclei (CMN) were defined as the number of cells containing MN per 1000 cells per subject. Care was taken while counting to avoid overlapping of the field of view and repeated counting of the same cells. The counting of MN was done by two independent observers who evaluated the slides twice at different intervals to minimize intra and inter observer bias.

2.4 Statistical analysis

Statistical analyses were performed using statistical package for social sciences version 20.0. T-test of variation was used to determine the differences in distribution of CMN between both populations. Mann-Whitney U and Kruskal-Wallis tests were used to assess differences in CMN based on occupational characteristics. All statistical tests were two sided using 0.05 alpha level of significance.

3. RESULTS AND DISCUSSION

3.1 Distribution of micronuclei frequency among abattoir workers and control subjects

The study showed that out of the 30 abattoir workers occupationally exposed to smoke from singeing animal hides, 18 of them had micronucleated buccal cells (Figure 1). After statistical analysis, we found that those exposed to the smoke were about 4.5 times more likely to have micronuclei in their buccal cells than the control individuals who are not occupationally exposed (RR = 4.50, 95% C.I.: 1.726 to 11.73). The participants occupationally exposed to the smoke had a significantly higher ($p = 0.0001$) number of buccal cells with micronuclei (1.43 ± 1.38) when compared to the unexposed control participants (0.17 ± 0.46) as shown in Figure 2.

These findings revealed that buccal cells of abattoir workers exposed to this smoke expressed micronuclei up to eight times more than the buccal cells of control participants. This increase was also found to be associated with the frequency of exposure to this smoke. El-Setouhy et al [2] reported a similar finding when he found that water pipe smokers had a two fold increase in the frequency of micronuclei when compared with non-smokers.

Abattoir workers in Nigeria prefer the use old tyres and wood as the major source of fuel for burning the hide of animal carcass. It is possible that combustion mixtures and particulate matter arising from either fuel sources play roles in the increased genetic damage expressed as micronuclei in the buccal cells of exposed abattoir workers.

Leon-Meija et al [9] is of the opinion that genotoxic changes due to occupational exposure to genotoxic substances may not be as result of a single substance, but may indeed be due to a complex mixture of elements. This may be the case in the present study because it is difficult to say with a degree of certainty, the particular substance causing the observed genotoxic changes among the exposed participants. Such particles and chemicals as metals, carbon monoxide, sulphur dioxide, benzene, phenols, styrene and other polycyclic aromatic hydrocarbons (PAH) are all produced by incomplete burning of organic materials and...
may have played a role in the observed genotoxic changes [10].

The genetic damage induced by such particles may not be due to the inherent genotoxic properties of combustion products of these fuel sources but may be as a result of long term inflammation induced by oxidative stress [11-13]. One of the major known molecular mechanisms of micronuclei induction is due to breakage of phosphodiester backbone of DNA by reactive oxygen or nitrogen species, which ultimately leads to DNA strand breaks. When these breaks are left unrepaired, they induce replication stress and hinder DNA replication leading to the formation of temporary or permanent breaks in DNA which end up forming micronuclei [14].

![Figure 1. A buccal cell with a micronucleus (shown by the arrow). Smear was stained with Giemsa stain. Magnification x400.](image)

**Figure 2.** Bar chart showing the distribution of mean total number of cells with micronuclei among abattoir workers exposed smoke from animal hides and the control participants (* = P < 0.05 using T test).

### 3.1 Effect of exposure characteristics on micronuclei frequency

Table 1 shows the mean CMN among those occupationally exposed to animal hide smoke and those who were not exposed based on age and occupational characteristics. Our results did not show any significant differences in the distribution of CMN among both groups based on age, number years in occupation, use of...
protection and cigarette smoking (P > 0.05). It was however observed that CMN was significantly higher among abattoir workers who were exposed to the smoke on daily basis than those exposed weekly, monthly or occasionally (P = 0.001).

The frequency of MN in cells was not age dependent as the exposed participants under 35 years and above 35 years did not have a significantly different level of MN expression. Similarly, the number of years spent in occupation as abattoir workers did not significantly affect the level of MN expression. Ascertaining the effect of using protective wears among the test participants would be difficult since only one participant admitted using protective gear among the exposed population. This supports the observation of Cadmus et al., [8], that most abattoir workers in Nigeria do not use any form of protection against smoke when working.

Most importantly, there was a significant relationship between frequency of exposure to the smoke and expression of micronuclei in buccal cells. Participants who were exposed daily had significantly higher frequency of MN expressed in their buccal cells than those exposed weekly, monthly or occasionally. This contrasts some previous studies reviewed which showed no significant relationship between frequencies of exposure to genotoxic substances and expression of micronuclei [2, 9, 15]. Also, there was no significant relationship relationship between cigarette smoking and frequency of micronuclei among occupationally exposed participants even though Khan and Sudha [16], report a higher degree of micronuclei and nuclear abnormalities among cigarette smokers who were also occupationally exposed to PAH.

The presence of micronuclei in the control subjects is indicative of the multiplicity of substances capable of inducing genetic damage in cells including drugs, pesticides, ionising radiation, cigarette smoking, heavy metals and chronic infections [2].

### Table 1: Distribution of mean total cells with micronuclei (CMN) among abattoir workers exposed to smoke from burning animal hides according to age and occupational characteristics.

<table>
<thead>
<tr>
<th>Exposed abattoir workers characteristics</th>
<th>N</th>
<th>CMN</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 35 years</td>
<td>13</td>
<td>1.23 ± 1.20</td>
<td></td>
</tr>
<tr>
<td>35 years and above</td>
<td>17</td>
<td>1.58 ± 1.50</td>
<td>0.51</td>
</tr>
<tr>
<td>Years in occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>15</td>
<td>1.27 ± 1.27</td>
<td></td>
</tr>
<tr>
<td>5 years and above</td>
<td>15</td>
<td>1.60 ± 1.50</td>
<td>0.54</td>
</tr>
<tr>
<td>Use of protection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>1.48 ± 1.38</td>
<td>0.40</td>
</tr>
<tr>
<td>Frequency of exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>21</td>
<td>2.05 ± 1.20</td>
<td></td>
</tr>
<tr>
<td>Weekly</td>
<td>5</td>
<td>0.00 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Monthly</td>
<td>4</td>
<td>0.00 ± 0.00</td>
<td>0.001*</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>1.37</td>
<td>0.67</td>
</tr>
</tbody>
</table>

CMN – mean total cells with micronuclei per 1000 cells presented as mean ± Standard deviation; *P < 0.05 using the Kruskal – Wallis test

4. CONCLUSION

In conclusion, an increased number of micronuclei was observed among abattoir workers exposed to smoke from singeing animal hides especially among those who are exposed daily without any form of protection. These individuals may thus be at risk of developing diseases associated defective genetic states such as malignancies. There is therefore a need to develop strategies to ensure that these workers are adequately protected from such exposures so as to prevent potentially carcinogenic genotoxic damage.
ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

OSO and PUA conceived and designed the study, NCA and TKU oversaw literature review, OBO wrote the protocol and first draft, ODS performed statistical analysis. All authors read and approved the final manuscript.

REFERENCES