Objective: This study assessed the cytogenetic damage associated with occupational exposure to paints by evaluating exfoliated buccal epithelial cells from car spray painters in Enugu metropolis using some biological markers.

Methods: A total of 352 apparently healthy males, comprising 200 car spray painters and a control group of 152 individuals, participated in the study. Buccal smears were obtained from each participant and were stained using hematoxylin and eosin technique. A total of 1000 cells per individual were scored under light microscopy to determine the frequencies of micronuclei (MN) and binucleate cells (BNC). Structured questionnaires were used to obtain relevant participant information. Expression patterns of Ki-67 and p53 genes on the buccal cells were determined by immunocytochemical methods.

Results: Car spray painters had significantly increased frequencies of MN and BNC (*p<0.05) when compared to the control subjects. Paint sprayers aged over 35 years had higher buccal cell MN frequency when compared to those <25 years. Furthermore, car spray painters who had worked for ≥15 years had higher frequencies of MN when compared to those who had worked for <5 years (*p<0.05). Smoking and alcohol consumption increased the MN frequency of the car spray painters (*p<0.05). There was no expression of p53 and Ki-67 genes in the buccal cells of both control and exposed subjects.

Conclusion: Car spray painters in Enugu metropolis may be occupationally exposed to substances capable of inducing genotoxic changes which manifested as increased frequency of MN in their buccal cells.

Keywords: Micronuclei, Binucleate cells, Buccal cell, Occupational exposure, Car spray painters, Immunocytochemical, Genotoxicity.

INTRODUCTION

Painting is an age-long profession. Painters are often occupationally exposed to substances in paints (such as solvents and metals) which may be deleterious to their health due to chronic poisoning [1,2]. Paints are a mixture of solvents, pigments, organic compounds, and other additives. Most of these compounds are volatile and easily spread into the environment as paint mists during spraying activities [3]. Different paints of varying chemistry have been in use for both domestic and industrial purposes. Paints and its constituents such as organic solvents, including aromatic hydrocarbons (mainly toluene), aliphatic hydrocarbons, alcohols, ketone and esters, and metals such as aluminum, cobalt, chromium, titanium, and lead may cause unfavorable cellular effects to occupationally exposed individuals [4,5]. Despite the fact that the International Agency for Research on Cancer has not considered some of these compounds contained in paints as being carcinogenic, some individual metals or their mixture may contribute to an increased risk of cancer in exposed individuals hence the classification of paints as a Group 1 carcinogen [6-8]. Furthermore, exposure to these organic solvents, metals, and other potentially mutagenic compounds in paints (such as phthalic acids and chlorophenols) is known to be capable of inducing DNA damage and chromosomal changes sometimes manifesting as micronuclei [1,9].

The examination of exfoliated buccal epithelial cells using the buccal cell micronuclei (MN) test as a biomarker serves as a minimally invasive method for bio-monitoring of human exposure to potentially genotoxic environmental pollutants [5]. In experimental animal models, MN assay has also been employed to monitor the damage of chromatin materials caused by exposure of animals to chemicals. A previous study documented an increase in the percentage frequency of micronucleus in animals treated with arsenite, which indicates chromosomal damage [10]. Another study also employed the in vivo micronucleus assay in bone marrow cells of male and female Sprague-Dawley rats to evaluate genotoxicity [11]. MN are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division [12]. An increased number of MN in exfoliated buccal cells is often an indication of genetic damage [13]. The presence of MN can be seen as an early warning sign for the potential risk of developing long-term health problems [14].

Car spray workers in Enugu metropolis, Nigeria constitute a major workforce with a considerable population. In the course of their work, these painters are exposed to paint mist or vapor primarily through inhalation, dermal absorption, and ingestion [15]. Mishandling of paints, inhalation of paint materials, and inadequate use of personal protective equipment (PPE) observed among some car spray painters have increased their risk of exposure to these harmful chemicals which are potential threats to health [16]. In view of this, the present study was, therefore, designed to evaluate cytogenetic damage of exfoliated cells of the buccal mucosa of car spray painters by determination of MN frequency using the MN test and binucleated cells.

METHODS

Ethical consideration

Approval for the study was obtained from the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital Ituku-Ozalla,
After incubation in the primary antibodies, the smears were washed with a Pasteur pipette and allowed to incubate at room temperature for 1 h. (1:100) (anti-Ki-67 and p53) were applied onto smears with the aid of smears were drained afterward, the exact portions of smears on slides with phosphate buffer saline (PBS) solution to prevent drying. The treated in protein block and biotin block solutions for 25 min in each then to distilled water for 15 s. Slides were arranged in slide racks and was performed according to the manufacturer’s instruction.

**Peroxidase/Diaminobenzidine detection immunohistochemistry kit** was employed for immunostaining while detection of immunoreactivity was negative for p53 immunoreactivities (**Fig.** 2a and b). The positive stain immune control section, however, showed p53 reactivity indicated using arrows (**Fig.** 2c).

**RESULTS**

**Effects of exposure on buccal cell nuclei**

Nuclear damage indices assessed in the buccal cells of study participants were MN and BNC (**Fig.** 1a-b) is representative micrographs showing normal buccal cell (**Fig.** 1a); cells with MN (**Fig.** 1b) and BNC (**Fig.** 1c).

| MN frequencies recorded. MN were counted by two independent observers who evaluated the slides twice at different intervals to minimize intra- and inter-observer bias.

**Immunocytochemical (ICC) staining and evaluation for p53 and Ki-67**

**ICC staining of buccal smears was carried out according to previously described methods** [18,19]. Monoclonal antibodies Ki-67 and p53 were employed. Expose Mouse and Rabbit Specific Horseradish Peroxidase/Diaminobenzidine detection immunohistochemistry kit was employed for immunostaining while detection of immunoreactivity was performed according to the manufacturer’s instruction.

**Procedure**

Smears were hydrated by passing through 50% ethanol for 15 s and then to distilled water for 15 s. Slides were arranged in slide racks and treated in protein block and biotin block solutions for 25 min in each solution. Thereafter, they were arranged on a staining rack and flooded with phosphate buffered saline (PBS) solution to prevent drying. The smears were drained afterward, the exact portions of smears on slides were carefully ringed with a hydrophobic pen, and diluted antibodies (1:100) (anti-Ki-67 and p53) were applied onto smears with the aid of Pasteur pipette and allowed to incubate at room temperature for 1 h. After incubation in the primary antibodies, the smears were washed with PBS, flooded with a secondary antibody for 25 min, washed with PBS, drained and diaminobenzidine DAB were applied for 5 min. Finally, the smears were washed with PBS; counterstained in Harris Hematoxylin for 5 min, washed in water and was differentiated by dipping 10 times in 1% acid alcohol. Slides were later washed and blued in tap water, dehydrated by passing through 70%, 90% and two changes of absolute ethyl alcohol for 15 s each, cleared in xylene and mounted in DPX. Ki-67 and p53 positive immune control sections were also stained alongside test and control smears. The ICC staining was semi-quantitatively scored, according to Zlobec et al. [18].

**Statistical analysis**

**Statistical analysis was performed using Statistical Package for the Social Sciences version 20.0.** Data obtained from the assay were expressed as the mean ± standard deviations. Student’s t-test (two-tailed) was used to compare nuclear abnormalities among test and control groups. The level of significance was set at *p* < 0.05. Overall effects of age, exposure duration, alcohol consumption, and smoking were determined using one-way analysis of variance, followed by post-hoc multiple comparisons.

**Variations of nuclear abnormalities (MN and BNC) based on demographics, lifestyle, and exposure characteristics**

Car spray painters were further subdivided according to age, occupational exposure factors, and lifestyle to assess the influence of these factors on the frequency of MN and BNC (Table 2). With respect to age, the result showed a statistically significant increase in the MN of car spray painters in the age group >35 years when compared with those in the lower age groups <25 years and 26–35 years.

**Effects of exposure on immunoreactivities for p53 gene**

Buccal cell cytology of both car spray painters and control participants were negative for p53 immunoreactivities (**Fig.** 2a and b). The positive stain immune control section, however, showed p53 reactivity indicated using arrows (**Fig.** 2c).

**Effects of exposure on immunoreactivities for Ki-67 gene**

There was also no expression of Ki-67 in the buccal cell nuclei of both car spray painters and control participants (**Fig.** 3a and b). The positive stain immune control section shows expression of Ki-67 (**Fig.** 3c).

**DISCUSSION**

Occupational and environmental exposure to hazardous chemical agents poses a great risk to human health and has become a global concern [20,21]. Buccal epithelial cells are often the first to come in contact with airborne pollutants in occupationally exposed individuals, especially when exposure is through inhalation or ingestion. These cells are capable of metabolizing proximate carcinogens into reactive products [22].

**RESULTS**

Effects of exposure on buccal cell nuclei

Nuclear damage indices assessed in the buccal cells of study participants were MN and BNC (**Fig.** 1a-b) is representative micrographs showing normal buccal cell (**Fig.** 1a); cells with MN (**Fig.** 1b) and BNC (**Fig.** 1c).

Table 1 compared the mean values of the frequencies of MN and BNC in control and exposed car spray painters. Car spray painters were found to have significantly higher MN (p = 0.000) and BNC (p = 0.000) when compared to the control subjects.

**Variations of nuclear abnormalities (MN and BNC) based on demographics, lifestyle, and exposure characteristics**

Car spray painters were further subdivided according to age, occupational exposure factors, and lifestyle to assess the influence of these factors on the frequency of MN and BNC (Table 2). With respect to age, the result showed a statistically significant increase in the MN of car spray painters in the age group >35 years when compared with those in the lower age groups <25 years and 26–35 years.

Car spray painters who have worked for 5–10 years, 11–15 years, and >15 years had significantly higher MN (p = 0.000) when compared with those who have worked for <5 years. Duration of exposure and use of PPE did not significantly affect the distribution of MN and BNC, though cars spray painters who worked >10 h daily had an increased frequency of MN and BNC though not statistically significant. Cigarette smoking and alcohol consumption significantly increased MN frequency (p = 0.000) of car spray painters.

**Effects of exposure on immunoreactivities for p53 gene**

Buccal cell cytology of both car spray painters and controls participants was negative for p53 immunoreactivities (**Fig.** 2a and b). The positive stain immune control section, however, showed p53 reactivity indicated using arrows (**Fig.** 2c).

**Effects of exposure on immunoreactivities for Ki-67 gene**

There was also no expression of Ki-67 in the buccal cell nuclei of both car spray painters and control participants (**Fig.** 3a and b). The positive stain immune control section shows expression of Ki-67 (**Fig.** 3c).

**DISCUSSION**

Occupational and environmental exposure to hazardous chemical agents poses a great risk to human health and has become a global concern [20,21]. Buccal epithelial cells are often the first to come in contact with airborne pollutants in occupationally exposed individuals, especially when exposure is through inhalation or ingestion. These cells are capable of metabolizing proximate carcinogens into reactive products [22].
In the present study, both car spray painters and the unexposed control group had MN and BNC in their exfoliated buccal epithelial cells. However, the car spray painters had significantly higher frequencies of buccal cell MN and BNC when compared to the unexposed control subjects. The result obtained from the present study corroborates with the findings of a preliminary study conducted on automobile spray painters in the coal camp mechanic village in Enugu [23]. More so, previous studies have documented similar increased levels of MN and other nuclear abnormalities in buccal epithelial cells of workers occupationally exposed to potentially genotoxic substances [9,24-26].

With reference to age, the result showed that car spray painters older than 35 years had the highest frequency of MN and BNC. Furthermore, there was a significantly higher MN frequency in the buccal cells of car spray painters who were older than 35 years when compared to those <35 years. This finding suggests that there may be an association between an individual's age and the frequency of MN, as previously documented [26]. In line with this finding, a previous study also reported a significant change in MN frequency due to age in a study involving automobile painters in South India [27]. In contrast; however, a previous study reported no significant change in MN frequency in relation to age among gas station attendants [16].

In the present study, a greater percentage of the exposed participants spent 5–10 h daily in their workplace, this may have also attributed to the significantly higher frequency of MN among those that have worked as car spray painters for 5–10 years, 11–15 years, and above 15 years when compared to those who have worked for <5 years. This suggests a positive correlation between the increased MN frequency and the number of years of exposure/Frequency of exposure which is similar to the findings documented by a previous study which showed an increased MN frequency with a positive correlation with increased period of exposure in motor garage workers [2]. Car spray painters who have worked for more than 15 years had a higher frequency of BNC when compared to those who have worked for <5 years. Even though the finding was not significant, it is of importance because an increase in BNC frequency of BNC is considered as an indicator of cytotoxicity [1].

The use of PPE such as goggles, overalls, gloves, and boots did not significantly alter the frequency of MN and BNC in car spray painters even though most of the workers sampled in this study did not strictly adhere to an adequate use of PPE while working. A previous study in Nigeria documented that only a few paint workers use some form of PPE despite public awareness on the use of PPE [2]. Regardless, adequate use of PPE among car spray painters is essential in preventing health hazards associated with occupational exposure.

According to previous studies [22,28], lifestyle factors such as alcohol consumption and smoking habits are considered as contributing factors for increased frequency of nuclear abnormalities in individuals who are exposed to genotoxic substances. The present study showed that alcohol consumption in addition to cigarette smoking habits...
Table 1: A comparison of MN and BNC frequencies in control subjects and car spray painters

| Groups               | Parameters (Mean±SD) | MN       | BNC       |
|----------------------|----------------------|----------|-----------|
| Control              |                      | 12.14±0.61 | 1.45±0.11 |
| Car spray painters   |                      | 5.35±0.67* | 4.95±0.11* |
| p-value              |                      | 0.000     | 0.000     |

Data expressed as mean±SD. *p<0.05 when compared with control. MN: Micronuclei; BNC: Binucleate cells; N: Number; MN: Micronuclei; SD: Standard deviations

Table 2: Frequency of MN and BNC in car spray painters and by demographics and exposure variables

| Characteristics                  | Nuclear abnormalities | n | MN       | BNC       |
|----------------------------------|-----------------------|---|----------|-----------|
| Age (<25)                        |                       | 22 | 48.8±2.44 | 5.00±0.44 |
| 26–35                            |                       | 142 | 5.28±0.70 | 4.92±0.12 |
| >35                              |                       | 36  | 5.17±1.16* | 5.06±0.11* |
| p-value                          |                       | 0.000 | 0.880     |
| Working experience (%)           |                       | 13  | 4.69±3.43 | 4.15±0.42 |
| <5                               |                       | 153 | 5.24±0.60* | 5.10±0.12 |
| 5–10                             |                       | 27  | 6.22±1.30* | 4.37±0.30* |
| >15                              |                       | 7   | 5.57±3.98* | 5.29±0.57* |
| p-value                          |                       | 0.000 | 0.228     |
| Duration of exposure (daily)     |                       | 185 | 17.40±0.23 | 5.10±0.18 |
| <5                               |                       | 15  | 17.82±0.69 | 5.90±0.56 |
| Yes                              |                       | 85  | 5.28±1.00 | 5.00±1.69 |
| No                               |                       | 115 | 5.08±0.96 | 4.91±1.45 |
| p-value                          |                       | 0.604 | 0.207     |
| Lifestyle habits (smoking and alcohol consumption) |       |   |           |           |
| Non-smokers+non drinkers         |                       | 66  | 5.10±2.21 | 4.71±0.20 |
| Smokers only                     |                       | 9   | 5.33±0.24 | 5.11±0.63 |
| Drinkers only                    |                       | 100 | 5.11±0.88 | 5.08±0.14 |
| Smokers+drinkers                 |                       | 25  | 6.12±1.72* | 5.00±0.34* |
| p-value                          |                       | 0.000 | 0.503     |

*p<0.05; N: Number; MN: Micronuclei; BNC: Binucleate cells

The expression of tumor suppressor gene, p53 and cell proliferation marker, Ki-67 has been widely used to monitor the progression of epithelial dysplasia of the oral cavity [35]. There was no expression of both p53 and Ki-67 genes in the buccal epithelial cells of car spray painters and control subjects. However, there was significantly increased MN frequency in the epithelial buccal cells of car spray painters in the present study, mosere, a previous study reported that increased MN frequency in peripheral blood lymphocytes of healthy subjects is a predictive biomarker of cancer risk, 12-15 years after the MN test was performed [36]. Another study documented a gradual increase in MN frequency of exfoliated buccal cells from normal mucosa to precancerous lesions then to oral squamous carcinoma, which suggested a link of this biomarker with neoplastic progression [37]. With the observed increase of MN frequency in buccal epithelial cells of car spray painters in the present study, periodical biological monitoring of these exposed car painters is therefore needful. Similar to our findings, a previous study on IHC staining for p53 and Ki-67 to characterize urothelial cells in urine cytology revealed a negative immunoreactivity for p53 and Ki-67 genes in subjects with normal cytology with median percentage values (first to third quartile) of p53 and Ki-67 being 0 (0–5) and 0 (0–1), respectively. The authors, however, recorded positive immunoreactivities for p53 and Ki-67 genes in subjects with urothelial carcinoma for 30 (10–80) and 20 (10–30), respectively [38].

CONCLUSION

The results obtained from the present study showed that car spray paint workers in Enugu metropolis occupationally exposed to genotoxins have increased MN and BNC frequencies, which may pose potential health risks with fatal consequences. There is, therefore, need to provide appropriate training for workers on the importance of using PPE and other safety practices so as to reduce the exposure to genotoxic agents and improve conditions of occupational safety.

ACKNOWLEDGMENT

The authors wish to thank the members of the teaching laboratory of the Department of Medical Laboratory Science, University of Nigeria Enugu Campus for the technical support and all the volunteers of this study for their maximum cooperation.

AUTHOR'S CONTRIBUTIONS

Anulka Onyemelukwe – conducted the study and drafted the manuscript, Peter Achukwu – designed and supervised the study, Nkiruka Azubuike – performed the statistical analysis, Uzoamaka Madakor – assisted in laboratory investigations, and Okechukwu Onwukwe – critically reviewed the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest for this research.

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