INNOVARE JOURNAL OF LIFE SCIENCE

Vol 7, Issue 4, 2019

ISSN - 2321-550X

Research Article

FREQUENCY OF NUCLEAR ABNORMALITIES IN BUCCAL CELLS OF ROAD CONSTRUCTION WORKERS IN ANAMBRA STATE, NIGERIA

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Received: 22 May 2019, Revised and Accepted: 25 June 2019

ABSTRACT

Objective: The objective of this study is to assess the frequency of nuclear damage in the buccal cells of road construction workers in Anambra state, Nigeria.

Methods: In this study, the frequency of nuclear abnormalities – cells with micronuclei (CMN), total MN (TMN), binucleate (BN) cells, cells with nuclear bud, and karyorrhectic cells, in exfoliated buccal cells of 33 road construction workers and 33 control subjects consisting of healthy persons was evaluated. Demographic data and exposure characteristics of the participants were obtained using a structured questionnaire. Buccal smears were obtained from both cheeks for each participant. The smears were stained using papanicolaou staining technique. At least 1000 cells in each smear were assessed and scored. The frequency of nuclear abnormalities in both road construction workers and control subjects was then compared.

Results: The results obtained show a significant increase in the frequency of CMN (p=0.005), TMN (p=0.008), and BN (p=0.006) in buccal cells of road construction workers compared with control subjects. Factors such as age, years in occupation, and work days per week, use of protective materials, on-going medication, smoking habit, and alcohol consumption did not significantly affect the frequency of nuclear abnormalities among the road construction workers.

Conclusion: Road construction workers may be exposed to substances capable of inducing nuclear damage in buccal cells.

Keywords: Road construction workers, Genotoxicity, Micronuclei, Asphalt, Bitumen.

INTRODUCTION

Exposure to hazardous substances is common among construction workers in road construction sites. These substances can occur as gases, vapors, fumes, and particles which include polycyclic aromatic hydrocarbons (PAHs) [1]. Exposure to PAHs can come from both occupational and environmental sources arising from the use of coal tar, bitumen and exposure to automotive exhaust, gases, tobacco smoke, and many other sources [2].

PAHs have remarkable mutagenic and carcinogenic properties; hence, exposure to bitumen fumes constitutes a major occupational health concern among road construction workers. Çelik *et al.* [3] in their investigation of the potential risk for developing cancer among road construction workers exposed to bitumen fumes determined that lung cancer frequency is more than 100. It is, therefore, important to carry out risk assessment studies to generate relevant data for protecting these individuals against potential harm.

Crude asphalt produced from bitumen contains the most widely distributed class of possible carcinogens present in the human environment [4]. Bitumen is made up of different chemical compounds many of which PAHs. PAHs are an important class of chemical hazards. These compounds or their metabolites can interact with DNA, resulting in covalent bonding between chemicals and biological macromolecules and consequent damage to DNA [5,6]. When heated, bitumen and bitumen containing products emit fumes. These emissions have been classified by regulatory bodies as genotoxic, carcinogenic, or irritant. Road construction workers are also exposed to chemical substances from coal tar, silica, organic solvents, and diesel exhausts. These have been shown to have harmful effects on road construction workers [7].

Examination of buccal cells for the presence of micronuclei (MN) and other nuclear abnormalities is generally accepted as a non-invasive tool for biomonitoring of the effects of exposure to potentially genotoxic substances on nuclear integrity [8,9]. From literature available to us at the time of the study, there is scarce documentation on the frequency of nuclear abnormalities among road construction workers in Nigeria. This study was thus designed to assess the frequency of nuclear abnormalities in the buccal cells of road construction workers.

METHODS

Ethical consideration

This study was conducted after obtaining Ethical Clearance Certificate from the Health Research and Ethics Committee of University of Nigeria Teaching Hospital, Ituku/Ozalla, with reference number NHREC/05/01/2008B-FWA00002458-1RB00002323.Before recruitment of participants and sample collection, the procedure was explained to the participants and they gave their approval by signing an informed consent form.

Study area and design

The study was carried out in three different road construction sites located in Nteje, Umueri, and Awka towns in Anambra state. The study lasted for 6 months from March 2018 to August 2018. A total number of 66 male participants who were apparently healthy were recruited into the study; 33 of them were road construction workers while the others were not. A case–controlled cross-sectional approach was adopted in the study.

Participants and data collection

Recruited participants were aged between 18 and 60 years for both the test and control groups. The test group included road construction

workers that have been on the job for at least 1 year. The control participants were apparently healthy individuals who are not road construction workers. Individuals who reported on-going ailments and those seen to have oral lesions were excluded from the study. Relevant demographic, lifestyle, and exposure characteristics such as age, body mass index (BMI), lifestyle, general health, smoking habits, use of drugs and treatments, and exposure to ionizing radiations (like X-rays) were obtained using a structured questionnaire administered before sample collection.

Collection of buccal smears and staining

Samples from each participant were clearly labeled to avoid losing or mixing up information. Before collection, the subjects were given water to rinse their mouths to remove unwanted debris. Samples were collected by gently scraping the inside of both cheeks with wooden spatulas. The exfoliated cells were smeared immediately on clean microscope slides, one for each cheek. This was followed immediately by fixation in 95% ethanol. A slightly modified method of papanicolaou staining method optimized before field studies was employed in this study [10].

Microscopy

Each slide was evaluated and scored independently by two individuals who had experience with buccal cell cytology using the ×40 objective. At least 1000 buccal cells per slide were scored for cells with MN (CMN), total MN (TMN), binucleate (BN) cells, cells with nuclear bud (NB), and cells with karyorrhectic (KH) nuclei using guidelines developed by Tolbert *et al.* [11]. Two slides for both the right and left cheeks were assessed per participant and the average number of cells with each observed nuclear abnormality recorded.

Statistical analysis

The Statistical Package for the Social Sciences was used for data analysis. Student's t-test was used to compare nuclear abnormalities between the road construction workers and control subjects. Kruskal–Wallis and Mann–Whitney U-tests were used as appropriate to determine the effect of various demographic, lifestyle, and exposure characteristics on the distribution of nuclear abnormalities among the road construction workers. The results obtained were expressed as mean ± standard deviation and comparisons were made with the level of statistical significance set at $p<0.05$.

RESULTS

Characteristics of the study population

Table 1 is a summary of the demographic characteristics of the study population consisting of 33 road construction workers and 33 control subjects. The road construction workers had a higher mean age and BMI. Among the road construction workers, there were slightly more individuals who smoked and consumed alcohol than those who did not. All control participants had attained tertiary education while majority of the road construction workers attained secondary education level.

Frequency of buccal cell nuclear abnormalities

Table 2 compares the frequencies of buccal cell nuclear abnormalities between road construction workers and control subjects. The road construction workers had significantly higher CMN, TMN, and BN than the control subjects ($p=0.005$, $p=0.008$, and $p=0.006$, respectively). The road construction workers also had higher NB and KH than the control participants though not significant.

Effects of exposure characteristics and lifestyle on frequency of nuclear abnormalities among road construction workers

The frequency of nuclear damage among the road construction workers was not significantly affected by any of the exposure and lifestyle characteristic assessed (Table 3). However, construction workers older than 35 years and who had worked for more than 5 years and more than 3 days per week had higher CMN, TMN, BN, and NB than those who were younger than 35 years and less occupationally exposed. In the same manner, smokers, alcohol consumers, and those on medication had a higher frequency of CMN and TMN. Surprisingly, construction workers who used personal protective equipment had higher CMN that those who did not.

DISCUSSION

The aim of this study was to determine the frequency of nuclear abnormalities in the buccal cells of road construction workers. During the course of their work, road construction workers are constantly exposed to various potentially toxic substances. Structural modifications in the DNA of target cells lead to genomic instability in the form of chromosomal abnormalities. Hence, the use of early diagnostic tests bordering on biomonitoring would be beneficial to check the progress of premalignant lesion to malignancy [12,13].

Occupational exposure to bitumen fumes emitted during hot application of asphalt carries the risk of exposure to hydrocarbon compounds and also a significant amount of PAHs which are known to be toxic [14,15]. Several studies human studies have shown that exposure to bitumen and its derivatives may lead to DNA adducts and strand breaks [16]. MN are formed when cells undergoing mitosis fail to incorporate chromosome fragments into daughter cells [17,18].

From the result of the micronucleus assay, it was observed that micronucleus is the most occurring nuclear abnormality in the study population. This is followed by BN cells, KH, and cells with nuclei bud. A significant increase in the frequency of MN occurrence among road construction workers is similar to the reports of the previous studies [3,19].

Among the factors assessed among the road construction workers, it was observed that age, number of years in the occupation, work days per week, on-going medication, smoking habit, and alcohol consumption did not significantly affect the frequency of nuclear abnormalities in road construction workers. The reason for this pattern of results is not known, especially when certain lifestyle factors such as smoking and alcohol consumption are known risk factors predisposing to DNA damage [8]. Some other authors have reported that there was no significant difference in the frequency of nuclear abnormalities, especially MN between smokers and non-smokers who were road construction workers [3,19]. Sudha *et al.* [14], in a study involving metal arc welders, however, reported a significant increase in MN frequency among smokers when compared to non-smokers.

The result of this study indicates that age, number of years in the occupation, and number of work days per week did not significantly

Table 1: Demographic characteristics of the study population. Age and BMI are expressed as mean±standard deviation while other characteristics are expressed as absolute numbers (and percentages of total)

Demographic characteristic	Road construction workers (n=33)	Control subjects (n=33)
	Mean±SD	Mean±SD
Age	37.76±12.05	23.13±3.68
BMI	27.73±4.47	22.43±4.78
	n (%)	n (%)
Educational level		
Primary	9 (27.27)	0 (0)
Secondary	18 (54.55)	0 (0)
Tertiary	6 (18.18)	33 (100)
Smoking habit		
Smokers	11 (33.33)	0 (0)
Non-smokers	22 (66.67)	33 (100)
Alcohol consumption		
No	11 (33.33)	18 (54.55)
Yes	22 (66.67)	15 (45.45)

BMI: Body mass index, SD: Standard deviation

Table 2: Comparison of the frequencies of nuclear abnormalities between road construction workers and control subjects

Groups	Nuclear abnormalities (mean±SD)				
	CMN	TMN	BN	NB	KH
Road construction workers	0.65±1.05*	1.29±2.13*	0.50±0.00*	0.05±0.14	0.06±0.15
Control subjects	0.09±0.29	0.19±0.64	0.00±0.00	0.00±0.00	0.02±0.09

*p<0.05 using the t-test statistic. Road construction workers n=33; control subjects n=33. CMN: Cells with micronuclei, TMN: Total micronuclei, BN: Binucleate, NB: Nuclear buds, KH: Karyorrhectic,SD: Standard deviation

Table 3: Factors likely associated with increased frequency of nuclear abnormalities among road construction workers (n=33)

Factors	Nuclear abnormalities (mean±SD)				
	CMN	TMN	BN	NB	KH
Age					
<35	0.54±1.05	0.82±1.70	0.61±1.32	0.04±0.13	0.07±0.18
Above 35	0.74±1.07	1.63±2.38	0.42±0.63	0.05±0.16	0.05±0.16
Years in occupation					
5 years and less	0.35±0.38	0.62±0.77	0.42±0.57	0.00±0.00	0.08±0.19
More than 5 years	0.85±1.29	1.72±2.60	0.55±1.17	0.08±0.18	0.05±0.15
Work days per week					
3 days or less per week	0.63±0.97	1.37±2.29	0.34±0.50	0.05±0.16	0.08±0.19
More than 3 days per week	0.68±1.19	1.17±1.97	0.71±1.37	0.04±0.13	0.04±0.13
Use of protective equipment					
No	0.60±0.95	1.30±1.89	0.37±0.55	0.07±0.18	0.07±0.18
Yes	0.69±1.15	1.28±2.36	0.61±1.22	0.03±0.11	0.06±0.17
On-going medication					
No	0.50±0.92	0.92±1.56	0.56±1.16	0.06±0.16	0.06±0.16
Yes	0.83±1.19	1.73±2.64	0.43±0.70	0.03±0.13	0.07±0.18
Smoking habit					
Non-smokers	0.64±1.17	1.25±2.29	0.66±1.14	0.05±0.15	0.07±0.18
Smokers	0.68±0.81	1.36±1.87	0.18±0.25	0.05±0.15	0.05±0.15
Alcohol consumption					
No	0.45±0.76	0.82±1.27	0.55±0.61	0.05±0.15	0.09±0.20
Yes	0.75±1.17	1.52±2.44	0.48±1.11	0.05±0.15	0.05±0.15

Significance set at $p<0.05$ using the Mann–Whitney U-test. CMN: Cells with micronuclei, TMN: Total micronuclei, BN: Binucleate, NB: Nuclear buds, KH: Karyorrhectic, SD: Standard deviation

increase the frequency of nuclear abnormalities. Gattás *et al.* [20], however, reported that increased occupational exposure and age are related to a significant increase in frequency of MN.

Several factors are known to affect the pattern of results in studies such as this. The pattern of results is often affected by overestimates of the micronucleus frequency because smoking, for instance, is known to cause nuclear degeneration and appearance of MN-like bodies in exfoliated cells, which may easily be confused with MN [8,21]. Furthermore, the increased frequency of cells with degenerating nuclei may occur due to normal cytotoxic processes and may not necessarily be associated with occupational exposure. Variables such as disease history, inherited mutations, dietary habits, and exposure to other potentially toxic substances may also affect the results [21-22].

CONCLUSION

From the results of the present study, it may be concluded that road construction workers might be exposed to certain potentially toxic substances during the course of their work. These substances may have genotoxic properties which were seen in this study as the occurrence of a higher frequency of MN in the exfoliated buccal epithelial cells of the road construction workers compared to the controls.

REFERENCES

1. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Bitumens and Bitumen Emissions, and Some N-and S-heterocyclic Polycyclic Aromatic Hydrocarbons. Lyon, France: International Agency Research Cancer WHO; 2013.
2. Khan MR, Sudha S. Evaluation of genotoxicity in automobile mechanics occupationally exposed to polycyclic aromatic hydrocarbons using micronuclei and other nuclear abnormalities. Iran J Cancer Prev 2012;5:87-92.
3. Çelik A, Yildirim S, Ekinci SY, Taşdelen B. Bio-monitoring for the genotoxic assessment in road construction workers as determined by the buccal micronucleus cytome assay. Ecotoxicol Environ Saf 2013;92:265-70.
4. Asphalt Institute. The Bitumen Industry-a Global Perspective: Production, Chemistry, Use, Specification and Occupational Exposure. Lexington: Asphalt Institute; 2008.
5. Haas EJ Willmer D, Cecala AB, Haas EJ, Cecala AB. Compendium of NIOSH Construction Research. Vol. 69. Ministry of Engineering Conference; 2002. p. 105-9.
6. Sellappa S, Mani B, Keyan KS. Cytogenetic biomonitoring of road paving workers occupationally exposed to polycyclic aromatic hydrocarbons. Asian Pac J Cancer Prev 2011;12:713-7.
7. van Thienen G, Spee T. Health effects of construction materials and construction products. J Appl Occup Sci 2008;14:2-23.
8. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, *et al.* 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;659:93-108.
9. 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:ZC82-5.
10. Ochei JO, Kolhatkar AA. Medical Laboratory Science: Theory and Practice. London: McGraw Hill Education; 2000.
11. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: Methods development. Mutat Res 1992;271:69-77.
12. Kumar M, Prasad UC, Chandolia B, Manjunath SM, Basu S, Verma S, *et al.* Can feulgen stain be a reliable biomarker over PAP stain for estimation of micronuclei score? J Clin Diagn Res 2016;10:ZC07-11.
13. Bloching M, Hofmann A, Lautenschläger C, Berghaus A, Grummt T. Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. Oral Oncol 2000;36:550-5.

14. 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;12:1041-4.
15. 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;24:302-7.
16. Kriech AJ, Schreiner CA, Osborn LV, Riley AJ. Assessing cancer hazards of bitumen emissions a case study for complex petroleum substances. Crit Rev Toxicol 2018;48:121-42.
17. Torres-Bugarín O, Zavala-Cerna MG, Nava A, Flores-García A, Ramos-Ibarra ML. Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells. Dis Markers 2014;2014:956835.
18. Nagarathna PK, Wesley MJ, Reddy PS, Reena K. Review on genotoxicity, its molecular mechanisms and prevention. Int J Pharm Sci Rev Res 2013;22:236-43.
19. Arul P. Application of liquid-based cytology preparation in micronucleus assay of exfoliated buccal epithelial cells in road construction workers. Indian J Dent Res 2017;28:413-7.
20. Gattás GJ, Cardoso Lde A, Medrado-Faria Mde A, Saldanha PH. Frequency of oral mucosa micronuclei in gas station operators after introducing methanol. Occup Med (Lond) 2001;51:107-13.
21. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, *et al.* Buccal micronucleus cytome assay. Nat Protoc 2009;4:825-37.
22. Diler SB, Ergene S. Nuclear anomalies in the buccal cells of calcite factory workers. Genet Mol Biol 2010;33:374-8.