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Cytogenotoxicity Levels In Buccal Mucosal Cells Of Paint Handlers: Micronuclei Induction And Immunocytochemical Profile (p53 And Ki67).

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ABSTRACT

Workers who handle paints are often occupationally exposed to potentially harmful substances. This study assessed the P53 and Ki67 immunocytochemical profile and the frequency of buccal cell nuclear abnormalities in paint handlers occupationally exposed to paint solvents and metals. A total of 251 apparently healthy individuals comprising 51 control subjects, 28 paint sellers, 63 building painters, 109 paint industry workers were enrolled in the study. Buccal smears were obtained from each participant and were stained using Haematoxylin and Eosin technique. A total of 1000 cells per individual were scored under light microscopy to determine the frequencies of micronuclei (MN) and other nuclear abnormalities. Structured questionnaires were used to obtain relevant demographic and exposure information about the participants. Immunocytochemical methods were used to assess the patterns of immunoreactivity of Ki-67 and p53 genes on the buccal cells. All paint handlers had significantly increased frequencies of MN and other nuclear abnormalities. Further analysis showed that the number of years spent in the occupation, alcohol consumption and cigarette smoking significantly affected the frequency of nuclear abnormalities the among paint handlers. P53 and Ki-67 proteins did not show immunoreaction in the buccal cells of both control and exposed subjects. Paint handlers in Enugu Metropolis may be exposed to substances capable of causing genotoxic changes which manifested in their buccal cells as increased occurrence of MN and other nuclear abnormalities.

Keywords: Painters, micronuclei, buccal cells, occupational health, immunocytochemical, genotoxicty, mutagens.

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INTRODUCTION

Paints are complex mixtures of solvents and metals that can cause health damages in exposed individuals [1]. Numerous paints are classified as hazardous substances because they contain potentially harmful constituents which may cause injury and illness in exposed individuals through inhalation of toxic vapors and mists, or dermal absorption of irritants [2]. In developing countries, like Nigeria, exposure to the potentially hazardous chemicals of paints is common [3, 4]. According to International Agency for Research on Cancer (IARC), aromatic hydrocarbons like benzene, toluene and xylene used as paint organic solvents and metals such as aluminum, cobalt, chromium, titanium, and lead used as pigments in paints are enlisted as hazardous substances to which paint handlers are exposed to [5, 6]. Some individual metals or a mixture of them can contribute to increased risk of cancer in exposed individuals hence the classification of paints as a Group 1 carcinogen [7, 8]. It is known that occupational exposure to organic solvents [9 – 11] and some metals [12, 13] can induce oxidative stress and DNA damages, as documented by some studies [14 – 17], though the exact mechanism of such damages is not fully understood. The induction of DNA damage and chromosomal changes by exposure to these organic solvents, metals, and other potentially mutagenic compounds in paints (such as phthalic acids and chlorophenols) is sometimes manifested as micronucleus [1, 18]. Micronuclei (MN) are whole chromosomes or fragments of chromosomes lagging behind at the anaphase stage in mitosis [19]. Examination of buccal cells for the presence of MN and other nuclear abnormalities serves as a non-invasive tool for biomonitoring of the effects of exposure to potentially genotoxic substances on the integrity of the nucleus [19, 20]. An increased number of MN in exfoliated buccal cells serve as an early warning sign for the potential risk of developing long-term health problems [21].

In Enugu metropolis, Nigeria, paint handlers rarely use personal protective equipment (PPE) and are often exposed to paint mist or vapor while painting or during production and this increases their risk of exposure to these harmful chemicals which are potential threats to health [2].

The paucity of information and scarce documented literature available as at the time of study prompted this research with a major objective to evaluate genetic damage in paint handlers who are directly exposed to harmful chemicals using buccal cell micronucleus frequency and other nuclear abnormalities as biomarkers.

METHODS

Ethical consideration

This research was conducted after obtaining ethical clearance certificate from the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital Ituku-Ozalla with reference number : - NHREC/05/01/2008B-FWA00002458-1RB00002323. Before recruitment, the details of procedures involved in the study were explained to the study participants, volunteers who consented to participate in the study signed an informed consent form.

Study area and design

The study was conducted in Enugu, the capital city of Enugu state, Nigeria, and lasted for 7 months from April 2018 to October 2018. The study adopted a case controlled, cross-sectional design.

Participants and data collection

The study participants were aged between 18 and 65 years and recruited from Enugu metropolis, South East, Nigeria. A total of 251 apparently healthy males and females, comprising 51 control subjects, 28 paint sellers, 63 building painters, 109 paint industry workers participated in the study. The control subjects were apparently healthy individuals who are not paint handlers while the test groups included paint handlers who have been on the job for at least 1 year. Individuals seen to have oral lesions and those who reported on-going ailments were excluded from the study. A structured questionnaire was given to the participants before sample collection to obtain relevant demographic, lifestyle, and exposure characteristics such as age, smoking habits, alcohol consumption.

Collection of buccal smear and staining

The participating individuals thoroughly rinsed their mouth with water to remove any unwanted debris prior to sample collection. Buccal cells were obtained by scrapping the inside of both cheeks gently with a sterile wooden spatula; the sample was suspended in a labeled universal container containing 95% ethanol which served as a fixative. The samples were prepared in a laboratory by transferring into centrifuge bottles and centrifuging at 5000 rpm for 5 min, the supernatants were decanted, and the sediments were smeared on poly-L-lysine charged grease free slides. They were allowed to air dry and stained with hematoxylin and eosin staining technique.

Microscopy and Evaluation

Each stained slide was evaluated and scored independently by two individuals with buccal cell cytology experience using ×40 objective of a light microscope. At least 1000 intact buccal epithelial cells per slide were scored for cells with MN, binucleate cells (BN), cells with nuclear bud (NB), and cells with karyorrhectic (KH) and karyolitic (KL) nuclei using the criteria described by [22].

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

ICC staining of buccal smears was carried out according to previously described methods [23, 24]. 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 seconds and then to distilled water for 15 seconds. 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 buffer 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 hour. 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) was 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 seconds 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.,* [23].

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.

RESULTS

Socio-demographic characteristics of study participants

Table 1 summarizes some demographic and lifestyle (smoking and alcohol consumption) of the study participants. Out of the 251 participants recruited into the study, 204 (81.3%) were males while 47 (18.7%) were females. Majority of the participants were aged between 26 and 35 years old and attained at least secondary

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education. There were more non-smokers – 200 (79.7%) than smokers – 51 (20.3) and more participants consumed alcohol (151 - 60.2%) than those who did not (100 - 39.8).

Table 1: Socio-demographic characteristics of the study population.

	GROUPS (n/% of total)			
characteristics	**Control**	**Paint sellers**	**Building painters**	**Paint industry workers**
Gender				
Male	31(12.4%)	27(10.8%)	63(25.1%)	83(33.1%)
Female	20(8.0%)	1(0.4%)	0(0.0%)	26(10.4%)
Total	51(20.3%)	28(11.2%)	63(25.1%)	109(43.4%)
Age				
18-25years	13(5.2%)	8(3.2%)	10(4.0%)	19(7.6%)
26-35years	23(9.2%)	9(3.6%)	25(10.0%)	52(20.7%)
>35years	15(6.0%)	11(4.4%)	28(11.2%)	38(15.1%)
Total	51(20.3%)	28(11.2%)	63(25.1%)	109(43.4%)
Educational level				
Primary	13(15.2%)	9(3.6%)	17(6.8%)	16(6.4%)
Secondary	21(8.4%)	16(6.4%)	39(15.5%)	81(32.2%)
Tertiary	17(6.8%)	3(1.2%)	7(2.8%)	12(4.8%)
Total	51(20.3%)	28(11.2%)	63(25.1%)	109(43.4%)
Smoking habit				
Yes	10(4.0%)	5(2.0%)	16(6.4%)	20(8.0%)
No	41(16.3%)	23(9.2%)	47(18.7%)	89(35.5%)
Total	51(20.3%)	28(11.2%)	63(25.1%)	109(43.4%)
Alcohol consumption				
Yes	34(13.5%)	15(6.0%)	45(17.9%)	57(22.7%)
No	17(6.8%)	13(5.2%)	18(7.2%)	52(20.7%)
Total	51(20.3%)	28(11.2%)	63(25.1%)	109(43.4%)

Frequency of buccal cell nuclear abnormalities among the participants

Table 2 shows a comparison of the frequency of buccal cell abnormalities between the control participants and pooled paint handlers. The paint handlers were observed to have significantly higher MN, BN, NB, KH and KL (p=0.013, P=0.000, p=0.000, p= 0.001, p= 0.001) respectively when compared with the control. The number of buccal cells with karyorrhectic nuclei were also higher in the participants who worked in paint industries though not statistically significant (p = 0.103).

Table 2: A comparison of the frequency of buccal cell nuclear abnormalities between control subjects and paint handlers.

		Nuclear abnormalities (mean ±SD)				
Groups	N	MN	BN	NB	KH	KL
Control	51	4.28±5.16	1.16±5.25	0.70±1.52	0.06±0.24	0.00±0.00
Paint handlers	200	16.66±4.60*	5.25±4.76*	2.58±2.83*	0.58±1.74*	0.41±0.29*
P-value		0.013	0.000	0.000	0.000	0.001

Values are presented as Mean ± Standard deviation, *significantly different from control with significance set at p <0.05.
MN- Micronuclei, BN- Binucleate cells, NB- Nuclear buds, KH- Karyorrhectic nuclei, KL- Karyolitic nuclei.

Buccal cell nuclear among the different categories of paint handlers

Table 3 compares the frequency of buccal cell nuclear abnormalities between the control participants and the different categories of paint handlers. All categories of paint handlers had significantly higher MN and BN when compared to the control participants (p = 0.001). Building painters and paint industry workers had a significantly higher frequency of NB compared to the controls (p = 0.001) while the paint sellers had a higher NB though not significant (p > 0.05). No significant differences were observed in the frequency of nuclear KH and KL in the different categories of paint handlers compared to the control subjects (P = 0.094 and P = 0.058 respectively).

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Table 3: A comparison of the frequency of buccal cell nuclear abnormalities between the various groups of paint handlers and control.

		Nuclear Abnormalities				
Groups	N	MN	BN	NB	KH	KL
Control	51	4.27±5.11	1.16±2.50	0.70±1.50	0.06±0.24	0.00±0.00
Paint sellers	28	14.54±6.40*	4.00±2.64*	1.60±1.90	0.21±0.50	0.00±0.00
Building painters	63	16.50±6.00*	5.00±4.11*	3.11±3.43*	0.62±1.34	0.05±0.21
Paint industry workers	109	17.42±5.00*	5.80±5.43*	2.60±2.60*	0.70±2.11	0.15±0.53
P-value		0.000	0.000	0.000	0.094	0.058

Values are presented as Mean ± Standard deviation, *significantly different from control with significance set at p <0.05. All categories of paint handlers had higher values of nuclear abnormalities when compared with the control. Paint industry workers had the highest frequency of MN and BN, followed by building painters, while paint sellers had the least.

Effects of demography, lifestyle and occupational exposure characteristics on frequency of buccal cell nuclear abnormalities among the paint handlers.

Table 4: The frequency of buccal cell nuclear abnormalities in paint handlers by demographics and exposure variables.

Characteristics	Nuclear abnormalities (mean ±SD)					
	N	MN	BN	NB	KH	KL
Age						
18-25 years	40	11.70±6.74	3.08±4.40	1.02±1.50	0.10±0.51	0.06±0.42
26-35 years	89	13.16±7.10	3.70±4.50	1.84±2.40	0.31±1.38	0.05±0.30
>35 years	71	16.80±7.43*	6.10±4.70*	3.30±3.24*	0.90±2.03*	0.10±0.40
P-value		0.000	0.001	0.000	0.006	0.346
Time in occupation						
1-5 years	90	14.63±5.32	3.81±4.54	1.52±1.90	0.14±0.61	0.03±0.32
6-10 years	74	17.82±5.10*	6.12±4.95*	3.00±2.90*	0.73±2.20*	0.14±0.50
11-15 years	28	19.61±5.22*	7.14±3.72*	4.20±3.24*	1.43 ±2.50*	0.20±0.50
> 15 years	8	19.90±5.70*	7.40±4.41*	5.00±4.55*	1.30±1.60*	0.13±0.40
P-value		0.000	0.001	0.000	0.003	0.265
Use of protective clothing						
Yes	75	17.25±4.83	5.80±5.30	2.51±2.72	0.89±2.45	0.16±0.55
No	125	16.40±9.50	4.96±4.40	2.65±2.91	0.40±1.10	0.06±0.29
P-value		0.064	0.201	0.373	0.061	0.072
Overall lifestyle habits						
Non smoker & non drinkers	76	14.29±6.67	4.26±3.70	1.93±2.51	0.42±1.41	0.07±0.36
Smokers only	10	17.71±5.72*	5.64±3.84	4.00±2.83	1.00±1.41	0.00±0.00
Drinkers only	71	14.34±6.42	4.26±3.39	2.03±2.57	0.35±1.21	0.07±0.38
Smokers & Drinkers	43	16.76±6.80*	4.83±3.78	3.02±3.31	0.85±2.37	0.11±0.37
P-value		0.009	0.340	0.060	0.267	0.907

Values are presented as mean ±SD, *p<0.05 when compared with other groups using one way ANOVA followed by *post hoc* multiple comparison.

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The nuclear damage among the paint handlers was significantly affected by age, time in occupation, cigarette smoking and alcohol consumption habits as shown in Table 4. The frequency of some of the nuclear abnormalities observed appear to be higher in older paint handlers. For instance, paint handlers above 35 years had significantly higher MN, BN, NB and KH when compared with those between the ages of 18-25 years ($P < 0.05$). Also, paint handlers who have been exposed to paints for a period of 6-15 years and above 15 years had significantly higher frequency of all nuclear abnormalities except KL compared to those who have handled paints for 5 years or less ($P < 0.05$). The use of PPEs did not affect the frequency of nuclear abnormalities among the paint handlers ($P > 0.05$). Paint handlers who used protective clothing surprisingly had a higher frequency of MN, BN, KH and KL. Paint handlers who smoked cigarette only and those with combined habit of cigarette smoking and alcohol consumption had a significantly higher MN frequency when compared to paint handlers who neither smoked cigarette nor consumed alcohol ($P < 0.05$). No significant differences were observed in the frequency of BN, NB, KH and KL even though paint handlers who consumed alcohol and smoked cigarettes appeared to have higher frequencies of these abnormalities ($P > 0.05$).

Immunoreactivity of p53 and Ki67 in the buccal cells of participants

All categories of paints handlers and control participants showed negative immunoreactivities for both p53 and Ki67 in their buccal cells (Fig 1).

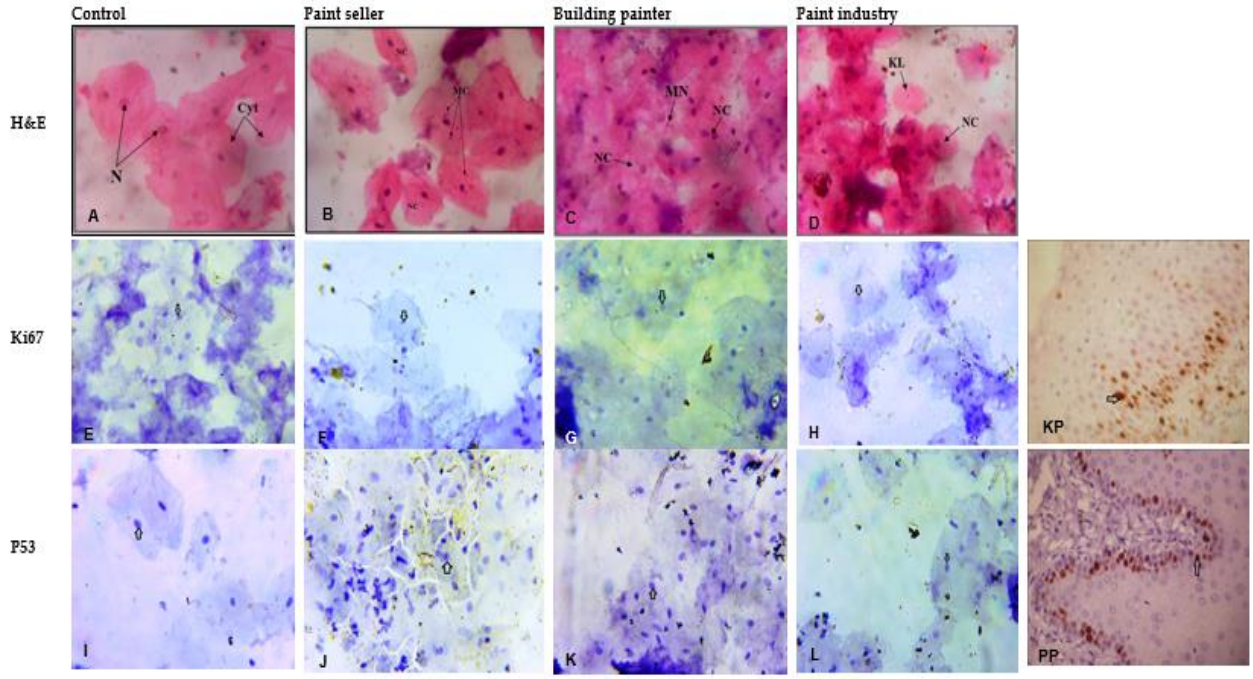

Figure 1: Light photomicrographs of exfoliated buccal epithelial cells of study participants - Control subjects (1A); showing normal cytoplasm (Cyt) and nuclei (N). Paint sellers (1B); showing presence of normal cells (NC) and micronuclei cells (MC) Building Painters (1C); showing presence of normal cells (NC), micronuclei cells (MN) and cells. Paint industry workers (1D); showing presence of normal cells (NC), karyolytic cells (KL), and nuclear buds (NB) (red arrow). Figure 1 E – F are photomicrographs of Ki67 stained slides showing negative immunoreactivity in the nuclei (arrows) of Control subjects (1E), Paint sellers (1F), Building painters (1G), and Paint industry workers (1H). Figure 1 I – L are photomicrographs of P53 stained slides showing negative immunoreactivity in the nuclei (arrows) of Control subjects (1I), Paint sellers (1J), Building painters (1K), Paint industry workers (1L). KP and PP are the positive control micrographs of Ki67 and P53 immunostains respectively. (Mag: ×400).

DISCUSSION

In the present study, the P53 and Ki67 imunocytochemical profile and the frequency of nuclear abnormalities in the buccal cells of paint handlers were assessed. Paint handlers in the course of their work are constantly exposed to potentially toxic substances which may induce genetic damage [15 – 17]. The use of buccal

cell micronucleus (MN) assay as an early diagnostic tool for detecting DNA damage is employed to investigate the genotoxicity of potential hazardous chemicals like the ones used in the production of paints [20, 25].

Inhalation, ingestion and contact with volatile organic solvents and metals by paint handlers during mixing of chemicals in the course paint production and painting activities increase their risk of exposure to hydrocarbon compounds, metals and a significant amount polycyclic aromatic hydrocarbons (PAH) which are known to be toxic [26, 27]. Occupational exposure to these potentially hazardous chemical agents is of great public health concern, hence the need to assess and monitor the genetic risks related to occupational exposure to these toxic substances.

The micronucleus assay performed in this study showed that micronuclei (MN) was the most predominant nuclear abnormality observed, followed by binucleate (BN) cells while the least observed abnormality was karyoltic (KL) cells. A significantly higher frequency of MN, BN, nuclear buds (NB) and karyorrhectic (KH) cells was observed in the pooled paint handlers when compared with the control subjects. Similar significantly higher frequencies of MN and other nuclear changes in the buccal epithelial cells of building construction workers, foundery and petrol station workers have been documented by previous studies [28 – 30].

It was also observed that paint industry workers had the highest frequencies of MN and BN cells while paint sellers had the least when compared to the other groups of paint handlers. This may be attributed to the fact that paint industry workers are in direct contact with these paint chemicals and inhalation during mixing is inevitable, unlike paint sellers who retail the already finished paint products mostly in sealed containers. More so, the pattern and levels of exposure of paint industry workers to individual paint components may differ from those of other paint handlers. This finding is similar to that of Diaz *et al*., [18], which documented a significantly increased MN frequency in paint industry workers in their study conducted in Cuba.

The findings of this study indicate that age, duration of exposure to paint, and certain lifestyle factors such as smoking and alcohol consumption significantly increased the MN frequency, while the use of protective equipment did not show any significant effect in the frequency of the nuclear abnormalities among the paint handlers.

The uniform progression in the mean value of the frequency of nuclear abnormalities (MN, BN, NB and KH) from younger to older paint handlers suggests that the older individuals are subjected to greater risks of developing potentially deleterious nuclear changes in their buccal cells which may lead to cancer on prolonged exposure [31]. This finding is in line with previous studies which documented increased MN frequency and other nuclear abnormalities in older individuals [32, 33]. However, Benites *et al.,* [34] did not document any significant difference in MN frequency according to the age of occupationally exposed workers in a study involving gas station attendants.

The frequencies of MN, BN, NB and KH was observed to be significantly higher in participants who have worked longer in an occupation requiring them to handle paints. This pattern suggests that most direct and indirect genotoxic substances usually require chronic exposure to exert its damaging effect [35]. According to Thomas and Fenech [36], binucleate cells may be associated with cell proliferation and is also considered an indicator of genotoxicity [28]. This suggests that paint handlers occupationally exposed to genotoxic chemicals in paints are at risk of genetic damage as revealed by the progressive higher levels of BN cell frequency in the buccal cells of paint handlers based on the years spent in the occupation as observed in the present study. The significantly higher NB frequency observed in workers that have spent greater than six (6) years when compared to workers that have spent 1-5 years in the occupation implies that the epithelial cells may be undergoing degeneration or exhibiting apoptosis due to the exposure. The frequency of KH cells was also significantly higher in paint handlers that have worked for 6-10 years, 11-15 years and more than 15 years than those that have worked for 1-5 years. This is suggestive that the nucleus may be undergoing degenerative changes due to exposure to chemical agents in paints, with frequency of KH cells progressively increasing uniformly with time in occupation. A previous study demonstrated a similar higher frequency of MN, NB and KH in petrol pump workers exposed to gasoline fumes [37]. On the contrary, Onwukwe *et al*., [38] in a previous study conducted on road construction workers exposed to bitumen documented that the number of years spent as road construction workers did not significantly increase the frequency of MN and other nuclear abnormalities.

The frequency of MN and other nuclear abnormalities were not significantly affected by the use of PPE such as gloves, boots, overalls and face masks among paint handlers, even though that the majority of workers sampled in this study did not adhere strictly to adequate use of PPE while working. Awodele *et al.,* [3] documented a similar non adherence to the use of PPE by paint handlers despite public awareness. Nevertheless, adequate use of PPE is very important in preventing work related health hazards due to occupational exposure.

Lifestyle factors such as smoking and alcohol consumption are known predisposing risk factors to DNA damage and most likely causing an increase in the frequency of MN in buccal cells [19]. In the present study, paint handlers who smoked cigarette only and those who consumed alcohol and smoked cigarette too had significantly higher frequency of MN when compared to paint handlers who neither smoked nor consumed alcohol. Benzene is used as a solvent in the production of paints and cigarette smoke also contains benzene – a known carcinogen [1, 39]. Celik *et al*., [28] also documented a distinct relationship between an increase in MN frequency and exposure to benzene and its metabolites. A study by Bishop *et al*., [40] described alcoholic beverages as containing mutagenic substances. A clear relationship between nuclear abnormalities in buccal epithelial cells and exposure to cigarette smoke and alcohol was documented by [41]. Alcohol consumption and cigarette smoking are implicative factors to the significantly higher MN frequency observed in paint handlers in Enugu metropolis exposed to occupational genotoxins.

Tumor suppressor gene; p53 and cell proliferation marker; Ki-67 expressions have been widely used to monitor the progression of epithelial dysplasia of the oral cavity [42]. The most common genetic changes observed in Oral Squamous Cell Carcinomas (OSCCs) are mutations in the p53 gene, these mutations lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and eventually in malignancy [24]. The result of this study showed that there was no immunoreactivty of both p53 and Ki-67 genes in the buccal epithelial cells of paint handlers and control subjects. A previous study by Bonassi *et al.*, [43] reported that increased frequency of MN in peripheral blood lymphocytes of healthy individuals is a predictive biomarker to cancer risk, 12-15 years after the MN assay was performed. With the increased frequency of MN and other nuclear abnormalities observed in the buccal epithelial cells of paint handlers in the present study, it is therefore needful to carefully bio-monitor paint handlers periodically. This finding agrees with the result of a previous study on the expression of p16, p53 and Ki-67 proteins in the progression of epithelial dysplasia of the oral cavity documented by Angeiro *et al*., [42], which observed a negative immunoreactivity for p53 and Ki-67 in all cases of mild dysplasia and non dysplastic cases, but positive in cases of moderate and severe dysplasia and squamous cell carcinoma.

CONCLUSION

In conclusion, the results of the present study showed that paint handlers had a significantly higher frequency of MN and other nuclear abnormalities compared to the controls; this implies a possibility of genetic damage as a result of adverse occupational exposure to potentially toxic chemicals in paints. It is also probable that long-term exposure to paints can lead to an increased susceptibility for development of health risks.

REFERENCES

[1] Cassini C, Calloni C, Bortolini G, Garcia SC, Dornelles MA, Henriques JA, Erdtmann B, Salvador M. Occupational risk assessment of oxidative stress and genotoxicity in workers exposed to paints during a working week. Int J Occup Med Environ Health. 2011;24(3):308.

[2] Mersal FA, Esmat OM. Structured teaching program on occupational health hazards among painting workers at elherafeyien city in Cairo. Biomed Nurs. 2018;4:1-1.

[3] Awodele O, Popoola TD, Ogbudu BS, Akinyede A, Coker HA, Akintonwa A. Occupational hazards and safety measures amongst the paint factory workers in Lagos, Nigeria. Saf Health Work. 2014;5(2):106-11.

[4] Moolla R, Curtis CJ, Knight J. Occupational exposure of diesel station workers to BTEX compounds at a bus depot. Int J Environ Res Pub health. 2015; (4):4101-15.

[5] Pinto D, Ceballos JM, Garcıa G, Guzman P, Del Razo LM, Vera E, Gomez H, García A, Gonsebatt ME. Increased cytogenetic damage in outdoor painters. Mutat Res. 2000;467(2):105-11.

[6] IARC. Painting, firefighting, and shiftwork. IARC Monograph Evaluation Carcinogenic Risks Humans 2010. 98:1–804.

[7] IARC (1989). Working Group on the Evaluation of Carcinogenic Risks to Humans. Some organic solvents, resin monomers and related compounds, pigments and occupational exposures in paint manufacture and painting. IARC Monogr Eval Carcinog Risks Hum; 1989. 47:1-442.
[8] Scélo G, Metayer C, Zhang L, Wiemels JL, Aldrich MC, Selvin S, Month S, Smith MT, Buffler PA. Household exposure to paint and petroleum solvents, chromosomal translocations, and the risk of childhood leukemia. Environ Health Perspec. 2009;117(1):133-9.
[9] Bayil S, Cicek H, Geyikli Cimenci I, Hazar M. How volatile organic compounds affect free radical and antioxidant enzyme activity in textile workers. Arhiv za higijenu rada i toksikologiju. 2008 Dec 8;59(4):283-7..
[10] Duan H, Leng S, Pan Z, Dai Y, Niu Y, Huang C, Bin P, Wang Y, Liu Q, Chen W, Zheng Y. Biomarkers measured by cytokinesis-block micronucleus cytome assay for evaluating genetic damages induced by polycyclic aromatic hydrocarbons. Mutat Res. 2009;677(1-2):93-9.
[11] Marczynski B, Pesch B, Wilhelm M, Rossbach B, Preuss R, Hahn JU, Rabstein S, Raulf-Heimsoth M, Seidel A, Rihs HP, Adams A. Occupational exposure to polycyclic aromatic hydrocarbons and DNA damage by industry: a nationwide study in Germany. Arch toxicol 2009;83(10):947-57.
[12] Mateuca R, Aka PV, De Boeck M, Hauspie R, Kirsch-Volders M, Lison D. Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. Toxicol lett. 2005 Apr 10;156(2):277-88.
[13] Mohammad I, Mahdi A, Raviraja A, Najmul I, Iqbal A, Thuppil V. Oxidative stress in painters exposed to low lead levels. Arh Hig Rada Toksikol; 2008; 59(3):161-9.
[14] Zhu CQ, Lam TH, Jiang CQ. Lymphocyte DNA damage in bus manufacturing workers. Mutat Res. 2001;491(1-2):173-81.
[15] Martino-Roth MG, Viegas J, Roth DM. Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test. Genet Mol Res. 2003;2(4):410-7.
[16] Testa A, Festa F, Ranaldi R, Giachelia M, Tirindelli D, De Marco A, Owczarek M, Guidotti M, Cozzi R. A multi-biomarker analysis of DNA damage in automobile painters. Environ Mol Muta¬gen. 2005;46(3):182-8.
[17] Aksoy H, Yõlmaz S, Çelik M, Yüzbaşıoğlu D, Ünal F. Genotoxicity study in lymphocytes of offset printing workers. J Appl Toxicol. 2006;26(1):10-5.
[18] Diaz S, Fonseca G, Fernandez I Analysis of lymphocyte and oral mucosa cell micronuclei in Cuban paint industry workers. Hereditas. 1990;113(1):77-80.
[19] Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutat Res. 2008;659(1-2):93-108..
[20] Banerjee S, Singh NN, Sreedhar G, Mukherjee S. Analysis of the genotoxic effects of mobile phone radiation using buccal micronucleus assay: a comparative evaluation. J Clin Diagn Res. 2016;10(3):ZC82.
[21] Smita V P, Sumangala P, Sampada K. Micronuclei - As A Biomarker Of Genotoxicity In Automobile Mechanics Of Western Maharashtra Asian J Pharm Clin Res. 2018; 11(8); 467-469
[22] Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat Res. 1992;271(1):69-77..
[23] Zlobec I, Steele R, Michel RP, Compton CC, Lugli A, Jass JR. Scoring of p53, VEGF, Bcl-2 and APAF-1 immunohistochemistry and interobserver reliability in colorectal cancer. Mol Pathol. 2006;19(9):1236-42..
[24] Humayun S, Prasad VR. Expression of p53 protein and ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. Natl J Maxillofac Surg. 2011;2(1):38.
[25] Kumar M, Prasad UC, Chandolia B, Manjunath SM, Basu S, Verma S. Can Feulgen Stain be a Reliable Biomarker over PAP Stain for Estimation of Micronuclei Score?. J Clin Diagn Res. 2016;10(10):ZC07.
[26] Sudha S, Kripa SK, Shibily P, Joseph S, Balachandar V. Biomonitoring of genotoxic effects among shielded manual metal arc welders. Asian Pac J Cancer Prev. 2011;12(4):1041-4.
[27] Klöslová Z, Drímal M, Balog K, Koppová K, Dubajová J. The relations between polycyclic aromatic hydrocarbons exposure and 1-OHP levels as a biomarker of the exposure. Cent Eur J Public Health. 2016; 24(4):302.
[28] Çelik A, Çavaş T, Ergene-Gözükara S. Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. Mutagenesis. 2003;18(5):417-21.
[29] Sellappa S, Prathyumnan S, Joseph S, Keyan KS. Micronucleus test in exfoliated buccal cells from chromium exposed tannery workers. Int J Biosci Biochem Bioinform. 2011;1(1):58.

[30] Singaravelu SR, Sellappa S. Assessment of genotoxicity in exfoliated buccal epithelial cells of foundry workers occupationally exposed to polycyclic aromatic hydrocarbons. Asian J Pharm Clin Res. 2013;6:339-42.
[31] Rafiq Khan M, Sellappa S. Elevated frequencies of micronuclei and other nuclear abnormalities in buccal epithelial cells of spray painters in South India. Int J Pharm Life Sci. 2013;4(5).
[32] Bolognesi C, Lando CE, Forni A, Landini EL, Scarpato R, Migliore L, Bonassi S. Chromosomal damage and ageing: effect on micronuclei frequency in peripheral blood lymphocytes. Age and ageing. 1999 Jul 1;28(4):393-7.
[33] Orta T, Günebakan S. The effect of aging on micronuclei frequency and proliferation in human peripheral blood lymphocytes. Ind. J. Hum. Genet. 2012;18(1):95.
[34] Benites CI, Amado LL, Vianna RA, da Graça Martino-Roth M. Micronucleus test on gas station attendants. Genet and Molec Res. 2006;5(1):45-54.
[35] Benedetti D, Nunes E, Sarmento M, Porto C, dos Santos CE, Dias JF, da Silva J. Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays. Mutat Res. 2013;752(1-2):28-33.
[36] Thomas P, Fenech M. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. Mutagenesis. 2008;23(1):57-65.
[37] Shaikh A, Barot D, Chandel D (2018). Genotoxic effects of exposure to gasoline fumes on petrol pump workers. Int J Occup Environ Med; 9: 79-87.
[38] Onwukwe OS, Azubike NC, Udeani TK, Onyemelukwe AO, Chukwuagu CF, Achukwu PU. Changes in haematological profile and frequency of nuclear abnormalities in buccal epithelial cells of automobile welders in Enugu, Nigeria. Res. J. Environ. Toxicol., 2018; 12:56-62.
[39] Zhang L, Eastmond DA, Smith MT. The nature of chromosomal aberrations detected in humans exposed to benzene. Critic Rev Toxicol; 2002; 32: 1 - 42.
[40] Bishop, JB, Witt KL, Sloane RA. Genetic toxicities of human teratogens. Mutat Res. 1997; 396: 9-43.
[41] Jyoti, S., Siddiquea Y.H., Khan S. Effect on micronucleus frequency and DNA damage in buccal epiththelial cells of various factors among pan masala and gutkhaoral science. Int J Oral Science; 2015; 12: 9-14.
[42] Angiero F, Berenzi A, Benetti A, Rossi E, Del Sordo R, Sidoni A. Expression of p16, p53 and ki-67 proteins in the progression of epithelial dysplasia of the oral cavity. Anticancer Res; 2008; 28:2535-9
[43] Bonnasi S., Neri, M., Lando C., Ceppi M., Lin Y., Chang W.P., Holland N., Kirsch-Volders M., Zeiger E., Fenech M., and Human collaborative Group 1 (2003). Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from Human MicroNucleus Project. Mutat Res 2003; 543, 155-166