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Relationship Between Behavioural Characteristics and Cytotoxicity in Undergraduate Subjects in Benin City, Nigeria

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ABSTRACT

Background of Study: Exfoliative cytology is the microscopic examination of desquamated cells from the epithelial surface, usually the mucous membrane. It also includes the study of cells that can be collected from body fluids such as sputum, saliva and pleural fluid. These exfoliated cells removed using specific instruments, can be studied quantitatively or qualitatively.

Methods: This research utilised a cross-sectional study to determine the relationship between cytotoxicity and behavioural characteristics in buccal smear among undergraduate students at the University of Benin. A total of one hundred and ninety-five (195) participants were included. Participants' socio-demographics were collected via questionnaires. The Papanicolaou staining technique was used and viewed using a microscope. However, α=0.05 and student T-test was used to assess the data.

Results: The findings indicated that Karyolysis was higher in the test group than the control group; however, this was not significant (P> 0.05); the Karyorrhesis was higher in the test group (0.81±0.44) than the control group (0.27±0.46) and was highly significant (P=3.3; P> 0.05). Also, the Pyknosis was higher in the test group than the control group and was significant. However, the comparison between male and female consenting participants and among age group showed no level of significance.

Conclusion: This study concludes that there is an association between oral sex and Pyknosis, indicating cytotoxicity. Also, biological factors (gender and age) and lifestyle factors (chewing gum, drinking hot beverages or playing musical instruments) have no relationship between cytotoxicity and behavioural characteristics in buccal smear of undergraduate subjects in this study.

Keywords: Buccal cells, Cytotoxicity, behavioural characteristics, Karyolysis, Karyorrhesis, Pyknosis

1. INTRODUCTION

All living things experience cell division except for cells that have finished somatic differentiation. In multicellular creatures, cell division enables the growth of hair and nails, buccal mucosa, the healing of wounds, cellular repair, somatic growth, and the genesis of reproductive cells. Still, in single-celled species, it results in a rise in the number of people [1] [2]. Cytotoxicity is the degree to which a substance can cause damage to a cell. A cytotoxic compound can cause cell damage or death through necrosis or apoptosis. Karyorrhexis is the destructive fragmentation of the nucleus of a dying cell whereby its chromatin is distributed irregularly throughout the cytoplasm. It is usually preceded by Pyknosis and can occur because of programmed cell death, cellular senescence, or necrosis [3]. The epithelium of the oral cavity undergoes exfoliation of its superficial cells. The cells of the deeper layer adhere to each other and are not normally shed unless there is a pathological condition or disease. These cells may lose their cohesiveness, and the cells in the deeper layer may also shed along with the superficial cells [2]. Cytotoxicity can result from spills, needle stick injuries, unintentional contact with cytotoxic substances without personal protective equipment, lack of training, insufficient controls, and poor communication.

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Mutation, carcinogenicity, and unfavourable reproductive outcomes like infertility, abortion, and poor newborn outcomes are some of the long-term health implications of cytotoxicity [4]. A buccal swab, also known as a buccal smear, is a way to collect DNA from the cells inside a person's buccal mucosa. Buccal swabs are a relatively non-invasive way to collect DNA samples for testing. Buccal means cheek or mouth. It is very common in clinical trials and law enforcement investigations, which can include or exclude individuals as suspects [5]. A buccal smear is a test where cells are taken from the cheek. Cells are collected by scraping the cheek with a cotton swab. Chemical agents may act by hampering protein or nucleic acid synthesis in the cell, weakening the membrane in the cell or impeding cellular energy production pathways [6] [7]. And many of these can be found in the buccal cavity. Environmental conditions also play a huge role as students may have been exposed to toxins and ionising radiation one way or the other. On the other hand, there are limited studies and data relating to the subject matter. This research evaluates the relationship between cytotoxicity and behavioural characteristics in buccal smear among undergraduate students in the University of Benin.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Papanicolaou stain, frosted end glass slides, preservative vials containing alcohol, coupling jars, sterile cotton swaps

2.1.2 Biological Material

The biological material used for this research was buccal cells collected from male students in the University of Benin residence halls.

2.2 Method

2.2.1 Research Design

This research utilised a comparative cross-sectional study design, with the aim to determine the relationship between cytotoxicity and behavioural characteristics in buccal smear among undergraduate students in the University of Benin. Ethical approval from the Edo State Ministry of Health Research Ethics committee in Benin City was obtained. Written informed consent was also obtained from the subjects, and voluntary participation offered no incentives. The purpose, process and all information regarding the study were conveyed to the subjects.

2.2.2 Study Area

This study was conducted in the various halls of residence at the University of Benin. Intending students who were much younger and had yet to be exposed to the University were selected as control.

2.2.3 Target Population

The study population included undergraduates in the various residence halls at the University of Benin, Benin city.

2.2.4 Sample Size/Sampling Technique

This cross-sectional study was conducted among 195 systematically selected undergraduate subjects comprising male and female occupants in residence halls. Multistage sampling was used to select the study subjects. Blocks were first selected, followed by a selection of rooms using a simple random sampling technique. Sampled subjects were then selected systematically using the number of subjects in a room as the sampling frame.

2.2.5 Sample Collection

Buccal cells were collected using a sterile cotton swab by scraping the upper quadrant of the cheek from 92 male and 103 female students and smeared on two glass slides for each. Smear on one glass slide was allowed to air-dry and then stained with PAP stain within 6 hours, while the other two were stained with Giemsa.

2.2.6 Data Collection

The tool of data collection was a self-developed, structured questionnaire. The questionnaire elicited information on socio-demography and lifestyle variables.

2.3 Statistical Analysis

Data obtained was presented as Mean ± S.D. (standard deviation) and was analysed using Statistical Processor System Support "SPSS" for Windows software to compare all the treated groups. LSD (least significant

difference) analysis was performed to assess the significance of the differences among various treated groups, with a significance level of $p < 0.05$.

3. RESULTS

The distribution of the chew gum habit showed that 29 (14.9%) chew gum often while 66 (33.8%) rarely did. Subconscious cheek chewing among the population showed equal distribution (46.7%) between the test and the control. Hot beverage lovers were more among the controls (48.9%) and the test (39.3%). The mean karyolitic cells of the test and control groups were (3.12±1.23) and (2.87±1.14), respectively. The mean karyorrhectic cell level in the control (was 0.27±0.46) and test (was 0.81±0.44) while the mean pyknotic cell in the control and the test were 1.53±0.81 and 4.13±0.98 respectively. The mean comparison between the groups for each cytotoxic feature showed a statistically significant mean difference in the pyknotic cells and Karyorrhectic cells. However, the mean difference in the karyolitic between groups was insignificant (p=0.97). The mean karyolytic cells count (3.23±1.66), Karyorrhesis (0.73±0.44), and Pyknosis (4.04±1.04) in the age group >20 were higher than the age group <20. The Mean difference in the Pyknosis was statistically significant ($p<0.05$), but the mean difference in the karyolytic and karyorrhexis count was statistically not significant ($p>0.05$). The average pyknotic (3.11±1.56) and karyolytic (2.76±0.86) count of male subjects was higher than the female, but the mean difference was not statistically significant (p-values >0.05). The mean karyorrhexis count was higher in females (0.71±0.42) than in males, but the mean difference was insignificant.

Table 3.1: Socio-demographic characteristics of the study population

Variable	Category	Frequency	Percentage
Gender	Female	103	52.8
	Male	92	47.2
	Total	195	100
Age	16 – 25	85	43.6
	25 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 3.2: Mean Cytotoxic features comparison between test and control groups

Variable	Control (n=45, Mean±SD	Test (n=150, Mean±SD	t-value	p-value
Karyolysis	2.87±1.14	3.12±1.23	0.04	0.97
Karyorrhesis	0.27±0.46	0.81±0.44	3.3	0.001
Pyknosis	1.53±0.81	4.13±0.98	4.38	<0.001

Table 3.3: Mean comparison of the cytotoxic features (Karyolysis, Karyorrhesis, and Pyknosis) between age groups.

Variable	≤19yrs (n=85, Mean±SD)	≥ 20 yrs (n=110, Mean±SD)	t-value	p-value
Karyolysis	2.85±1.23	3.23±1.66	0.6	0.55
Karyorrhesis	0.62±0.49	0.73±0.44	0.47	0.64
Pyknosis	2.85±0.94	4.04±1.04	2.42	0.02

Table 3.4: Mean comparison of the cytotoxic features (Karyolysis, Karyorrhesis, and Pyknosis) between gender

Variable	Female (103, Mean±SD)	Male (n=92, Mean±SD)	t-value	p-value
Karyolysis	3.02±1.42	3.11±1.56	0.12	0.91
Karyorrhesis	0.71±0.42	0.65±0.39	0.26	0.79
Pyknosis	2.34±1.12	2.76±0.86	0.35	0.73

Table 3.5: Mean buccal cavity Cytotoxic features distributions across the behavioural status of the respondents

Variables	Categories	Karyolysis	Karyorrhesis	Pyknosis
Chew Gum	Rarely (n=66)	2.88±1.46	0.71±0.46	3.89±1.06
	Sometimes (n=100)	3.02±1.37	0.68±0.50	3.54±1.00
	Frequently (n=29)	3.63±1.96	0.62±0.40	2.69±0.85
	p-value	0.75	0.97	0.34
Chew Cheek Subconsciously	No (n=104)	3.46±1.72	0.57±0.40	3.76±0.94
	Yes (n=91)	2.60±1.89	0.81±0.55	3.27±1.08
	p-value	0.17	0.27	0.36
Chewing Cheek Frequency	Rarely (n=58)	2.53±1.20	0.93±0.63	3.33±1.11
	Regularly (n=33)	2.73±1.20	0.61±0.41	3.18±1.06
	p-value	0.81	0.41	0.86
Hot Beverage	No (n=28)	2.89±1.54)	0.54±0.42	3.25±1.23
	Seldomly (n=86)	2.84±1.27	0.62±0.42	3.79±1.18
	Often (n=81)	3.36±1.68	0.80±0.53	3.36±0.76
	p-value	0.73	0.64	0.68
CHEW OR HOLD OBJECTS	No (n=91)	3.19±1.64	0.57±0.42	3.68±1.13
	Yes (n=104)	2.95±1.32	0.78±0.50	3.40±0.91
	p-value	0.71	0.35	0.6
ORAL SEX	No (n=150)	3.21±1.56	0.59±0.40	3.16±0.90
	Yes (n=45)	2.49±1.17	1.00±0.68	4.78±1.26
	p-value	0.32	0.19	0.03
How often (Oral Sex)	Once (n=8)	4.38±0.99	0.63±0.63	4.75±1.01
	Few times (n=30)	1.80±1.03	1.20±0.76	5.30±1.36
	Many Times (n=7)	3.73±1.36	0.57±0.39	2.57±1.03
	p-value	0.14	0.63	0.36
Musical Instruments	No (n=154)	3.10±1.49	0.76±0.52	3.72±1.01
	Yes (n=29)	2.93±1.41	0.39±0.25	2.83±1.06
	p-value	0.82	0.17	0.16

Table 3.6: Multivariate analysis of the Pyknosis-associated predictors

Variables	B	95% CI for B	B	t-value	p-value
Constant	1.46	1.52 - 4.07		0.902	0.368
Age	0.011	0.14 - 0.16	0.011	0.139	0.889
Gender	0.56	1.615 - 0.504	0.076	1.034	0.303
Groups	2.53*	1.266 - 3.795	0.292	3.945	<0.001
Oral Sex	1.53*	0.315 - 2.737	0.176	2.485	0.01
adj. R^2	0.102				

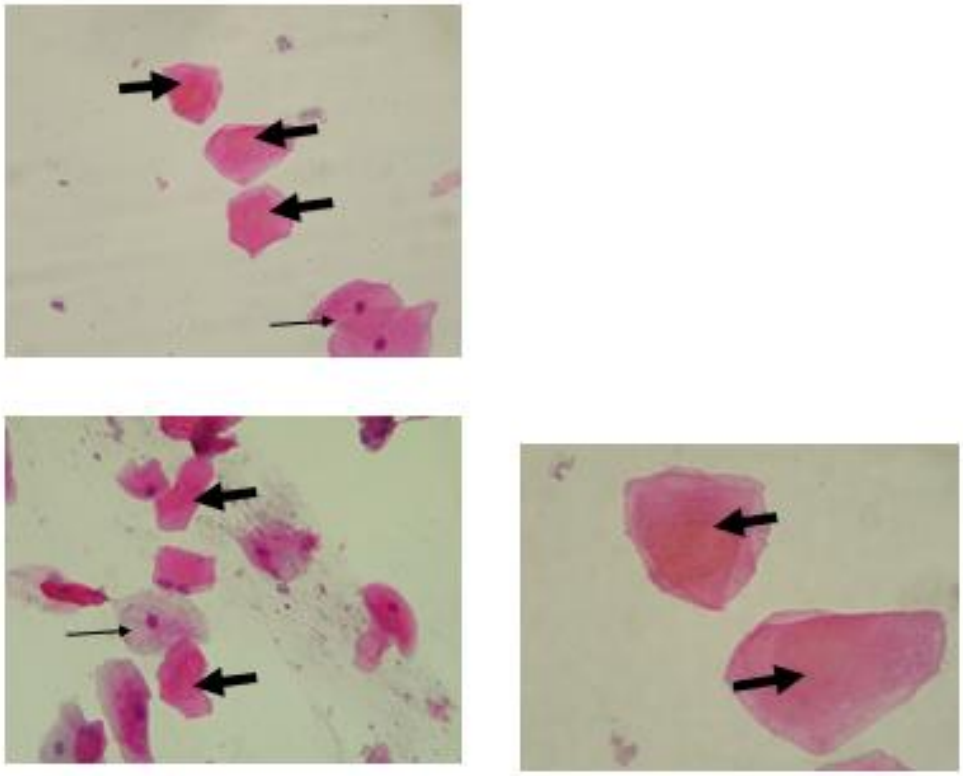

Figure 3.1: Karyolysis x400&1000 Magnification. Cytological photomicrograph showed Normal cells (thin arrow) and cell(s) that have undergone Karyolysis (thick arrow) without nuclear material.

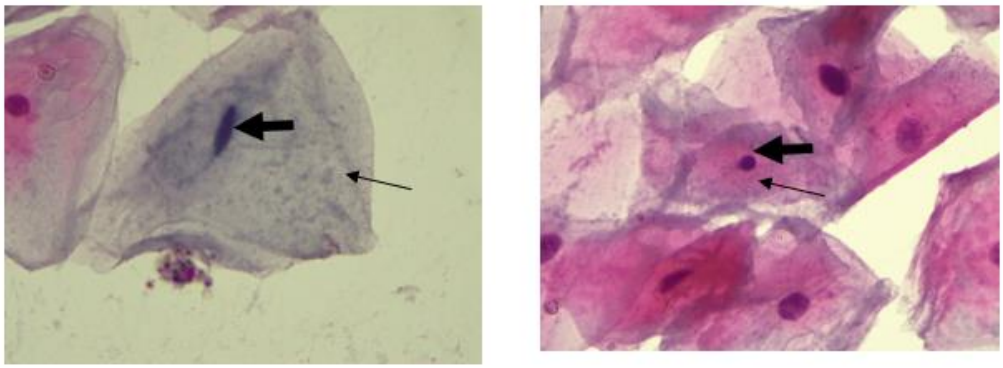

Figure 3.2: Pyknosis at x1000 magnification. Cytological photomicrograph showed cell(s) with pyknotic nucleus (thick arrow) and the cytoplasm (thin arrow).

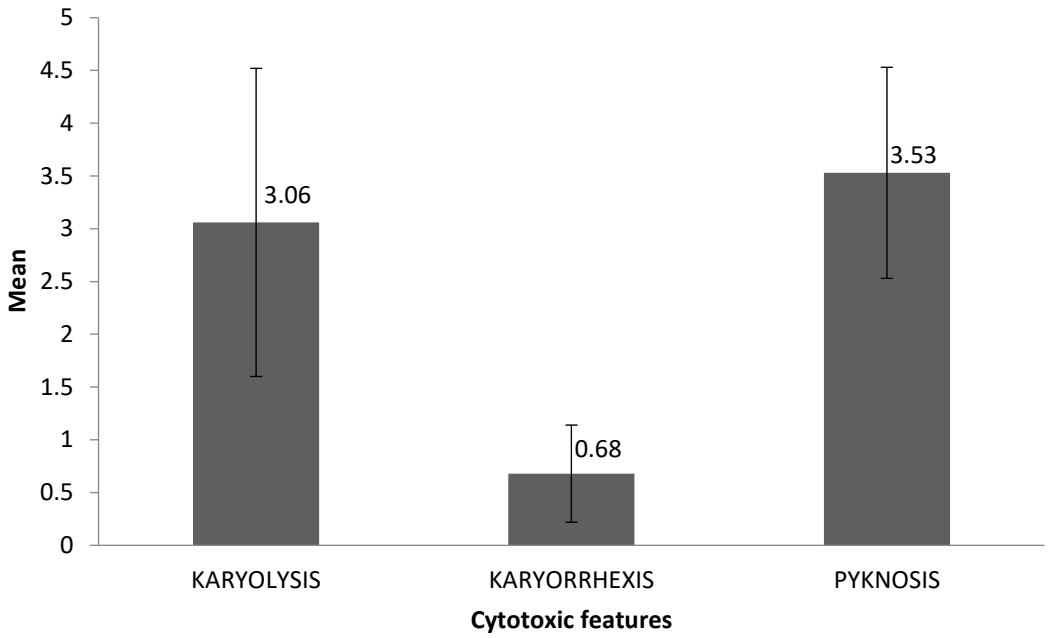

Figure 3.3: The Average buccal cavity cytotoxic feature features

4. DISCUSSION

The social demographic of the participants in the study presented a gender distribution of 52.8% females and 47.2% males, the age distribution of 43.6% of participants being less than 20 years of age whilst the other 56.4% were above 20 years of age with only 1% of participants being married. The test subjects for this study accounted for 76.9% (150) of participants, including all participants who had spent not less than one session as a student at the University of Benin, whilst the control group were the newly admitted students into the University (Jambites) accounting for about 23.1% (45) of participants. Karyorrhexis, Karyolysis and Pyknosis are the cytotoxic features investigated in this study. Results showed that the mean karyolitic cells of the test and control groups were 3.12±1.23 and 2.87±1.14 respectively, and the mean karyorrhexis cell level in the control and test groups were 0.27±0.46 and 0.81±0.44 respectively, while the mean pyknotic cell levels in the control and the test groups were 1.53±0.81 and 4.13±0.98 respectively. Comparison of these results ultimately indicated a statistically significant difference ($p < 0.05$) between the karyorrhexis and pyknosis cells levels of the control groups and those of the test groups with p-values of 0.001 and <0.001 for karyorrhexis and Pyknosis respectively. However, there was no statistically significant difference in the levels of karyolysis cells in the test and control groups (p-value of 0.97). Also, a statistically significant difference was observed (p-value =0.70) in the pyknosis levels of the different age group classifications employed. Participants under the age of 20 (≤ 19 years) showed a mean pyknosis cell value of 2.85±0.94, whilst participants above the age of 20 (≥ 20 years) presented a mean pyknosis cell value of 4.04±1.04. However, the mean difference in karyolytic and karyorrhexis count was not statistically significant (p-values of 0.55 and 0.64, respectively) between the age groups. Comparisons of the average karyolysis, karyorrhexis and pyknosis levels between participants' genders (males and females) did not yield any statistically significant result (p-values of 0.91, 0.79 and 0.73, respectively), as such a correlation between gender and cytotoxicity in buccal cells does not exist. The mean comparison of the buccal cavity cytotoxic features across the various behavioural status of the respondents shown in Table 3.5 showed that the mean difference between the categories across the variable was not statistically significant, with each variable presenting p-values > 0.05 except for the significantly higher pyknotic cells in the respondent who engage in oral sex. 45 of the 195 participants (test and control group inclusive) engaged in oral sex. They presented a mean pyknotic cell count of 4.78±1.26, whilst the remaining 150 participants who did not engage in oral sex presented a mean pyknotic cell count of 3.16±0.90. The analysis of these groups was statistically significant, with a p-value of 0.03. The result of the multiple regression analysis for karyorrhexis that employed the group variable (jambites and undergraduate) and important biological variables like Age and Gender did not significantly predict karyorrhexis $F(3,191) = 1.71$, $p=0.17$, Adj $R2=0.017$. The predictors accounted for 1.7% variance in the karyorrhexis; the "Group" variable statistically significantly predicted karyorrhexis while other variables held constant. The karyorrhexis count of the Test group was 0.63 times the control while other variables were held constant. There was a 0.02 decrease in karyorrhexis count for a year increase in age, which was not statistically significantly different. The

karyorrhectic cell count of the male was 0.10 lesser than the female, while other variables were held constant. Neeraj and Nisha [8] noticed significant mean values of karyorrhectic cells in people with diabetes were 0.42 ± 0.81, while in control, it was 0.02 ± 0.14. Jajarm et al. [9] did an exfoliative cytology study of 10 type 2 diabetics and 10 control individuals and showed the occasional karyorrhexis in diabetic cases compared to control [9]. Shariff et al. [10] did a study on 10 diabetics and 10 controls and studied 20 buccal cells. They found prominent karyorrhexis in the study but were statistically not evaluated [10]. It should be noted, however, that the results obtained by these previous studies are not entirely in conjunction with the results obtained from this study, as a test for diabetes was not carried out on any of the participants. The result of the multiple regression analysis for Pyknosis employed the same independent variables with the addition of oral sex because it was a statistically significant variable. These independent variables could significantly predict pyknosis $F(4,190) = 6.49$, $p<0.001$, Adj $R2=0.102$. The variables accounted for 10.2% of variances in the pyknotic cell count in the population. The group status and the oral sex statistically significantly ($p<0.05$) predicted Pyknosis independently while other variables were held constant. The pyknotic cell count of the test group was 2.53 times of the jambite while other variables were held constant. The pyknotic cell count in the respondents who performed oral sex (yes) was 1.25 times more than those who didn't, while other variables are constant. No other study mentions a correlation between Oral sex and Pyknosis. Pyknosis, karyorrhexis and Karyolysis are cytotoxic features usually characterising cell death that denotes abnormalities in repair mechanisms for genetic damage indicating genome instability, as the latter leads to apoptosis. This phenomenon might be further explained by the efficiency of the cell cycle checkpoints, which might also result in the occurrence of micronuclei since cells with micronuclei might be induced to death and, consequently, are not visualised.

5. CONCLUSION

This study concludes that there is an association between oral sex and pyknosis, indicating cytotoxicity. Also, biological factors such as gender and age as well as behavioural characteristics such as chewing gum, drinking hot beverages or playing musical instruments have no relationship between cytotoxicity and behavioural characteristics undergraduate subjects in this study. However, further studies should be done to substantiate these claims.

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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 ABA 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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