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Association Between Lifestyle and Genotoxicity in Undergraduate Subjects in Benin City, Nigeria

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ABSTRACT

Background of Study: Genotoxicity describes the characteristic of chemical substances known as genotoxins that harm the genetic material inside a cell, resulting in mutations that may predispose to cancer.

Methods: This research utilized a cross sectional study design aimed at determining possible association between lifestyle factors and genotoxicity of undergraduate subjects in Benin City. One hundred and ninety five (195) subjects, involving one hundred and fifty (150) undergraduates as study subjects and forty-five (45) intending students as control participated. Questionnaires were used for data collection while a wooden spatula was used to obtain buccal epithelial cells for micronuclei investigation. The presence of micronuclei (MN) was assessed under light microscopy and a total of 200 cells per sample were scored.

Results: The results showed that there was no significant statistical difference ($p>0.05$) between the mean micronuclei (Mni) cells counts of the non-cigarette smokers and former smokers; However, the average Mni count for Shisha smokers was statistically significantly lower than non-shisha smokers. The average Mni count between alcohol consumers and non-consumers was not statistically significant. The mean micronuclei (Mni) count per 200 cells was (5.56±1.30) in undergraduate compared to intending students (3.72±1.39), the mean Mni in age group ≥ 20yrs was 4.32±1.39 while the average Mni for ≤ 20yrs of age was (2.82±1.29) with each of these groups being statistically significant (p-values =0.02).

Conclusion: There is a clear correlation between lifestyle factors and genotoxicity in undergraduate subjects in this study as observed from the increase in buccal cell micronucleus.

Keywords: Buccal cells, Genotoxicity, Genotoxins, Lifestyle factors, Micronucleus.

1. INTRODUCTION

Genotoxicity is a term used to describe the property of chemical compounds called genotoxins that damage the genetic material within a cell, resulting in mutations that may lead to cancer. The somatic cells of the organism or the germ cells that will be passed on to following generations may change as a result of these heritable changes caused by genotoxicity [1]. Apoptosis or DNA repair can stop the manifestation of genotoxic mutations, but sometimes the damage cannot be repaired, which results in mutagenesis. The epithelium of the oral cavity undergoes exfoliation of its superficial cells. The cells of the deeper layer are adherent to each other and are not normally shed unless there is a pathological condition or disease. The cells may lose their cohesiveness and the cells in the deeper layer may also shed along with the superficial cells [2]. This damage can occur as a result of environmental exposure to genotoxins, lifestyle factors (eg, alcohol, smoking, drugs, and stress) [3], and it has become essential to harvest these cells and investigate the underlying condition that may have led to the exfoliation of the harvest these cells and investigate the underlying condition that may have led to the exfoliation of the basal layer, including other deeper layers [4, 5]. Students these days have taken up the habit of drinking, smoking and other lifestyle factors just for fun disregarding the adverse effects these might cause. Not only can these cause stress on organs such as the liver, lungs, etc when continued consistently, it can also damage the oral mucosa, may cause genomic damage or even lead to oral cancer. Environmental

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conditions also play a huge role as students may have one way or the other been exposed to toxins and ionizing radiation. On the other hand, there are limited studies and data relating to the subject matter. This research will educate students and expose them to the extent at which certain life styles may impair the functionality of buccal cells and the pathological conditions that may be induced resulting from certain lifestyles.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Frosted end glass slides, coplin jars, staining racks, wooden spatula, 95% alcohol, stains used; Papanicolaou stain, May-Grunwald Giemsa stain

2.1.2 Biological Material

The biological material used for this research was buccal cells collected from the inner walls of the cheek.

2.2 Methods

2.2.1 *Research Design*

This research utilized a cross sectional study design to determine the association between lifestyle and genotoxicity in the buccal smear of undergraduate subjects in the University of Benin, Benin City. Ethical approval from Edo State Ministry of Health Research Ethics committee in Benin City was obtained. Written informed consent was also obtained from the subjects and participation was voluntary offering no incentives. The purpose, process and all information regarding the study was clearly conveyed to the subjects.

2.2.2 Study Area

This study was conducted on the various halls of residence in University of Benin. Intending students who were much younger and have not been exposed to the University were selected as control.

2.2.3 Target Population

The study population included all undergraduate subjects in the various halls of residence, University of Benin, Benin city.

2.2.4 Sample Size/Sampling Technique

This cross-sectional study was conducted among 150 systematically selected undergraduate subjects comprising both male and female who are occupants in the halls of residence. Multistage sampling was used to select the study subjects. Blocks were first selected, followed by selection of rooms using simple random sampling technique. Sampled subjects were then selected systematically using the number of subjects in a room as the sampling frame.

45 Control subjects were systematically selected among adolescents who are intending students and are yet to be given admission to the University.

2.2.5 Sample Collection

The subject was first asked to rinse his/her mouth with water to remove food particles. Buccal epithelial cells were then collected twice from each subject by scraping against the upper and lower quadrant of the inner walls of the cheek using a wooden spatula. Both sides of the cheek were scraped to ensure collection of enough cells. These were then smeared onto two separate glass slides; one dipped into a coupling jar containing 95% alcohol to fix and the other air dried.

2.2.6 Data Collection

The tool of data collection was a self-developed, structured questionnaire validated by my supervisor. The questionnaire elicited information on socio-demography, oral health status and lifestyle factors (which included smoking and alcohol consumption).

2.3 Statistical Analysis

Data obtained were subjected to descriptive, non-parametric and regression statistics using the Statistical Package for the Social Sciences (SPSS version 22 software). $P< 0.05$ was considered as significant.

3. RESULTS

The results showed that the mean micronuclei (Mni) cells counts of the non-cigarette smokers (5.17±1.37) was higher than the Mni cell count for both the current (4.33±1.61) and former smokers (3.36±1.32); but

there was no significant statistical difference (p>0.05); However, the average Mni count for Shisha smokers (4.02±1.29) was statistically significantly lower than non-shisha smokers (5.67±1.37). The average Mni count in alcoholic consumer (5.02±1.44) was higher than non-alcoholic consumer (5.00±1.35) but not statistically significant. The mean micronuclei (Mni) count per 200 cells was (5.56±1.30) in undergraduate compared to the Jambite (3.72±1.39), the mean Mni in age group ≥ 20yrs was 4.32±1.39 while the average Mni for ≤ 20yrs of age was (2.82±1.29) with each of these groups being statistically significant (p values =0.02).

Table 3.1: The Academic Level of the Study Population

Variable	Category	Frequency	Percentage
Group	Jambite	45	23.1
	Undergraduate	150	76.9
	Total	195	100
Level	Jambite	45	23.1
	100	41	21
	200	11	5.6
	300	49	25.1
	400	19	9.7
	500	30	15.4
	Total	195	100

Table 3.2: Sociodemographic characteristics of the study population

Variable	Category	Frequency	Percentage
Gender	Female	103	52.8
	Male	92	47.2
	Total	195	100
Age	0-19	85	43.6
	20 above	110	56.4
Marital status	Married	2	1
	Single	193	99
Religion	Christian	187	95.9
	Muslim	8	4.1
	Total	195	100

Table 3.3: Dental care distribution across the population

Variables	category	Frequency	Percentage
Brush Teeth	>1	60	30.8
	once	135	69.2
	Total	195	100
MOUTH WASH	No	136	69.7
	Yes	59	30.3
ORAL PROSTHESIS	No	179	91.8
	Yes	16	8.2

Table 3.4: The average micronuclei per 200 cells count across the sociodemographic

		Micronuclei		
Variable	Category	Mean±SD	t-value	p-value
Groups	Jambite (n-=45)	3.72±1.39	2.36	0.02
	Undergraduate(n=150)	5.56±1.30		
Sex	Female(n=103)	4.12±1.39	1.44	0.15
	Male (n=92)	3.13±1.37		
Age group	≤19yrs (n=85)	2.82±1.29	2.29	0.02
	≥ 20 yrs (n=110)	4.32±1.39		

Table 3.5: Mean comparison between the dental care variables' category

Variables	Categories	Micronuclei/200 cells count	t-value	p-value
Brushing Teeth	>1 (n=50)	5.32±1.44	0.58	0.56
	once (n=135)	4.87±1.34		
Mouth Wash	No (n=136)	5.52±1.46	2.24	0.012
	Yes (n=59)	3.83±1.12		
ORAL PROSTHESIS	No (n=179)	5.16±1.39	1.40	0.16
	Yes (n=16)	3.38±1.06		

Table 3.6: Mean comparison of the Mni count across the associated risk factors.

Variables	**Categories (n)**	**Mean±SD Micronuclei/200 cells count**	Test Statistics	p-value
Cigarette Smoking	Never (n=169)	5.17±1.37	0.86	0.42
	Current (n=15)	4.33±1.61		
	Former (n=11)	3.36±1.32		
C. Smoking Frequency	Daily (n=4)	2.25±1.64	0.71	0.51
	Weekly (n=7)	4.71±2.13		
	Rarely (n=4)	5.75±0.49		
Smoking Shisha	No (117)	5.67±1.37	2.41	0.02
	Yes (78)	4.02±1.29		
Alcohol Consumption	No (n=110)	5.00±1.35	0.03	0.97
	Yes (n=85)	5.02±1.44		

Table 3.7: Regression Analysis of biological variables and micronuclei predictors

Variables	B	95% CI for B	β	t-value	p-value
constant	2.69	-1.12 - 6.49		1.39	0.16
Age	0.13	-0.08- 0.33	0.1	1.25	0.21
Sex (male)	-1.34	-2.75 - 0.07	-0.14	1.88	0.06
Groups (undergrad)	1.88	0.15 -0 3.61	0.16	2.15	0.03
mouth wash (yes)	-1.49	-2.95 – (-0.03)	-0.14	2.02	0.04
Smoke Shisha (yes)	-1.72	-3.09 – (-0.35)	-0.17	2.48	0.01
adj. R^2	0.078				

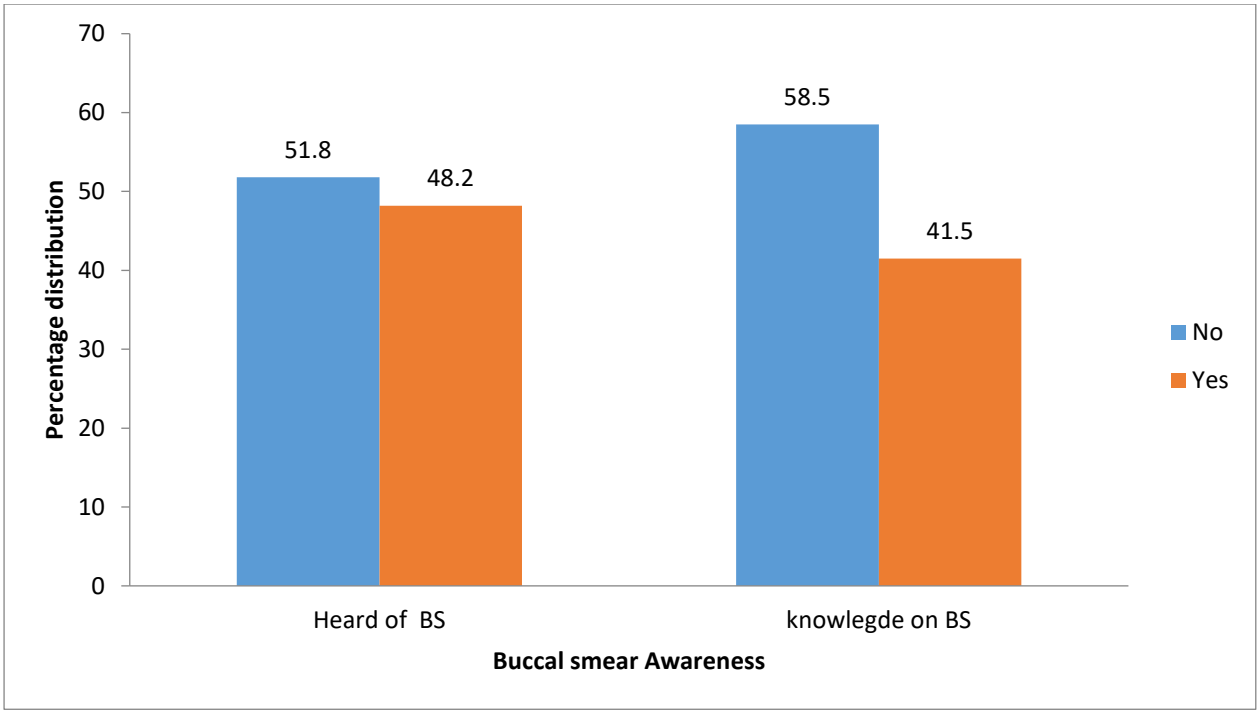

Figure 3.1: Bar Chart showing distribution of the level of awareness on buccal smear by the participant.

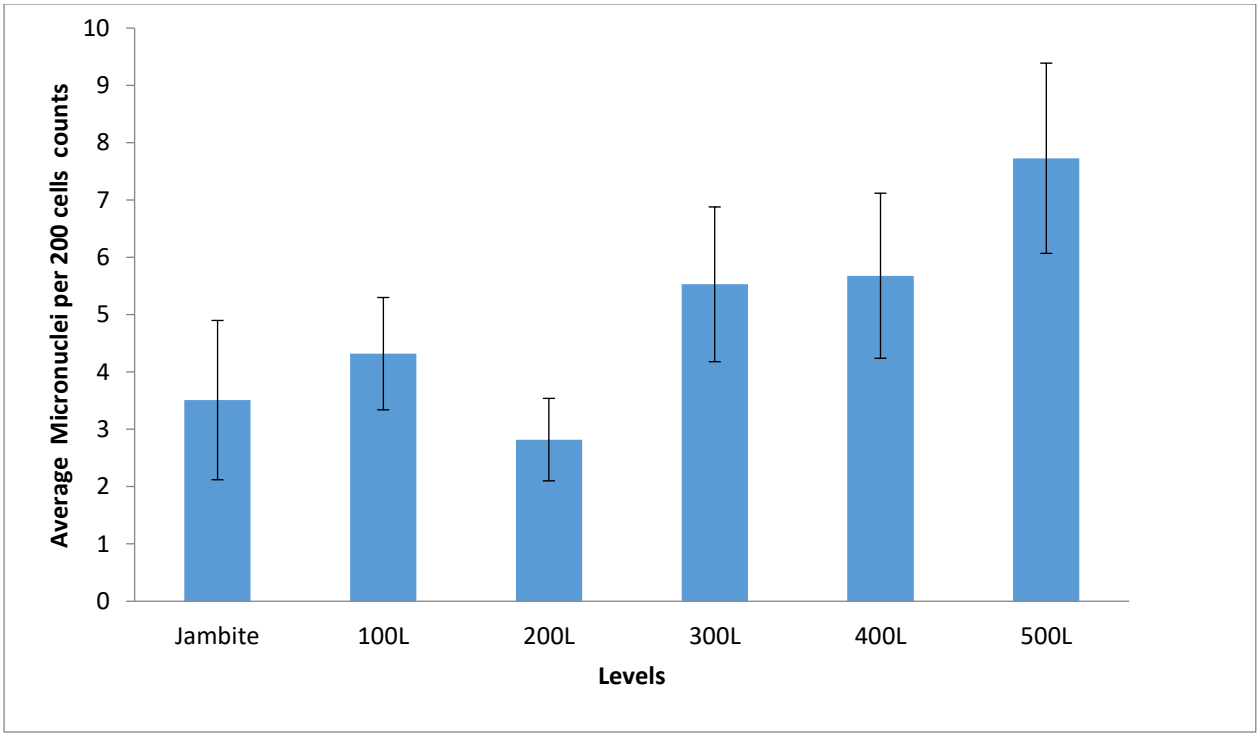

Figure 3.2: Comparison of Average Mni/ 200 cells across the Participant according to academic year levels

4. DISCUSSION

The epithelium of the oral mucosa maintains itself by continuous renewal of cells. The new cells which are produced by mitosis migrate from the basal layer to replace shed cells in the surface epithelium [6]. Buccal cell changes such as the presence of micronuclei are generally seen as practical biomarkers of cancer risk in humans. Upon exposure to genotoxins, the basal layer of the mucosa which contains stem cells that may express genetic damage are affected resulting to chromosome breakage/loss and

Micronuclei (MN) formation [7]. The results of this research yielded astonishing results with the exclusion and inclusion criteria employed in the screening of participants presenting a sociodemographic with 52.8% of respondents being females whilst the other 47.2% male, 56.4% of the study population being higher than 20 years of age whilst the other 43.6% being less than 20 years of age, and only 1% of the study population were married. However, the vast majority of the participants had no prior encounter and base knowledge of buccal cell cytology. The results obtained from the research and experiment showed that dental care variables were established across the various participants of the study leading to the formation of groups and categories for analysis. Mean Mni cells counts of 4.87±1.34, 3.83±1.12, and 3.38±1.06 were observed in subjects who brush once daily, use mouth wash, and use oral prosthesis. These Mni cell count values were lower than the cell count of subjects who brush more than once daily (5.32±1.44), do not use any kind of mouth wash (5.52±1.46) and oral prosthetic non-users (5.16±1.39) respectively. A p-value of 0.012 was observed between subjects who were mouth wash users and those who did not, clearly showing a statistically significant difference ($p<0.05$). This is in agreement with the findings of Karthik [8] who observed that chronic exposure to mouth rinses can cause genotoxic damage to buccal epithelial cells. Their study evaluated the genotoxicity of non-alcoholic mouth washes/rinses on the buccal cells via a micronucleus test and the results of which showed that micronucleate cells ($P < .00$) and MN ($P < .00$) were higher in individuals exposed to chlorhexidine (CHX), followed by chlorine dioxide (ClO2), potassium nitrate (KNO3), and sodium fluoride (NaF), and amine fluoride (AmF) which are extensive components of mouth washes against the control group exposed to normal saline. However, the results obtained from the analysis of the oral prothesis groups and the frequency of teeth brushing groups in our present study did not show any significant significance thus eliminating the chances of a correlation with formation of micronuclei. In this study, the analysis of buccal micronuclei cells count against Cigarette/weed smoking which is an associated risk factor for genotoxicity was carried out. The mean Mni cells count of the non-cigarette smokers (5.17±1.37) was higher than the Mni cells count for both the Current (4.33±1.61) and Former smokers (3.36±1.32); but there was no significant statistical difference ($p>0.05$). This result disagrees with Fenech (2009) who after adjustment for age and sex, stated that individuals with high cigarette usage (>30) had statistically greater MN compared to non-smokers. This difference in results could very well be attributed to the difference in frequency of cigarette/weed smoking (dosage) of participants in both studies seeing as Fenech's results was for individuals with high usage smoking around 30 cigarettes per day. However, the lack of significant difference observed in our study conforms with the comprehensive analysis of 24 databases (3501 non-smokers, 1409 current smokers, and 800 former smokers) for the effects of smoking on MN induction that was conducted as part of the HUMN project [9] with most laboratories equally showing no significant differences between smokers and non-smokers. The pooled analysis of this HUMN project interestingly, indicated an overall decrease for all smokers compared to controls [9]. Surprisingly, whilst no significant difference was observed between cigarette/weed smokers and non-smokers, the opposite was the case for Shisha smokers. 44.6% of the participants smoked shisha; the mean Mni cell count for Shisha smokers is 4.02±1.29 whilst that Mni cell count for non-shisha smokers is 5.67±1.37 depicting a significant statistical difference with a p value of 0.02. These results implied a direct correlation between smoking of Shisha and formation of Micronuclei in the buccal mucosa cells and is consistent with the findings of kalan et al., [10], who reported a significant difference (p-value = 0.00) between the mean Mni cells count of water pipe (Shisha) smokers (1.94±0.39) and non-smokers (1.68±0.35) [10]. These finding are also consistent with the study carried out by El-Setouhy et al., [11]. It can be inferred from this results that the belief stating that smoking Shisha is harmless in comparison to cigarette/weed smoking is false and non-factual because the smoke from the Shisha pipe still contains toxic materials such as carbon-monoxide (CO) and various heavy metals and some studies show that the carboxyhaemoglobin levels of Shisha smoking is a third times higher than cigarette [12]. Alcohol consumption as a lifestyle factor did not pose a risk of genotoxicity in the buccal smear. From the results obtained, the average Mni count in alcoholic consumers (5.02±1.44) was a tad bit higher than Mni count for non-alcoholic consumers (5.00±1.35) but presenting a p-value of 0.97 there was no significant difference. This result is consistent with Ladeira et al., [1] who noted higher means of Mni count in drinkers (0.18±0.068) and in smokers (0.2±0.074) in comparison with non-drinkers (0.11±0.105) and non-smokers (0.08±0.08) without statistical significance (Mann-Whitney test, $p>0.05$). However, a strong synergistic effect between alcohol consumption and cigarette smoking was noticed showing statistical signification ($p=0.043$) with a stronger effect from tobacco smoke [1]. An association between increase in the formation of buccal cells micronucleus and age was observed as the result showed that the mean micronuclei (Mni) count per 200 cells was higher (5.56±1.30) in undergraduate compared to the Jambite (3.72±1.39) who were the control group. Also, the mean Mni in agegroup ≥ 20 yrs was 4.32±1.39 which was almost 2 times greater the average Mni for under 20 years of age (2.82±1.29). This

presented a statistically significant (p-values = 0.02) difference in both the academic status groups (Jambite and Undergraduate) and the Age groups (≤19yrs and ≥ 20 yrs). However further research is required in this area as sufficient literature for this age range are lacking. A multivariate regression model was employed to test the effect of the confounding variables and other important biological variables and the results of this analysis showed that the group of variables (Age, sex, Groups, Mouth wash and shisha smoking) statistically significantly predicted micronuclei level F (5,189) = 4.25, p=0.001. adj. R2 =0.078. The combined variables can account for 7.8% variance in the micronuclei value of the oral mucosal cavity. Groups, mouth wash and shisha smoking significantly ($p<0.05$) independently predict Mni while other variables are held constant. For a year increase in age there is 0.13 increase in micronuclei, not statistically significantly different. The micronuclei count of the male was 1.34 lower than female while other variables were held constant, the difference was not statistically significant. The micronuclei value of the mouth wash user was 1.49 significantly lower than non-user when other variables were held constant. While other variables were controlled, the micronuclei of the shisha smokers were 1.72 significantly lesser than non-smoker. Also, the micronuclei value of the undergraduate was 1.88 statistically significantly higher than the Jambite. The R2 value from this model also shows that 78% of the amount of Mni cell counts can be attributed to the independent variables ultimately affirming that use of mouthwash and smoking of shisha leads to genotoxicity via the formation of buccal cells micronucleus.

5. CONCLUSION

These findings show a clear correlation between lifestyle and genotoxicity in buccal smear of undergraduate subjects in the University of Benin as observed from the increase in buccal cell micronucleus. This study could serve as a yardstick to educate students and expose them to the extent at which certain lifestyles may impair the functionality of buccal cells and the pathological conditions that may be induced resulting from these lifestyles.

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Conflict of interest:
Authors declare that no conflicting interest exists

Contribution of the authors:
EBO and **OI** conceived the research ideas. All authors contributed to the following: design of the study, field exercise, acquisition of samples and data, laboratory investigation and data analysis. **OI** and **BED** conducted literature search and drafted the manuscript. All authors critically revised the manuscript for valuable intellectual content while **EBO** read and approved the final draft. All authors read and approved the final revised manuscript. All authors made significant contributions that warrant authorship. **IO** acted as corresponding author on behalf of others.

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