Exfoliative Cytology of Buccal and Gingival Mucosa in Diabetes Mellitus Type 2

Ary Andini[1,*], *Irmawan* Farindra[2], *Nety Mawarda* Hatmanti[3], *Uliyatul* Laili[4], *Naila* Fitriya[1], *Shinta Nuriya* Munawaroh[1], *Nurul* Hidayah[1], and *Nini* Karimah[1]

[1]Diplome of Medical Laboratory Technology, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia
[2]Bachelor of Medicine, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia
[3]Bachelor of Nursing, Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia
[4]Bachelor of Midwifery, Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia

Abstract. Patients' diabetes mellitus is prone to an increase of bacteria in the oral cavity which can cause abnormalities to the buccal and gingival mucosa cells. This study aims to analyze the relationship between high blood glucose levels in Diabetes Mellitus Type 2 and the condition of buccal and gingival cytology cells by using Giemsa stain and Periodic Acid Schiff (PAS). This research was an observational study with cross sectional approach used 16 respondents of Diabetes Mellitus Type 2 with blood glucose level was 321.87±91.86 mg/dl. The observation of buccal cells showed that 10 people had normal cells (62.5%) and 6 people had weak damage (37.5%). The gingival cells condition found that 8 people had normal cells (50%), 6 people had weak cell damage (37.5%) and 2 people had moderate cell damage (12.5%). The results of epithelial cells by PAS staining showed normal conditions in 10 people (62.5%), slightly damaged in 3 people (18.8%), and moderately damaged in 3 people (18.8%). Based on statistical analysist results was obtained no significant relationship between blood glucose level and abnormality of buccal and gingival mucosa, p-value = 0.105 (buccal), p-value = 0.151 (gingival), but significant relation in epithelial cells stained with PAS (p-value = 0.048). In the future, prospective and comparative studies can be conducted to observe the development of PAS staining in individuals with diabetes mellitus (DM). This could assist in exploring fluctuating changes related to blood glucose control

1 Introduction

People with diabetes mellitus (DM) often experience various disorders or abnormalities in the epithelial tissue within the oral cavity [1]. These complications primarily include periodontal diseases such as periodontitis and gingivitis. Diabetes mellitus is a systemic

* Corresponding author : aryandini@unusa.ac.id

disease that can manifest in the oral cavity, particularly in individuals with type 2 diabetes. Uncontrolled diabetes can impact white blood cells, including neutrophils, monocytes, and macrophages, which play a vital role in the body's defense against infections. Consequently, diabetes patients may become more susceptible to infections due to a reduced ability to combat bacteria [2]. An increase in the quantity of bacteria in the oral cavity of diabetes patients can lead to abnormalities in the periodontal tissues, including the gums [1,2]. Several previous studies have identified diabetes as a risk factor for the prevalence of gingivitis. This assertion is further supported by earlier research conducted by Windiyaarti (2003) at Banjarnegara Regional General Hospital, where the prevalence of gingivitis among type 2 DM patients was found to be 96.7%. The high prevalence of gingivitis in type 2 diabetes patients underscores their susceptibility to this condition [3].

Cytological examination of the oral cavity is conducted through microscopic examination by gently rubbing a cytobrush over the surface of the gums (gingiva). This examination method offers several advantages, including being painless, cost-effective, and delivering rapid results [4]. During cytological examination, staining is performed using various dyes, including Papanicolaou, Giemsa, and Periodic Acid Schiff (PAS). Giemsa staining, commonly used in cytological examination, reveals cell morphology, including the nucleus and cytoplasm. This staining procedure is straightforward, cost-effective, and typically takes 25-30 minutes [5]. Papanicolaou staining is a polychromatic staining technique that combines hematoxylin staining to color the cell nucleus and other dye components for the cytoplasm [6]. In contrast, PAS staining is employed to identify neutral carbohydrate compounds and carbohydrate-producing cells [7,8]. Additionally, this staining method helps provide more detailed information regarding the presence of other factors such as bacteria and fungi in cytopathological preparations. However, further investigation is needed to determine the most suitable staining method for specific diagnostic purposes. Additionally, there is a potential research gap in exploring the reliability and sensitivity of cytological examination in detecting various oral pathologies, considering the mentioned advantages of being painless, cost-effective, and providing rapid results. Further studies could focus on optimizing these techniques for enhanced diagnostic accuracy and applicability in clinical settings.

Building on this information, the researchers aimed to analyze the exfoliative cytopathological characteristics of smears from the buccal mucosa and gingiva using giemsa, and PAS stain in individuals with indications of diabetes mellitus. This research is significant because there has been limited comprehensive research on exfoliative cell cytopathology testing of the gingiva and mucosa in individuals with indications of DM, as well as a lack of comparison regarding the quality of staining methods. This research will facilitate scientific exploration into whether indications of DM or elevated blood glucose levels influence buccal and gingival mucosal cell abnormalities and how the choice of staining method impacts the quality of cytopathological examination results.

2 Method

2.1 Materials

This research is an observational study employing a cross-sectional approach to examine exfoliative cytopathology in the mucosa and gingiva. The study included 16 respondents with diabetes mellitus (DM) who underwent glucose level testing using Point of Care Testing (POCT) [9].

Glucose levels are determined using Auto Check glucose strips and Auto Check POCT. The cytology assay with giemsa staining required object glass, cover glass, distilled water,

absolute methanol, giemsa solution, xylene, and enthelan. Meanwhile, PAS staining utilized 50%, 70%, 80%, and 95% alcohol, xylene, PAS dye, distilled water, sulfite water, object glass, cover glass, and enthelan. Sampling of gingival and buccal mucosa was performed using a wooden tongue

2.2 Cytology with Giemsa Staining

Giemsa staining involved immersing the specimen in a methanol absolute as fixative solution for 5 minutes. The prepared specimens were then air-dried before being placed in Giemsa solution for 30 minutes. Afterward, they were rinsed with running water, air-dried again, and immersed in xylol for 3 minutes. Finally, 1-2 drops of enthelan were applied to the preparation, which was then covered with a cover glass and examined under a microscope [5,10].

Giemsa Staining Cytopathological images of the mucosal and gingival epithelium in individuals with indications of diabetes were observed using a microscope at magnifications of 400×. The results were then recorded. Interpretation of the cytology preparations was performed, and the results were assessed based on the following categories, as shown in Table 2. Additionally, the quality of the preparations was compared by assigning a score to four parameters for each staining method, ranging from 0 to 3 [10].

Score		Description
0	:	No atypical or abnormal findings
1	:	Atypical cytological features, but no evidence of malignancy
2	:	Cytological features suggestive of malignancy, mild to moderate dysplasia
3	:	Cytological features of malignancy with severe dysplasia V: Cytological features of malignancy

2.3 Cytology with Periodic Acid Schiff (PAS)

The gingiva cells were fixed in 10% neutral buffer formalin (NBF), The tissues embedded in paraffin blocks were prepared and sectioned microtomi by histopathological techniqu [8, 11], The PAS staining procedure began with the deparaffinization of gingival slide in xylol solution for 3-5 minutes, repeated three times. Rehydration of the preparations was carried out using graded alcohol solutions with decreasing concentrations (50%, 70%, 80%, 95%) for 3-5 minutes each, followed by rinsing with running water. The preparations were then oxidized in 1% periodic acid for 5 minutes and rinsed with distilled water three times, each for 5 minutes. Next, they were immersed in Schiff's solution for 15 minutes, followed by rinsing with sulfite water for 5 minutes [8,11,12].

PAS Staining showed images of carbohydrates in gingival cytology preparations [8,12] were observed using a microscope at magnifications of 400×. The results were recorded, and the interpretation of the cytology preparations was conducted, with results assessed based on the following categories [8,13]:

Colour Index		Description
-	:	negative reaction
+	:	weak reaction
++	:	moderate reaction
+++	:	strong reactions

2.4 Data Analysis

Data collected from cytological observations of the mucosal and gingival epithelium in individuals with diabetes mellitus were gathered in the past. Subsequently, data analysis was carried out using the Statistical Product and Service Solution (SPSS) application program. The Spearman test was employed for data analysis.

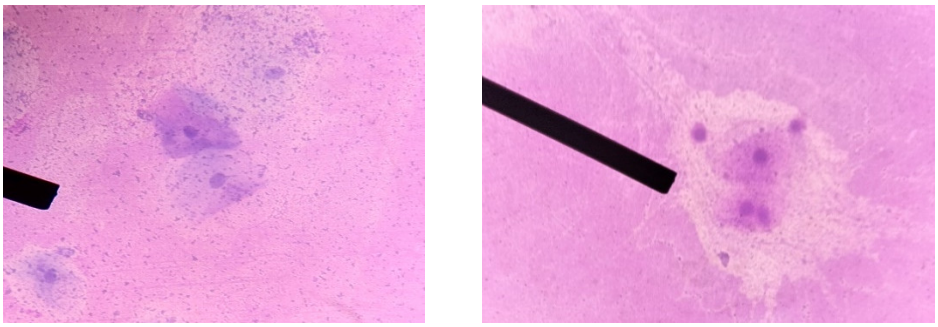
4 Results and Discussion

Respondent characteristics were obtained by distributing respondents based on age, gender and history of diabetes. Presented in the following Table 1.

Table 1. Respondents' characteristic

Categories	Group	N	(%)
Age	20-40 years	4	25%
	40-60 years	12	75%
Gender	Man	4	25%
	Woman	12	75%
Hereditery	Presence	10	62,5%
	Absence	6	37,5%
Blood glucose	>200	16	100%
	<200	0	0%

Microscopic observations of buccal cytology were conducted. The cytology preparations, which had been fixed and treated, were subsequently stained using Giemsa methods. The following are the results of microscopic observations of normal buccal and gingiva epithelial cells through Giemsa staining could be seen Figure 1.



(a) **(b)**

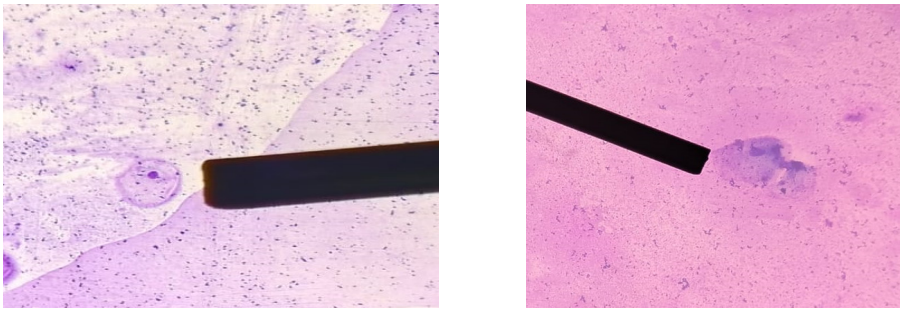
Fig 1. Buccal epithelial cells (a) Normal ephitelial cells through Giemsa staining, magnification 400×. b) Abnormal buccal epithelial cell binuclei with Giemsa staining, magnification 400×

The buccal cytology assay of the individual revealed diabetes through Giemsa staining. This was observed under a microscope at 400× magnification, revealing epithelial cell damage characterized by the presence of two nuclei. The image below demonstrates the quality of Giemsa dye, with both the cell nucleus and cytoplasm appearing clear and brightly colored. Giemsa staining produced nuclei with a purplish-blue hue and purple cytoplasm in normal cells. Based on results study abnormalities of buccal epithelial cells with Giemsa staining in 16 samples. The research results indicated that stage 0 was found in 10 samples (62.5%), while stage 1 was found in 6 samples (37.5%). Abnormal cells included dyskaryosis with enlarged, binucleated, and irregular nuclei.

Table 2. Distribution of abnormalities of buccal cells in DM

Categories of Abnormality cells	N	%
0	10	62.5
1	6	37.5
2	0	0
3	0	0

Swab samples of gum tissue were collected from individuals with diabetes indications and then processed as preparations, which were subsequently fixed with appropriate reagents. Observations of these preparations were conducted under a microscope with 400× magnification. The observed results revealed the presence of damaged or abnormal epithelial cells, characterized by karyolysis, karyorrhexis, and binucleation. Abnormality cells of gingiva cells could be seen in Figure 2. The cytological findings in gingiva of DM respondents obtained stage 0 was found in 8 preparations with a percentage of 50% (Table 3). In stage 1, 6 samples were found with a percentage of 37.5%. In stage 2, 2 samples were found with a percentage of 12.5%.



(a) **(b)**

Fig 2. Gingiva epithelial cells (a) Normal ephitelial cells through Giemsa staining, magnification 400×. b) Abnormal epithelial cell with Giemsa staining, magnification 400×.

Table 3. Distribution of abnormalities of gingiva cells in DM

Categories of Abnormality cells	N	%
0	8	50.0
1	6	37.5
2	2	12.5
3	0	0

Table 4. Distribution of abnormalities of gingiva cells in DM through PAS staining

Categories of Abnormality cells	N	%
-	9	62.5
+	3	18.8
++	3	18.8
+++	0	0

Table 5. Correlation between abnormalities in oral epithelial cells and blood glucose

Variables	Mean of Blood Glucose	P-Value*
Buccal cells (Stained Giemsa)		0.105
Gingiva cells (Stained Giemsa)	321.87±91.86 mg/dl	0.151
Gingiva cells (Stained PAS)		0.048*

*Significancy p-value <0,05, Spearman test

Based on the examination results, the average glucose level of the respondents reached 321.87 ± 91.86 mg/dl, which exceeded 200 mg/dL (Table 5). This falls into the category of diabetes mellitus condition [9]. Diabetes Mellitus (DM) can lead to significant oral and dental health issues. Diabetic patients are at a higher risk of experiencing various problems in the oral cavity, including candidiasis, mandible issues, and xerostomia. Changes in the morphology and function of oral mucosal cells can be observed through microscopic examinations using cytology techniques [14].

Observations using Giemsa staining on 16 sample preparations also yielded positive results, with 93.75% of the samples showing well-stained cell nuclei and cytoplasm. However, 37.5% of the samples exhibited cell clumping, cytoplasmic artifacts, and air bubbles in 100% of the samples. Giemsa staining resulted in blue-colored cell nuclei and purple cytoplasm, consistent with findings from other studies [15]. Giemsa staining showed that 62.5% of samples were undamaged, while 37.5% showed damage, specifically dyskaryosis characterized by hyperchromatic and irregular nuclei [16]. These findings are associated with increased free radicals and protein glycation in diabetes [14].

Buccal epithelial cell damage, such as dyskaryosis and binucleation, can be attributed to xerostomia and insulin deficiency. Xerostomia leads to the dehydration of oral epithelial cells, rendering them vulnerable to trauma and cell loss, potentially resulting in binucleated cell nuclei. Insulin deficiency can also affect cell growth and the nucleus-to-cytoplasm ratio [16].

Examination of gingival epithelial cells in control samples revealed normal-sized cells with intact nuclei and cytoplasm. However, in individual samples with indications of diabetes, morphological changes were observed, including binucleation, karyolysis, and karyorrhexis. These findings align with previous research demonstrating changes in gum epithelial cells in diabetes patients [15].

PAS staining is used to examine the presence and abnormalities of carbohydrates such as glycogen in a tissue or cell. As a potent oxidizing agent, periodic acid reacts with the 1.2 glycol linkage of carbohydrates in tissue sections to produce aldehyde, which is then colored with Schiff's reagent to form scattered red to magenta particles in the cytoplasm [17]. PAS staining was found to experience a significant increase in patients with diabetes mellitus (DM) in the buccal mucosa and gingiva. This indicates that in individuals with DM, there are structural changes in buccal and gingival cells in the oral area. This will impact an increased risk of dental caries and plaque formation.

5 Conclusion

In summary, there is no significant relationship observed between blood glucose levels and the presence of abnormal cells in the buccal and gingival tissues, but significant on gingival cells through PAS staining. In the future, it is hoped that this research can be further developed by considering aspects of correlation with clinical parameters in patients with diabetes mellitus (DM). Subsequently, prospective and comparative studies can be conducted to observe fluctuating changes related to glucose control in patients with DM. Additionally, there is a need to explore the mechanisms underlying changes in staining on the oral mucosa in individuals with DM.

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