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cytological and cytomorphological comparative study of oral mucosa in diabetes mellitus and nondiabetics in Misan Governorate.

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ABSTRACT:

This study compared type I and type II diabetic patients with a healthy control group using microscopic qualitative analysis of oral epithelial cytological smears in the possible early phase of diabetes. The cheek, ventral tongue mucosa, buccal, and palate smears were used for the superficial and profound cytological examination of the oral alterations. The type, duration, and consequences of diabetes were linked with cytological alterations of the oral cell population stained with PAP stain (Papanicolaou), PAS (Periodic acid-Schiff), hemotoxylin, and eosin. To detect changes, the experiment was conducted on 100 samples: 50 healthy people, 50 people with type I diabetes, and type II diabetes. They were taken from different areas, stained, and then examined with an optical microscope

Keywords: Oral epithelium, Cytological smears, cytomorphological, diabetes mellitus, nuclear diameter; nuclear-cytoplasmic ratio.

1.Introduction:

Exfoliative cytology is the microscopic analysis of shed or desquamated cells from the mucous membrane or other epithelial surface. The study of cells obtained by scraping the surface of the tissue or from bodily fluids like saliva, sputum, etc. is also included in this) Das Bijoy Kurnar, Mallik.,2000) Using exfoliative cytology, cells from the different layers of the epithelium) Ilayaraja et al.;2018). Oral cytology has become a useful diagnostic tool for oral lesions due to developments in quantitative oral exfoliative cytology. Morphometric cytometry can help improve the accuracy of diagnosing oral lesions, diabetes, and periodontal disease. (Cowpe JG, Longmore, 1981) Accurate measurement of several cell metricsCell size, including nuclear diameter (ND), cytoplasmic area (CyA), and nucleus-to-cytoplasm ratio (N: Cy), can be measured by morphological examination of cells using a light microscope.(Sumanthi J et al.; 2012).Inappropriate hyperglycemia, which results from the body's inability to use insulin or from the pancreatic beta cells' failure to make it,

characterizes a collection of metabolic illnesses known as diabetes mellitus (DM). (World Health Organization, 2009).

An increasing body of research indicates that diabetes increases the risk of periodontitis, oral premalignancies, and perhaps oral cancer. tissue can cause a systemic inflammatory response that can worsen diabetes, worsen cardiovascular outcomes, and increase mortality. critical role in the diagnosis and treatment of oral pathologies, as well as in the counseling of diabetics to maintain good dental health.(skamagas et al,2008).

The study aimed to evaluate the morphological changes in oral epithelial cells from people with type 1 and type 2 diabetes to non-diabetic people using exfoliative cytology and cell counting.

Materials and Methods:

smears of oral mucosa:

The age range for males and females used in the study is between 1-70 years. After acquiring patient consent and getting approval from the Scientific Research Ethics Committee at the University of Misan, samples were gathered from the Center for Diabetes and Endocrinology in Maysan Province. Four areas of the mouth's mucosa were brushed to collect these samples. This study included 100 people, who were divided into two groups.

Group 1 (the control group non-diabetes mellitus) consisted of 50 people, Next, the individuals were divided into four groups based on their age and gender, On the other hand, there were 50 individuals in Group 2, who had diabetes mellitus. A total of four ages and gender categories were used to classify the participant

Sample Collection:

A brush was used to collect swabs from the oral cavity to four areas: cheeks, gums, tongue, and palate. The smears were spread on a clean glass slide and let dry for a few seconds. Specimens were quickly fixed to avoid cell dehydration and shrinkage and maintain their structural integrity. To this end, glass slides were submerged in increasing amounts of ethanol: 100%, 95%, 80%, 70%, and 50%. The slides were stained using Papanicolaou stain (PAP), PAS (Periodic acid-Schiff), hematoxylin, and eosin. Afterward, the slides were placed under a light microscope once they had dried. Cell morphological changes were studied by measuring the diameter of the nucleus (ND), the cytoplasm(CYD) diameter, and the ratio of the nucleus to the cytoplasm for people with diabetes and then comparing them with people without diabetes.

Statistical Analysis:

The data were processed statistically using SPSS (Statistical Package for Social Science) software to determine the relationship between the number of calculated cells and the occurrence of diabetes. The level of significance was set as $P \leq 0.05$

Results:

Nuclear Diameter:

The current results showed a significant difference of about 0.05 between the nucleus diameters of people, depending on age, between those with diabetes and those without diabetes, as shown in **Table 1**, where the rate of ND in the non-diabetic group increased significantly when compared to the

other group. When compared, analysis results indicated a significant increase in the diameter of the non-affected nucleus compared to the diabetic group. The age F pr ratio is equal to (0.417), while the F pr ratio for those with diabetes is (0.174). Also, results demonstrated that the age groups 0-15 and the age groups 46-70 made no significant differences in the sense that A is similar to B, while the age groups 16-30 and 31-45 had significant differences, as A was greater than B.

Table 1**:** The cytomorphometric method Comparison of ND between two groups of oral diabetic patients and the control group Regarding age

Age	Nuclear Diameter of non-diabetics(µm)(A)	Nuclear Diameter of diabetics(µm) (B)
0-15	9.6	9.125
16-30	10.3	1.045
31-45	13.326	1.03
46-70	1	1

Standard errors of means

Table age	Diabetics
rep. 2	4
d.f. 3	3
e.s.e. 3.11	2

Analysis of variance

Variate: Nuclear

Table 1: Comparison of Nuclear diameter among the different age groups

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
age	3	75.29	25.10	1.30	0.417
Diabetics	1	60.64	60.64	3.14	0.174
Residual	3	57.89	19.30		
Total	7	193.83			

DF: Degrees of freedom, SS: Sum of squares, MS: Mean square, *P≤0.01 considered statistically significant

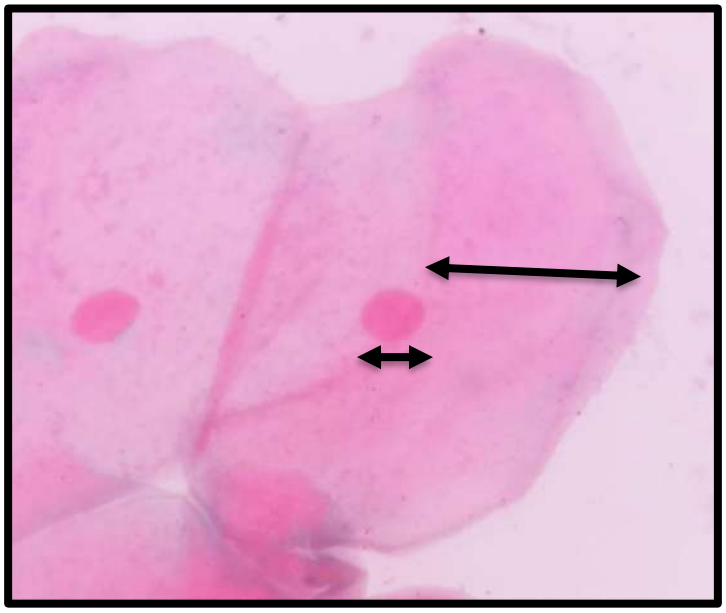


Figure: PAS stain of cytological smear from the oral cavity of the non-diabetic group (100 x) showing

Cytoplasm Diameter:

The current results showed significant differences in cytoplasmic diameters in all age groups, where A is larger than B when comparing the control group with the group with diabetes, according to age groups, as shown in Table 2.

Table 2: Method of measuring the cellular appearance and comparing CYD between two groups of oral diabetes patients and the non-diabetic group, according to age.

Age	Cytoplasm Diameter of non-diabetics(µm)(A)	Cytoplasm Diameter of diabetics(µm) (B)
0-15	504.375	54.275
16-30	4.7272	5.4545
31-45	6.2884	5.076
46-70	85.5422	5.6428

Standard errors of means

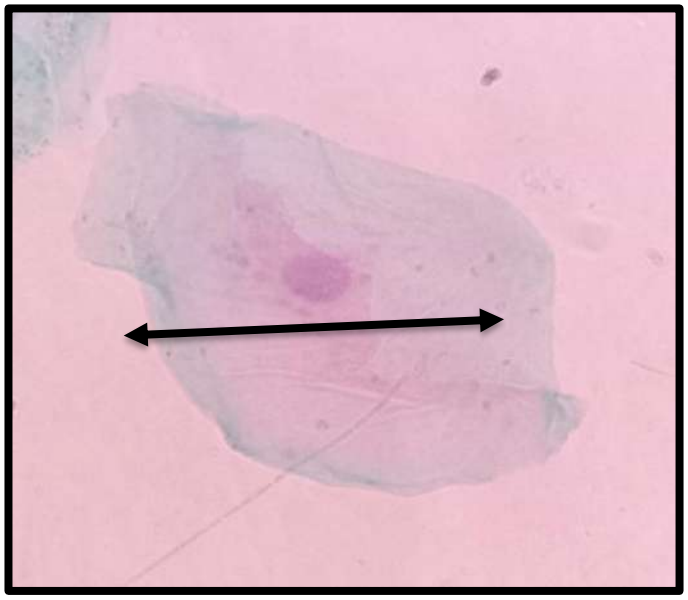
Table	age	Diabetics
rep.	2	4
d.f.	3	3
e.s.e.	107.5	76.0

Analysis of variance

Table 2: Comparison of **cytoplasmic** diameter among the different age groups

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
age	3	103975.	34658.	1.50	0.374
Diabetics	1	35177.	35177.	1.52	0.305
Residual	3	69311.	23104.		
Total	7	208463.			

(DF): Degrees of freedom, (SS): Sum of squares, (MS): Mean square, *P≤0.01 considered statistically significant



Figer: PAP stain of cytological smear from the oral cavity of the non-diabetic group (100 x) showing cytoplasm diameter.

Nuclear: Ratio of Cytoplasmic Diameter (N: CR)

Current findings showed a statistically significant increase in the results of the ratio of nucleus to cytoplasm in people with and without diabetes in the age groups between 0-15, where A is greater than B, that is, there are significant differences of 0.05, while the rest of the age groups from 16-70 are not significantly affected, as shown. In Table 3.

Table 3**:** Cytoplasmic cell measurement method Comparison of the nuclear/cytoplasmic ratio between two groups of oral diabetes patients and the control group and dependence on age

Age	Cytoplasm \ Nuclear Ratio of non-diabetics(µm)(A)	Cytoplasm \ Nuclear Ratio of diabetics(µm)(B)
0-15	23.985	16.797
16-30	1.9364	2.218
31-45	2.6457	2.096
46-70	2.7357	2.271

Standard errors of means

Table	age	Diabetics
rep.	2	4
d.f.	3	3
e.s.e.	1.746	1.235

Analysis of variance

Table 3: Comparison of nuclear-cytoplasmic ratio using one-way ANOVA test between age groups

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
age	3	490.187	163.396	26.80	0.011
Diabetics	1	7.842	7.842	1.29	0.339
Residual	3	18.290	6.097		
Total	7	516.320			

(DF): Degrees of freedom, (SS): Sum of squares, (MS): Mean square, *P≤0.01 considered statistically significant

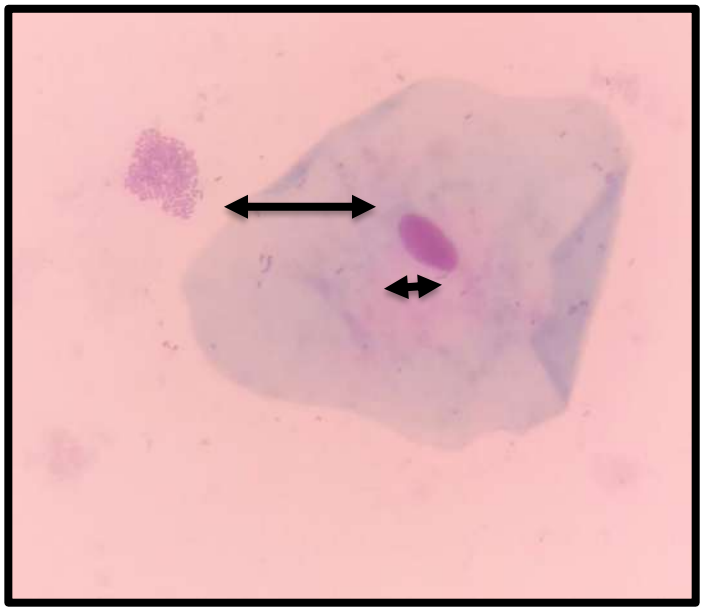


Figure: hemotoxylin-eosin stain of cytological smear from the oral cavity of the diabetic group (100 x) showing nuclear: cytoplasmic diameters ratio

Standard errors of means

Table	age	Diabetics
rep.	2	4
d.f.	3	3
e.s.e.	1.746	1.235

Discussion:

the patient's hyperglycemia and signs of type 2 diabetes (Baban and Garib, 2013). As far as we are aware, In this study, the morphological and cellular alterations in the cells of the cheek, gingiva, lateral border of the tongue, and oral mucosa in patients with type 1 and type 2 diabetes are investigated. The increasing narrowing of the artery lumen, which lowers the rate of cell turnover and perfusion of the afflicted tissue, is the cause of the delayed keratinization of the epithelium. This delay in epithelial development results in an increase in the number of mature cells, which have a big nucleus as a main component (Alberti et al., 2003; Martin and Michael, 2003; Kumar et al., 2003; Jagaram et al., 2008). Xerostomia brought on by decreased salivary flow might be the second cause. The present study also evaluated cytoplasm diameter (CyD). The results showed that individuals with both managed and uncontrolled diabetes exhibited statistically significant decreases in their mean CyD between study groups in the buccal mucosa and lateral border of their tongues when compared to control patients. This is in line with findings by Prasad et al. (2010), who noted that uncontrolled hyperglycemia was associated with a clear and noticeable decrease in cytoplasmic diameter. agree with Shareef and colleagues (2008) as well. A related study discovered a statistically significant drop in the area of the cytoplasm, which might be the result of dehydration-induced cell shrinkage. The results of Ogden et al. (1999), He supported this claim by noting a drop in CyD in individuals with alcoholism and suggesting that this may be because of their apparent dehydration. The somewhat greater increase in nuclear diameter in comparison to cytoplasmic diameter may help to explain this occurrence.

The N: CR values matched the findings of Prasad et al.'s (2010) investigation, which found a connection between the increase in ND and N: CR and the degree of diabetes, as assessed by Hb1Ac. Also, this result is consistent with previous research (Alberti et al., 2003; Shareef et al., 2008). However, in contrast to the results of Jajarm et al. (2008), who claimed that the mean N: CR in the diabetic group was much lower than in the control group.

The cause of age-related variations in ND, CD, and N/C ratios, independent of gender, is cellular senescence. As people age, their ability to renew themselves decreases, leading to the build-up of senescent cells. This, in turn, is influenced by a variety of environmental variables, which raises the ND and N/C ratio.

Based on these findings, there are significant differences between people with diabetes and those without diabetes following age groups, as there is no difference between the diameter of the nucleus for the age groups 0-15 and 46-70, although the groups 16-30 and 31-45 have significant differences between them and the diameter. There are differences in cytoplasm for all age groups. As for the ratio

of nucleus to cytoplasm, all age groups are equal except for the age group from 0-15, statistically significant differences appear between healthy people and people with diabetes.

Conclusion:

The findings of this study indicate emerging differences in the diameter of the nucleus, cytoplasm, and the ratio of nucleus to cytoplasm for people with diabetes and those without diabetes

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