Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 2 Year 2024 Page 1590-1593

Assessment Of Oral Mucosal Epithelium In Type 2 Diabetic Patients Using Exfoliative Cytology: A Preliminary Study

Neha Tyagi[1], Oly Banerjee[2,3], Tiyesh Paul[3], Titlee Majumder[4], Sandip Mukherjee[5,*]

[1]Department of Chemistry, Krishna College of Science and Information Technology, Bijnor, Uttarpradesh-246701.
*[2] ,[5] *Department of Physiology, Serampore College, 9 William Carey Road, West Bengal-712201*
[2,3,4]Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, Bara Kanthalia, West Bengal 700121

***Corresponding author:** Dr. Sandip Mukherjee*
**Department of Physiology, Serampore College 9 William Carey Road, Hooghly-712201, West Bengal, India Email ID: sandip@seramporecollege.ac.in*

***Abstract*:**

Diabetes mellitus (DM) has emerged as a worldwide health concern. By vigilantly tracking the well-being of individuals affected by diabetes, it becomes possible to thwart diabetic complications. Our objective was to scrutinize changes in the structure and cellular characteristics of buccal epithelial cells in type 2 DM patients through the utilization of oral exfoliative cytology techniques. This endeavor seeks to establish the significance of such analysis in the context of public health screening, as well as the diagnosis and monitoring of diabetes mellitus. A total of 72 type 2 diabetic individuals and 46 control individuals underwent oral smears collection from two distinct oral locations: the buccal mucosa and tongue dorsum. These oral smears were subsequently stained with Papanicolaou solution. A comprehensive assessment of both quantitative and qualitative alterations was conducted on each slide. Specifically, 50 well-defined cells per slide were meticulously examined under a microscope, and photographs were subjected to morphometric analysis. Diabetic group exhibited significantly greater cytoplasmic and nuclear areas compared to control. Conversely, the cytoplasmic/nuclear ratio was lower in the control group compared to the diabetic group. Moreover, at both smear sites, a higher proportion of cells displayed nuclear changes in the diabetic group. These findings suggest that diabetes mellitus can induce discernible modifications in the oral epithelium, which can be detected through the exfoliative cytology method employed in this study. Consequently, this method holds promise as a viable approach for assessing this disease as well as progression.

Key words: Type 2 diabetes mellitus, buccal epithelial cells, exfoliated cytology, oral health.

Introduction

Diabetes Mellitus (DM) is the chronic metabolic disorder primarily characterized as persistent hyperglycaemia. According to the old Egyptian manuscript, it is one of the oldest diseases known to the human race which is archaeologically evident-about 3000 years ago (Lakhtakia, 2013). It is one of the most important noncommunicable diseases and a major global health problem of great concern today (Karthik *et al.,* 2015). The prevalence of DM has increased exponentially over the past decade globally which often being interpreted as syndrome of disordered metabolism with inappropriate hyperglycaemia due to either an absolute or relative deficiency of insulin secretion or a reduction in the biologic effectiveness of insulin (or both) and found to be associated with disturbances in the metabolism of carbohydrates, protein, and lipids.
Diabetes mellitus with its ever-increasing global prevalence has emerged as one of the most important and challenging health issues confronting the human population of the present world. According to International Diabetes Federation (IDF), 537 million adults (20-79 years) are living with diabetes worldwide and the total number of people with diabetes is predicted to rise to 643 million by 2030 and 784 million by 2045 (Sun *et al.,* 2022). A large number of patients still remain undiagnosed in India. Significant number of patients show the signs of diabetes related complications at the time of diagnosis, thus resulting in increased morbidity and mortality, all this imposing a great economic burden on the country (Karthik *et al.,* 2015).
The oral cavity complications in diabetes is well-documented and includes xerostomia, tooth loss, gingivitis, periodontitis, odontogenic abscesses, soft tissue lesions of the tongue and oral mucosa (Rohani, 2019). Few studies in the recent past have focused on cytological analysis of oral smears in diabetes patients, in an attempt to better understand the oral changes at a cellular level. Both qualitative and quantitative parameters were studied in the oral epithelial cells and results showed diabetes-induced alterations were detectable by microscopy and cytomorphometry. Therefore, cytology can be used as a technique to detect early stage of diseases even in the absence of clinical features. Exfoliative cytology can be considered as a very powerful diagnostic tool in case of diabetes. It is a straightforward painless and non-invasive diagnostic technique in comparison to other diagnostic examinations. So, it is relatively easy to get to diabetes suspicion through a good anamnesis and a simple clinical examination of the patients.
In this backdrop, the current study was proposed to assess cytological changes in buccal epithelial cells of type 2 diabetic individuals of West Bengal and also to associate these changes with the diabetes pathophysiology.

Materials and methods

Study area and study population

This study was conducted across three districts in West Bengal, namely Kolkata, Hooghly, and Howrah. The study involved the recruitment of 72 individuals with type 2 diabetes, who provided their consent to participate. The control group comprised 46 healthy individuals with glycemia values below 120 mg/dL and no personal history of diabetes.
To assess the cellular changes induced by diabetes mellitus (DM), exfoliative cytology was employed for the examination of oral mucosa. Exclusion criteria were applied to eliminate individuals who smoked, consumed alcohol, had a diagnosis of anemia, a history of liver transplant, kidney failure, or any other systemic condition. Additionally, patients undergoing drug treatment for non-diabetic control were excluded, as prior research has indicated that cellular and nuclear sizes can be influenced by these factors.

Sample collection

Participants from both the study and control groups were briefed on the procedures, and written consent was acquired from each individual. A thorough clinical examination was conducted on all subjects to evaluate oral hygiene and to rule out the presence of any other oral or systemic diseases with oral manifestations.

Cytomorphometric Assessment

Following a comprehensive clinical examination, participants were instructed to rinse their mouths with normal saline. Smears were then collected from the buccal mucosa (BM) and the dorsum of the tongue of each subject using a wooden spatula moistened with distilled water. These smears were subsequently transferred onto grease-free glass slides and fixed with 95% ethyl alcohol.

For cytomorphometric analysis of cells, two smears from both sites (buccal mucosa and tongue) were stained with Papanicolaou stain. The stained slides were examined under a compound light microscope to visualize and analyze cellular morphology.

Statistical Analysis

GraphPad PRISM 10.0 was used for the statistical analysis. Data were presented as Mean±SD. Student's t test was carried out to calculate the variations in the various parameters. P-value less than 0.05 was considered statistically significant for all tests.

Results

The age of participants in both groups ranged from 40 to 58 years. The age range for the DM patients was 46-57 (mean age 51±6.9) years and for the control groups, the age range was 40-58 (mean age 49±9.2) years and a male: female ratio of 1.2:1 in the groups. The duration of the disease ranged from 5 to 12 years.

Table 1: Morphometric analysis of buccal and tongue dorsum smears obtained from type 1 diabetic patients and control individuals.

Parameters	**Control group**	**Diabetic group**	**Significance level***
	Mean±SE	**Mean±SE**	
Nuclear area (μm^2)			
Buccal dorsum	91.36±10.22	51.20±6.23	P<0.001
Tongue dorsum	79.23±5.19	37.22±3.10	P<0.001
Cytoplasmic area (μm^2)			
Buccal dorsum	1562.65±111.40	2099.12±127.33	P<0.001
Tongue dorsum	1552.91±99.20	2110.09±119.25	P<0.001
Cytoplasm to nuclear ratio			
Buccal dorsum	51.11±3.64	41.44±2.12	P<0.01
Tongue dorsum	50.12±2.36	39.33±2.08	P<0.01

* Significance level based on Student's t test.

Table 1 summarizes the various parameters including total cytoplasmic area, total nuclear area and nuclear to cytoplasm ratio. Results revealed that mean nuclear area was higher in diabetic subjects than in controls in both recorded sites, i.e., tongue and buccal mucosa. The same trend of higher values was also seen in the total cytoplasmic area in diabetic group compared to controls. However, cytoplasm to nuclear ratio was higher in controls than diabetic subjects both in tongue as well as buccal mucosa.

Discussion

Diabetes mellitus (DM) is a complex metabolic disorder characterized by diverse forms, each originating from distinct pathophysiological mechanisms. However, these forms often present as a disorder with overlapping and challenging-to-differentiate characteristics, as noted by Banday et al. (2020). The conventional use of invasive techniques in diabetic patients faces challenges due to fluctuations in blood glucose levels and the inherent variability of the disease.

Recent advancements in quantitative techniques have brought about a reevaluation of the potential role of cytology in oral diagnosis. This is particularly relevant in light of the limitations associated with invasive approaches. Analyzing significant changes in the mucosa of individuals with diabetes can offer clinicians a more precise understanding of the disease. The identification of these changes not only contributes to a more accurate portrayal of diabetes but also aids in the early stages of diagnosis and screening for diabetic patients.

As per a study conducted by Sankhla et al. (2014), diabetes has an impact on the morphology of oral mucosa, potentially resulting in compromised tissue repair and retardation of tissue growth. The use of cytomorphometric analysis on cells obtained from diabetic patients holds promise as a non-invasive diagnostic tool. This suggests the potential for it to evolve into a routine investigation for the diagnosis of

diabetes, providing clinicians with valuable insights into the disease through morphological changes in oral tissues.

In this current investigation, quantitative changes were observed in oral mucosal cells of patients with diabetes mellitus (DM) when compared to those of normal, healthy individuals. Noteworthy morphological alterations were identified in the nucleus and cytoplasm of oral epithelial cells among type 2 diabetic patients. The reported cellular changes primarily involved superficial and intermediate squamous cells of the oral squamous epithelium. Importantly, these morphological and nuclear morphometric changes in the buccal mucosa of the type 2 diabetic group were attributed to diabetes rather than factors such as age, smoking habits, or systemic diseases. This assertion is supported by the fact that the control and DM subjects were matched for age and smoking habits. The study's results revealed a significant increase in both nuclear area and nuclear perimeter in the diabetic group compared to the control group. This aligns with the findings of Prasad et al. (2010), who similarly reported an increase in nuclear diameter in uncontrolled diabetes, reinforcing the consistency of our observations with their study. Furthermore, several prior studies have conducted morphometric analyses of oral epithelial cells in type 2 diabetic patients, consistently noting a marked increase in nuclear area (Alberti *et al.*, 2003; Jajarm *et al.*, 2008; Shareef *et al.*, 2008). The collective evidence from these studies, including ours, underscores the relevance of morphometric alterations in oral epithelial cells as potential indicators of diabetes-related changes.

In conclusion, our present study indicates that type 2 diabetes induces detectable morphological and nuclear morphometric alterations in oral epithelial cells. These changes were successfully identified through microscopic examination and cytomorphometric analysis using the exfoliative cytology method. However, it is essential to note that further research in this field is warranted, particularly with a larger sample size. This would enable a comprehensive comparison of the observed cellular changes associated with type 2 diabetes to similar changes induced by other diseases. Such extended investigations will contribute to a more nuanced understanding of the specificity and diagnostic potential of these cellular alterations in the context of various health conditions.

References:

1. Alberti, S., Spadella, C. T., Francischone, T. R., Assis, G. F., Cestari, T. M., & Taveira, L. A. (2003). Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *32*(9), 538–543.
2. Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna journal of medicine*, *10*(4), 174–188.
3. Jajarm, H. H., Mohtasham, N., Moshaverinia, M., & Rangiani, A. (2008). Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method. *Journal of oral science*, *50*(3), 335–340.
4. Karthik, K. R., Malathi, N., Poornima, K., Prakash, S., Kadhiresan, R., & Arunmozhi, U. (2015). Evaluation of Glycemic Control in Type 2 Diabetes Mellitus using Cytomorphometry of Buccal Cells and Correlation with Glycosylated Hemoglobin. *Journal of international oral health : JIOH*, *7*(2), 20–24.
5. Lakhtakia R. (2013). The history of diabetes mellitus. *Sultan Qaboos University medical journal*, *13*(3), 368–370.
6. Prasad, H., Ramesh, V., & Balamurali, P. (2010). Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. *Journal of cytology*, *27*(4), 113–117.
7. Rohani B. (2019). Oral manifestations in patients with diabetes mellitus. *World journal of diabetes*, *10*(9), 485–489.
8. Sankhla, B., Sharma, A., Shetty, R. S., Bolla, S. C., Gantha, N. S., & Reddy, P. (2014). Exfoliative cytology of buccal squames: A quantitative cytomorphometric analysis of patients with diabetes. *Journal of International Society of Preventive & Community Dentistry*, *4*(3), 182–187.
9. Shareef, B. T., Ang, K. T., & Naik, V. R. (2008). Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients. *Medicina oral, patologia oral y cirugia bucal*, *13*(11), E693–E696.
10. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, *183*, 109119.