ORIGINAL RESEARCH

To determine the cytomorphometric study of oral squamous cells in diabetes individuals

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ABSTRACT

Aim: To determine the cytomorphometric study of oral squamous cells in diabetes individuals. **Material and methods:** Random blood glucose levels were determined for each patient on the day of the cytological investigation. Quantitative analysis used PAP stain, whereas qualitative analysis included both H and E and PAP stains. The inclusion criteria for healthy persons were adult adults without a history of diabetes or any other disease. The research included 80 cases, including a study group of type 2 diabetes patients (Group 1) and a control group of healthy adults (Group 2). The study and control groups were separated based on gender into Group 1A, Group 1B, and Group 2A. Group 2B included 20 men and 20 females aged between 35 and 75 years. Smears were taken from keratinized (T, P) and non-keratinized (BM, FOM) mucosa. CA and NA were determined using Image J analysis. **Results:** On intergroup gender comparison of diabetic men and females with control males and females a lower mean CA was reported. The results were significant for T and FOM in men between different groups and for BM, T, and FOM in females between different groups. Furthermore, the average NA was higher and the average CA:NA was lower, with statistically significant findings seen during intergroup gender comparison. The whole result was statistically significant, except for CA at sites BM and P in men, and for site P in females. When comparing genders within the same group of diabetics, the outcome was not statistically significant. The result was not statistically significant in the control group, except for the coefficient of performance and for temperature in the event of negative affect and coefficient of performance to negative affect ratio. **Conclusion:** Exfoliative cytology may be a more patient-friendly method for glucose monitoring in type 2 diabetics compared to invasive blood monitoring approaches. Therefore, it may be used as a tool for widespread screening for diabetes mellitus in public health initiatives. Studying quantitative and qualitative alterations in oral mucosa using exfoliative cytology in type 2 DM patients might enhance our comprehension of the disease process at a cellular level.

Keywords:PAP, Type 2 DM, Exfoliative cytology, Cytomorphometric

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of Diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels and oral cavity [1].

It is the prevalent non-communicable illness globally and ranks as the fourth to fifth most frequent cause of mortality in wealthy nations. The worldwide number of individuals with diabetes is projected to increase from 150 million to 220 million by 2010 and perhaps reach 300 million by 2025. India, among developing nations, saw the most significant growth in recent years. It is projected that India would have the highest number of diabetes individuals globally by 2025[2]. Various abnormalities and consequences are linked to diabetes mellitus. The typical symptoms include of excessive thirst, increased appetite, frequent urination, tiredness, weakness, irritability, weight loss, and itching. Complications associated with the oral cavity mostly include periodontal disorders (Periodontitis and gingivitis), salivary dysfunction resulting in

decreased salivary flow and alterations in saliva composition, taste dysfunction, and oral fungal and bacterial infections [3].Various clinical and paraclinical approaches may be used to identify oral mucosal alterations. Incisional or excisional biopsy is the most accurate method for definitively diagnosing disorders in the oral mucosa [4]. Exfoliative cytology is a valuable diagnostic technique used in mass screening programs for high-risk adult populations. Exfoliative cytology is a straightforward, uncomplicated, and noninvasive clinical procedure that has the potential to be established as a standard screening method for diabetes mellitus due to its ease, painlessness, and cost-effectiveness. It may be performed chair-side during a standard dental checkup [5].Oral exfoliative cytology was previously restricted by its subjective interpretations and often false negative outcomes [6]. A brief search on search engines shows that other specialized processes may be conducted on exfoliated cells, such as IHC and image analysis, highlighting the potential of this field in disease detection. Image analysis methodology allows for quantifying nuclear and cell alterations related to aberrant cells, providing an objective method to distinguish them from normal cells. The findings demonstrate that quantitative histomorphometric approaches may identify traits that regular histological investigation might miss[7,8].

MATERIAL AND METHODS

Random blood glucose levels were determined for each patient on the day of the cytological investigation. Quantitative analysis used PAP stain, whereas qualitative analysis included both H and E and PAP stains. The inclusion criteria for diabetes mellitus included patients meeting any of the following diagnostic criteria: (1) Fasting blood glucose levels between 130 and 200 mg/dl, (2) Random blood glucose levels of ≥200 mg/dl, (3) Postprandial blood glucose levels of ≥200 mg/dl, or individuals diagnosed with type 2 diabetes for no longer than 5–10 years, in good health and oral hygiene, without any oral lesions, and not taking medication such as oral hypoglycemic agents or insulin. The inclusion criteria for healthy persons were adult adults without a history of diabetes or any other disease. Exclusion criteria included medical history or records showing conditions other than type 2 diabetes, such as systemic diseases, malignancy, nutritional deficiency, and reduced immune function; physiological conditions like pregnancy and lactation; and habits like smoking, alcohol consumption, and tobacco use.

The research included 80 cases, including a study group of type 2 diabetes patients (Group 1) and a control group of healthy adults (Group 2). The study and control groups were separated based on gender into Group 1A, Group 1B, and Group 2A. Group 2B included 20 men and 20 females aged between 35 and 75 years. Smears were taken from keratinized (T, P) and non-keratinized (BM, FOM) mucosa. Patients were instructed to rinse their oral mucosa with saline solution, followed by cleaning and drying the mucosa using a cotton swab to eliminate surface debris and excess saliva. Eight smears were created using a wet wooden spatula by gently scraping at each place, then transferred onto clean, dry glass slides. The slides were thereafter immersed in 95% ethyl alcohol for a duration of 15 minutes. Four out of the eight smears were stained with PAP, while the other four were stained with H and E. 50 unfolded cells with appropriate staining were chosen in each PAP-stained smear using systematic sampling at 40× magnification, shifting the microscope stage in a specific pattern to prevent duplication of cells from various areas. CA and NA were determined using Image J analysis. Binucleation, inflammation, cytoplasmic vacuolation, karyorrhexis, karyolysis, pyknosis, microbiological colonies, and Candida were evaluated in each PAP and H&Estained smears.

The data was manually inputted into the Statistical Package for the Social Sciences (SPSS) version 21.0 for analysis. The data was analyzed using descriptive and inferential statistics to get the mean and standard deviation. Intergroup and intragroup gender means were comparedusing Student's t-test with a 95% confidence interval.

RESULTS

Table 1 displays the average values of CA, NA, and CA:NA ratio for the study and control groups across four locations. Statistical analysis revealed that Group 1 had a lower mean CA, higher NA, and lower CA:NA ratio across all four locations. All findings were statistically significant except for CA. On intergroup gender comparison of diabetic men and females with control males and females a lower mean CA was reported. The results were significant for T and FOM in men between different groups and for BM, T, and FOM in females between different groups. Furthermore, the average NA was higher and the average CA:NA was lower, with statistically significant findings seen during intergroup gender comparison. The whole result was statistically significant, except for CA at sites BM and P in men, and for site P in females as shown in Tables 2. When comparing genders within the same group of diabetics, the outcome was not statistically significant. The result was not statistically significant in the control group, except for the coefficient of performance and for temperature in the event of negative affect and coefficient of performance to negative affect ratio [Table 3]. In diabetics, gender does not influence morphometric alterations in the cell. However, the control group exhibited variations in all metrics.

Table 1: Comparison of cytomorphometric parameters in Type 2 diabetic (Group 1) and Control (Group 2)

Cytomorphometric	Type 2 diabetic		Control		
	Mean	Sd	Mean	Sd	P value
CA					
BM	3683.24	24.54	3812.76	24.98	0.01
T	2684.45	22.57	3182.78	23.67	0.01
FOM	2897.65	26.77	3243.37	22.37	0.01
P	2678.36	23.98	2699.55	21.66	0.12
NA					0.01
BM	87.23	4.23	63.56	4.76	0.01
T	74.57	3.54	55.72	4.88	0.01
FOM	89.37	3.55	59.12	3.76	0.01
P	73.66	3.38	54.48	4.37	0.01
CA: NA					0.01
BM	41.34	3.56	61.36	3.57	0.01
T	36.22	3.77	56.87	3.66	0.01
FOM	33.24	4.11	54.47	3.57	0.01
P	37.27	3.58	50.11	3.58	0.01

Table 2: Comparison of cytomorphometric parameters in Type 2 diabetic Male and Control male and Type 2 diabetic Female and Control Female

	Male					Female				
Cytomorphometric	Type 2 diabetic		Control			Type 2 diabetic		Control		
	Mean	Sd	Mean	Sd	P value	Mean	Sd	Mean	Sd	P value
CA										
BM	3622.29	25.65	3867.33	23.65	0.12	3603.04	22.65	3789.14	21.67	0.02
T	2644.48	26.54	3125.24	25.54	0.01	2612.25	23.54	3102.18	22.58	0.01
FOM	2876.22	27.61	3201.76	23.61	0.01	2837.85	24.12	3213.02	22.69	0.01
P	2637.36	24.58	2605.76	21.58	0.22	2638.13	27.22	2679.15	26.08	0.32
NA										
BM	87.08	4.23	63.16	4.11	0.01	86.99	4.15	63.26	4.54	0.01
T	74.43	3.54	55.32	3.04	0.01	74.17	3.59	55.42	3.98	0.01
FOM	89.18	3.76	59.02	3.06	0.01	89.07	3.56	59.02	3.54	0.01
P	73.32	3.69	54.28	3.12	0.01	73.26	3.49	54.28	3.89	0.01
CA: NA										
BM	41.08	4.56	61.06	4.27	0.01	41.04	4.11	61.16	4.76	0.01
T	36.32	3.98	56.57	3.78	0.01	36.12	3.99	56.57	3.59	0.01
FOM	33.06	3.69	54.17	3.55	0.01	33.04	3.54	54.27	3.72	0.01
P	37.07	3.98	50.01	3.88	0.01	37.07	3.09	50.01	3.09	0.01

Table 3 Comparison of cytomorphometric parameters in Type 2 diabetic Male and Female

Cytomorphometric	Type 2 diabetic Male		Type 2 diabetic Female		
	Mean	Sd	Mean	Sd	P value
CA					
BM	3622.29	25.65	3603.04	22.65	0.11
T	2644.48	26.54	2612.25	23.54	0.23
FOM	2876.22	27.61	2837.85	24.12	0.21
P	2637.36	24.58	2638.13	27.22	0.42
NA					
BM	87.08	4.23	86.99	4.15	0.24
T	74.43	3.54	74.17	3.59	0.13
FOM	89.18	3.76	89.07	3.56	0.14
P	73.32	3.69	73.26	3.49	0.21
CA: NA					
BM	41.08	4.56	41.04	4.11	0.34
T	36.32	3.98	36.12	3.99	0.34

FOM	33.06	3.69	33.04	3.54	0.21
P	37.07	3.98	37.07	3.09	0.25

DISCUSSION

The current research aimed to discover oral epithelial alterations in type 2 diabetes patients by utilizing exfoliative cytology with cytomorphometry and morphological techniques on BM, T, FOM, and P, and comparing them with the normal mucosa of healthy persons. The average of the parameters CA, NA, CA:NA, and morphological traits were computed and evaluated. Intergroup and intragroup gender comparisons were conducted. Quantitative analysis revealed lower average CA, higher average NA, and lower average CA:NA in diabetics compared to controls across all four locations, with statistically significant differences seen except for CA at site P. Several writers have reported similar findings [9-14], but the mean difference in CA measurements in their investigations was not statistically significant. In this research, there was a substantial reduction in mean coronary artery size in individuals with diabetes. However, when comparing those with diabetes to those without, there was no statistical difference in coronary artery size. There are other reasons for the sustained competitive advantage in both groups.

Type 2 diabetes mellitus is a condition that is influenced by psychological factors and is associated with aging. This leads to reduced blood flow to tissues and diminished cell regeneration. Reduced tissue perfusion is caused by the constriction of blood arteries, leading to ischemia and atherosclerosis, which in turn reduces the amount of nutrients reaching the cell. Additionally, reduced cell turnover creates a stressful environment for the cell [12]. A cell typically maintains appropriate proportions and amounts of various cellular components via genetic and enzymatic control. Therefore, dormant enzymes in a cell may be triggered as needed.

During a stressful state like diabetes mellitus, a significant portion of ATP in the cell is used up, leading to the production of a notable quantity of cAMP as a byproduct of ATP breakdown. Cyclic adenosine monophosphate (cAMP) functions as an enzyme activator for phosphorylase enzyme, regulating intracellular ATP levels and supporting cell activity under stress. Cyclic adenosine monophosphate (c-AMP) quickly triggers the glycogen breakdown enzyme and phosphorylase, releasing glucose molecules for fast metabolism to replenish ATP stores and give energy. ATP storage aids in maintaining cellular homeostasis by serving as a compensation strategy for cell function[15].

Another measure evaluated was sodium concentration, which was elevated in those with diabetes. Sustained high blood sugar levels lead to increased glycation of proteins, lipids, and nucleic acids, resulting in the buildup of advanced glycation end products in the walls of major blood vessels and the basement membrane of small blood vessels. As a result, ischemia and atherosclerosis occur, leading to a gradual reduction in the diameter of the blood artery, reduced tissue blood flow, and diminished cell renewal. This results in a delay in the keratinization process of the epithelium. The postponement in cell differentiation results in a rise in the quantity of cells that typically possess a prominent nucleus[16].

Xerostomia may be the second component, caused by decreased salivary flow. Reduced salivary flow is associated with high blood sugar levels, lack of hydration, medications such as diuretics, duct membrane disorders, and hormonal, microvascular, and neuronal abnormalities. Xerostomia results in heightened oral mucosa injury, leading to cell depletion. The basal cells' activity is increased to replace lost cells by expanding the actively dividing cell population, which consists of cells with distinct and big nuclei[13].

The third component may be the elevated glucose concentration inside the cell, which promotes cell development due to the crucial role glucose plays in metabolic activities. Therefore, a vigorously dividing cell displays a noticeable and sizable nucleus [13]. Suvarna et al[13]. found that when a cell is actively developing, a rise in NA is accompanied by a corresponding increase in CA. Contrary to this, Frost said that in a cell that is actively expanding, the cytoplasmic volume decreases while the nuclear contents rise due to replication[17]. This creates the impression of a bigger nucleus relative to the cytoplasm. Some writers have proposed that inflammation may lead to elevated levels of NA, particularly in immature cells[18].

The research demonstrated differences in cell and nuclear size across the four locations examined. A strong link was seen in some findings of cell area and nuclear area between bone marrow and frontal orbital muscle, as well as between tonsils and pancreas in both groups. This association may explain the resemblance in the kind of mucosa, namely nonkeratinized (buccal mucosa, floor of mouth) and keratinized (tongue, palate). Cowpe et al[19] said that predicting cell size from nuclear size, and vice versa, is not feasible. The CA:NA ratio showed substantial change with age and places in the study, leading the researchers to deem it an unacceptable criterion. Patel et al.[20] and Franklin et al.[21] have highlighted the importance of the CA:NA ratio. They said that it is an appropriate metric for distinguishing between different research populations and also has the benefit of linking nuclear volume to cellular volume, perhaps reflecting the considerable changes that take place in a cell[20].

The research conducted an intergroup gender comparison for all criteria. In diabetic men and women, the average CA size was lower compared to that of control men and women. The results were

significant for T and FOM in men between different groups, and for BM, T and FOM in females between different groups. Furthermore, the average NA was higher while the average CA:NA was lower, and the findings were statistically significant during gender comparison across groups. The whole finding was statistically significant, except for CA in men and BM and P in females. The difficulty in scraping the palate may be causing variable outcomes.
A statistically insignificant outcome was seen when comparing genders within the diabetes group. Most factors did not provide significant results in intragroup gender comparison in the control group, except for P in the case of CA and for T in the case of NA and CA:NA. In diabetics, gender does not impact morphometric alterations in the cell. However, the control group exhibited variations in all metrics. Cowpe et al.[19]'s sex-related survey on normal oral squames found no significant differences in CA, NA, and CA:NA between male and female control groups. Patel et al.[20] found morphometric variations in gender comparison, perhaps attributed to sexual dimorphism and hormonal differences, unlike the current research.
Degenerative alterations in the cytoplasm and nucleus were qualitatively seen and evaluated, showing greater prominence in diabetics compared to the control group. Cytoplasmic vacuoles, karyorrhexis, karyolysis, pyknosis, perinuclear halo, and nuclear vacuoles were only seen in individuals with diabetes. Candida was not detected in any of the smears from the study population.
Morphological alterations in the oral mucosa of diabetes individuals result from several factors. Type 2 diabetes mellitus is influenced by several variables including genetics, nutrition, lifestyle, psychological, and social situations, while being often associated with age. Extended periods of high blood sugar lead to the formation of advanced glycation end products, which play a crucial role in the development of diabetes mellitus and contribute to cellular aging [22]. Cellular aging results in a gradual reduction in the ability of cells to divide and their overall lifetime. Prolonged exposure to external forces leads to the advancement of changes in shape, cellular and molecular harm, such as irregular lobed nuclei, pleomorphism, and cytoplasmic vacuolization [22]. An aging cell displays nuclear degeneration, including karyolysis due to DNAse activity, pyknosis with nuclear shrinkage and condensed chromatin, and karyorrhexis involving fragmentation of the nucleus, nuclear vacuolization, and perinuclear halo formation.
Diabetes mellitus also leads to decreased epithelial cell growth and renewal, as well as a decrease in the enhancing impact of insulin and IGF-1. Changes in microvasculature leading to less cellular nutrition might also account for these morphological modifications. Furthermore, oral hypoglycemics might cause adverse effects include lactic acidosis, cellular enlargement, coarse nuclear chromatin, and changes in nuclear size. Some writers argue that insufficient metabolic regulation of diabetes is associated with specific oral symptoms. Some suggest that the increased susceptibility to infections in diabetes might be attributed to changes in the immune response, namely decreased chemotaxis and phagocytosis, as well as microcirculation issues resulting from reduced blood flow. Diabetic people are more susceptible to oral infections and changes[23].
Xerostomia causes changes in the structure of the mucosa, leading to dryness and atrophy. This may result in mucositis and increased susceptibility to opportunistic infections owing to heightened inflammatory response to microbial colonization[14]. There is a known neutrophil chemotactic impairment in DM. To address this shortage, a positive feedback process leads to heightened inflammation. Adverse hormonal, microvascular, and neuronal alterations might contribute to increased inflammation[14]. Amir et al[24]. said that hyperglycemia, a common result of type 2 diabetes mellitus, worsens inflammation and encourages the buildup of RAGE and TLR4 ligands in the oral mucosa by causing advanced glycated end product production and microbial colonization. The current investigation did not detect any Candida, however previous research by Guggenheimer et al[10]. and Jajaram et al.[9] indicated that individuals with diabetes had an increased susceptibility to Candidal infections because of their impaired immune system. Type 2 diabetes mellitus negatively impacts the structure of the oral mucosa, leading to impaired tissue function that increases the risk of oral infections and degeneration of epithelial cells. Therefore, exfoliative cytology, combined with computer-aided morphometry advancements, can serve as an effective diagnostic tool due to its simplicity and noninvasiveness. The research concludes that the average CA, NA, and CA:NA, together with other morphological alterations, might serve as a useful diagnostic tool for DM.

CONCLUSION

Exfoliative cytology may be a more patient-friendly method for glucose monitoring in type 2 diabetics compared to invasive blood monitoring approaches. Therefore, it may be used as a tool for widespread screening for diabetes mellitus in public health initiatives. Studying quantitative and qualitative alterations in oral mucosa using exfoliative cytology in type 2 DM patients might enhance our comprehension of the disease process at a cellular level.

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