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Journal of Bioscience and Applied Research
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New cytological method to diagnose Diabetes mellitus from oral mucosa

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DOI: 10.21608/jbaar.2024.357660

ABSTRACT

In this study, microscopic qualitative analysis of the oral epithelium cytological smears in the potential early phase of diabetes was performed and a comparison was made between type I and II diabetic patients and a healthy control group. The cytological assessment of the oral changes was realized on superficial and profound smears, from cheek and ventral tongue mucosa and buccal and palate. It was stained with PAP stain (Papanicolaou), and cytological changes of the oral cell population were correlated with the type, duration, and complications of diabetes. 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 to calculate the number of cells and compare them. The results showed that the number of cells in healthy people is much higher than in people with diabetes. The increase in the cheek area and the decrease in all areas of the oral cavity. The diagnosis was made according to age groups and based on the sugar level and cumulative blood sugar. The results proved a statistical relationship between the sugar level and the number of cells in the oral cavity, considered a modern diagnostic method for diabetes.

Keywords: Oral epithelium, Cytological smears, Type I diabetes, Type II diabetes, new method, diagnosis

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Introduction

A class of metabolic disorders known as diabetes mellitus is defined by hyperglycemia brought on by deficiencies in either insulin action or secretion, or both. [1,2]. The primary characteristic of diabetes is hyperglycemia, which increases the risk of microvascular damage, retinopathy, nephropathy, and neuropathy. It is linked to a lower life expectancy, considerable morbidity from certain microvascular diseases associated with diabetes, a higher risk of macrovascular consequences (such as peripheral vascular disease, ischemic heart disease, and stroke), and a worse quality of life [3,4,5].

Diabetes affects the function of the oral mucosa because of changes in salivary quantity and type, altered dietary intake, and lowered immune system activity. Diabetes patients may experience oral symptoms such as dry mouth, candidiasis, increased tooth decay, gingivitis inflammation, periodontitis, and peri-apical abscesses [6,7]. There are various ways to assess the oral mucosa of diabetics, and one of the most common ones is to use a biopsy, either incisional or excisional. However, using a biopsy is typically avoided because it can be aggressive and cause the patient to experience psychological issues [8]. A method that is less invasive, less expensive, and moderate on oral tissues would seem to be the

Received: March 5, 2024. Accepted: May 30, 2024. Published: June 23, 2024

most effective. Due to its affordability, benign nature, and lowest influence on the patient's dental health, cytology whether exfoliative or brush seems to be the ideal method. This technique, originally used for the quick identification of precancerous lesions, involves cell counting. Today, it is used for the diagnosis and evaluation of the quantitative and qualitative changes in epithelial cells of the oral mucosa that are suspected [9,10].

Thus far, only one study [11] has reported on the comparison of cytomorphometric changes of the oral mucosa of people with diabetes types I and II, and few studies have examined changes in the oral mucosa of diabetic patients and reported the replacement change in epithelial cells of the oral mucosa by cytology method [11,12]. There are several ways to diagnose diabetes, by measuring HbA1c, but this method has the disadvantage that the person must fast for no less than 8 hours, because the diagnosis cannot be made in most patients who come in the afternoon appointments or if they eat before the morning appointment. In addition to this, it is done by obtaining venous blood, and there is another way to diagnose diabetes, which is measuring the FPG level, as it only requires venous blood, as this method is considered to be easier than the HbA1c method [13]. A new method has been discovered to diagnose diabetes based on the number of cells taken from the oral cavity. This is considered an easy, simple, harmless, and quick method that does not require venous blood.

This study aims to investigate the relationship between the number of cells calculated from the oral mucosa and different areas of the oral cavity and the incidence of diabetes. Furthermore, the study indicates the possibility of adopting this relationship as a novel method for diagnosing diabetes.

Materials and Methods

smears of oral mucosa

The age range for males and females used in the study is between 0-70 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) consists of 50 people, Next, the individuals were divided into four groups based on their age and gender: 0–15, 16–30, 31–45, and 46–70. On the other hand, there were 50 individuals in Group 2, who had diabetes mellitus. A total of four age and gender categories were used to classify the participants: 0–15, 16–30, 31–45, and 46–70.

Sample Collection

A brush was used to collect swabs from the oral cavity to four areas: the cheek, gums, tongue, and palate. The smears were spread on a clean glass slide and left to dry for a few seconds. Specimens were quickly fixed to avoid cell dehydration and shrinkage and maintain their structural integrity. For this, the glass slides were submerged in increasing amounts of ethanol: 100%, 95%, 80%, 70%, and 50%. The slides were stained using Papanicolaou stain (PAP). Afterward, the slides were placed under a light microscope once they had dried. The Ocular Stage lens was operated to accomplish cell counting, with non-diabetic cell counts per square millimeter. However, since diabetics have a lower cell count overall, their cell counts were determined over a broader region than 1 square millimeter.

Serological Test (Blood Sugar)

The blood sugar level was measured for people with diabetes and non-diabetes, where blood was drawn using (TOSOH) device Sampling times were in the morning.

Statistical Study

The data were processed statistically using the SPSS (Statistical Package for Social Science) program to

determine the relationship between the number of calculated cells and the incidence of diabetes. The level of significance was set as P ≤0.05.

Results

The results of the current study indicated a significant difference at the level of (0.05) in the number of squamous cells shed from the oral cavity between the patients with type 1 and 2 diabetes compared to non-diabetic individuals. An increased number of cells was observed in the cheek area, whereas a decrease was noted in the gum area. The results showed significant differences in the cell numbers that were observed across all areas of the oral cavity, as illustrated in **Tables 1** and **2** and as shown in **Figures 1** and **2**.

The numbers of cells in the mucous layer of the mouth in the cheek, gums, tongue, and palate areas were diagnosed according to age groups and based on the level of sugar and cumulative sugar, as shown in **Table 2**. From the results of this table, it is clear that there is an important statistical relationship at the P ≤0.05 level between the number of cells calculated from the oral cavity and the sugar level according to age groups. The results for the age groups from 0 to 30 had a diabetes level ranging between 200, while the cumulative sugar level was 10 and the number of cells ranged from 10 - 15. As for the results for age groups, they show 30 - 70, the sugar level is between 250, the cumulative sugar level is 10, and the cell counts range from 15 - 20.

1-(0-30), FBS 200, HbA1C 10 ⟶ 10-15

2-(30-70), FBS 250, HbA1C 10 ⟶ 15-3

Table (1): The table includes the number of cells according to the areas of the mouth for people with non-diabetics and diabetics.

Region	Count of non-diabetics	Count of diabetics
Tongue	203	36
Cheek	257	42
Gum	192	29
Palate	216	38
Significance *P<0.01, NS = No Significance X2 Chi-square at 11.161, d.f 3, p-value .011		**Significance **P<0.01 NS = No Significance X2 Chi-square at 2.448, d.f 3, p-value .485**

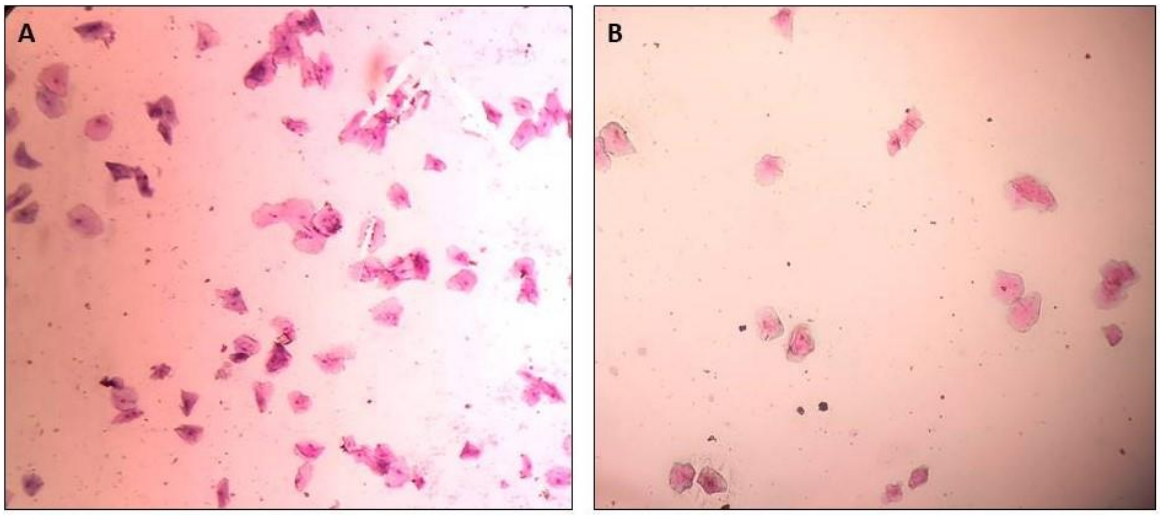

Figure 1: a comparison between shed cells in A healthy patient and a B diabetic patient, notice the scanty number of cells in a diabetic patient in comparison with a healthy one.

Table (2): shows the cumulative sugar, diabetes level, and cell numbers for people with diabetes.

Age	**FBS Test**	HbA1C	Number cell
0-15	202.94	9.26	15.4
16-30	205.79	10.34	10.4
31-45	262.29	9.43	14.6
46-70	250.69+	10.56	20.6

Table (3): The correlation between the number of cells, FBS, and HbA1C

Correlations		
	Count of diabetics	
FBS	**Correlation**	**0.413**
	P- value	**0.008**
HbA1C	**Correlation**	**0.012**
	P- value	**0.940**

1-There is a significant correlation between FBS and the number of cells (0.413)

2-There is no significant correlation between HbA1C and cell count (0.012)

Discussion

Results from this study indicate that exfoliative cytology offers significant advantages over conventional cytology in clinical practice. First, some aspects of the materials and methods used will be discussed. Cell smears were collected from selected areas of the palatine mucosal [14], the comparison of these two cell collectors is of interest since so far there have been very few investigations on different cell collectors in the literature. Previous studies compared, for example, a Cytobrush brush with a dermatological curette [15], with a wooden tongue spatula [16,6], or with a metal spatula [17]. In a study by Reboiras-López et al., three different sampling devices (Cytobrush, dermatological curette, OralCDx Brush) were used to perform liquid-based cytology [18].

The brush was stronger and stiffer, as were the cells obtained during smear sampling. It was necessary to ensure that the cell collector was rotating at a constant and modest contact pressure when swabs were taken [19]. Ignoring this may lead to uneven mucus cell loss, for example. In addition, smear sampling was consistently performed to prevent uneven elimination of mucosal cells. Furthermore, even though there are rules for proper smear sampling, the same investigator performed the smear sample every time since individual variations may occur between practitioners [20]. The following findings may be summed up by comparing the cytological appearance of the smears from the two utilized cell collectors.

One way to utilize the PapCone Brush was in conjunction with traditional methods of cell preparation. One benefit of this technique was the quick processing of the preparations at the Clinical and Experimental Oral Medicine section's laboratory following smear collection by directly rolling out the on-glass slides, followed by air drying, fixation, and staining. It is possible to view the glass slides made with the traditional procedure right away [21]. The gingiva and palate mucosa of the oral cavity include keratinized mucosa, while the buccal mucosa contains non-keratinized mucosa. Because insufficient amounts of keratinized gingiva cause gingival inflammation, keratinized gingiva around teeth and dental implants is essential for promoting oral hygiene and maintaining the health of periodontal and peri-implant tissues [22,23]. Extensive studies have shown to describe the patterns of shedding of oral mucous cells, but little shedding in people with diabetes, especially in the gum area and in the mouth, the duration of sampling, and obtaining many studies, it was found that the rate of cell shedding in people with diabetes is lower than in healthy people [16,24].

Conclusion

These results indicate that diabetes can produce changes in the number of oral epithelial cells, which can be detected by microscopic examination and counting the number of cells in each oral swab, which can be used as a new method for diagnosing diabetes, or the diagnosis of diabetes can be adopted using smear cells from the mouth of people with diabetes after calculating The number of cells shed from the oral mucosa from the cheek area, in which the number of cells is greater than the rest of the mouth in one area.

Funding:

This study did not receive any funding.

Competing interests:

The authors declare that they have no competing interests.

References

1- American Diabetes Association (ADA). Diagnosis and Classification of Diabetes Mellitus. Diabetes care. 2007; 30: S42-S47.

2- Guyton C, Hall JE. Textbook of Medical Physiology. 12th ed. ElsevierSaunders. Philadelphia, 2012.

3- World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate

hyperglycemia: report of a WHO/IDF consultation. Geneva, World Health Org., 2006.

4- Pawar, V., Sontakke, A., Pawar, S. Interconnection between oxidative stress and type 2 diabetes mellitus. *Journal of Bioscience and Applied Research*, 2023; 9(4): 192-198. doi: 10.21608/jbaar.2023.325677

5- Ahmed, H., Ali, N. Evaluation of Serum Ferritin in Type-2 Diabetes Mellitus Sudanese Patients. *Journal of Bioscience and Applied Research*, 2022; 8(3): 132-137. doi: 10.21608/jbaar.2022.247840

6- Ogden GR, Cowpe JG, Green M. Cytobrush and wooden spatula for oral exfoliative cytology. A comparison. Acta Cytol. 1992;36:706–10.

7- Newman MG, Takei HH, Klollevold PR: *Carranza's clinical periodontology. 10th ed.* ST Louis: Saunders; 2006.

8- Spafford MF, Kich WM, Red AL: Detection of head and neck squamous cell carcinoma among exfoliative oral mucosal cells by microsatellite analysis. *Clin Cancer Res* 2001, 7: 6–7.

9- Mehrota R, Gupta A, Sirgh M: Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. *Mol Cancer* 2006, 5: 5–11. 10.1186/1476-4598-5-5

10- Alberti S, Spadella CT, Francischone TR: Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. *J Oral Pathol Med* 2003, 32: 536–543.

11- Hoseinpour Gollo, M., Moslemi Naeini, H., Liaghat, G. H., Torkamany, M. J., Jelvani, S., & Panahizade, V. (2008). An experimental study of sheet metal bending by pulsed Nd: YAG laser with DOE method. *International Journal of Material Forming*, *1*, 137-140.

12- Dafer, R. M., Rao, M., Shareef, A., & Sharma, A. (2008). Poststroke depression. Topics in stroke rehabilitation, 15(1), 13-21

13- Gavin III, J. R., Alberti, K. G. M. M., Davidson, M. B., & DeFronzo, R. A. (1997). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care*, *20*(7), 1183.

14- Hellwig E, Klimek J, Attin T. Einführung in die Zahnerhaltung: Prüfungswissen Kariologie, Endodontologie und Parodontologie. In: Freiburg, Gießen. 6th ed. Zürich: Deutscher Zahnärzte Verlag. 2013; p. 457–58.

15- Navone R. Cytology of the oral cavity: a re-evaluation. Pathologica. 2009; 101:6–8.

16- Miller CS, Danaher RJ. Asymptomatic shedding of herpes simplex virus (HSV) in the oral cavity. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105:43–50. Doi: 10.1016/j.tripleo.2007.06.011.

17- Queiroz JB, Lima CF, Burim RA, Brandao AA, Cabral LA, Almeida JD. Exfoliative cytology of the oral mucosa: comparison of two collection methods. Gen Dent. 2010; 58:196–9

18- Reboiras-López MD, Pérez-Sayans M, Somoza-Martín JM, Antúnez-López JR, Gándara-Vila P, Gayoso-Diz P, et al. Comparison of three sampling instruments, Cytobrush, curette and OralCDx, for liquid-based cytology of the oral mucosa. Biotech Histochem. 2012; 87:51–8.

19- Reichardt T, Remmerbach TW. Tumoren in der Mundhöhle – zahnärztliche Aspekte der Früherkennung. Zahnmedizin up2date. 2016; 10:67+-- Navone R. Cytology of the oral cavity: a re-evaluation. Pathologica. 2009; 101:6–8.

20- Soud, Lamia Ibrahim. "Salivary IgA Concentration and Oral Parameters in Early Onset

Type I Diabetic Patient." Al-Anbar Medical Journal 9, no. 1 (2011).

21- Tozoglu U, Murat Bilge O: Exfoliative cytology of type I diabetic patients. *Eur J Gen Med* 2009, 7: 264–268.

22-Schrott, A. R., Jimenez, M., Hwang, J. W., Fiorellini, J., & Weber, H. P. (2009). Five-year evaluation of the influence of keratinized mucosa on peri-implant soft-tissue health and stability around implants supporting full-arch mandibular fixed prostheses. *Clinical oral implants research*, *20*(10), 1170-1177.

23-Lang, N. P., & Löe, H. (1972). The relationship between the width of keratinized gingiva and gingival health. *Journal of Periodontology*, *43*(10), 623-627.

24- Abed, Ahmed Taleb, and Nada Mohammed Hasan Al-Ghaban. "Acceleration of Ulcer Healing by Local Application of Myrrh Oil: An Experimental Study." Al-Anbar Medical Journal 19, no. 2 (2023): 128-134.