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Original Research

Cytomorphometric evaluation of exfoliated cells of the tongue and it's correlation with clinical findings in type II diabetic patients

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Abstract

Background and aims: Exfoliative cytology is a non-invasive diagnostic method that has been used for decades to analyze cellular and nuclear alterations. Diabetes mellitus (DM) is a common metabolic illness that results in chronic hyperglycemia, as well as significant cellular alterations in the oral mucosa, particularly in the tongue which is often neglected. Hence we carried out a quantitative and qualitative analysis of the exfoliated epithelial cells of the tongue and clinical changes that manifested in the tongue associated with uncontrolled and controlled type 2 diabetes mellitus (T2DM). **Materials and methods:** Tongue smears were obtained from 125 in uncontrolled T2DM (group I), 125 in controlled T2DM (group II) and 30 control individuals (group III) along with the clinical examination of the tongue. PAP stained smears were subjected for the analysis of nuclear area (NA), cytoplasmic area (CA) and cytoplasmic area to nuclear area ratio (C/N) using a digital analyzer system and PAS stained smears were subjected for the assessment of Candida organisms. **Results:** Cytomorphometric assessment of the tongue smears showed increased nuclear area, decreased cytoplasmic area and decreased mean cytoplasmic area to nuclear area ratio among the study group and control group, PAS stained smears showed positivity for candida organisms in 53.6% in group I and 44.0% in group II. Clinical correlation with cytomorphometric changes among groups I and II did not show any statistical significance in the present study. **Conclusion**: The results obtained from our study emphasize the role of exfoliative cytology as a diagnostic tool in contributing to the general understanding of the changes in the tongue in patients with uncontrolled and controlled T2DM.

Keywords: cytology, diabetes mellitus, oral diagnosis, oral examination, tongue.

Background and aims

Diabetes mellitus (DM) is a severe, chronic condition that has a profound effect on the lives and well-being of individuals, families, and communities worldwide [1]. DM is a metabolic disorder that is caused by impaired insulin synthesis, insulin action, or a combination of the two. It is characterized by sustained hyperglycemia and alterations in carbohydrate, lipid, and protein metabolism [2].

Diabetes mellitus has primary complications such as ketoacidosis also termed diabetic "coma", progressive illness of the kidney and retinal capillaries, peripheral nerve damage, and excessive arteriosclerosis [3]. Diabetes mellitus will often show distinctive clinical signs such as thirst, polyuria, blurred vision, and weight loss, and the long-term consequences of DM comprising of the progressive development of retinopathy with the potential for blindness, nephropathy with an increased tendency for renal dysfunction, or neuropathy with increased risk of foot ulcers, amputation. Patients with diabetes are at an increased risk of developing cardiac, peripheral vascular, and cerebrovascular disease [2].

Diabetes can increase the risk of oral manifestations such as periodontitis, which is considered the sixth manifestation of DM. Xerostomia, burning sensation, angular cheilitis, recurrent ulcers, infections, and oral lichen planus are all the most common oral defects found in patients with diabetes. Additionally, uncontrolled diabetic patients have a decreased response to infections produced by bacteria, fungi, and viral agents as a result of hyperglycemia and ketoacidosis [4].

Glossitis, atrophic tongue lesions, median rhomboid glossitis, geographic tongue, coated and fissured tongue, glossodynia and burning mouth sensation, and altered taste sensation are all described as tongue abnormalities that occur more frequently in diabetes individuals [5, 6].

Numerous pathological disorders affecting the oral mucosa can be distinguished clinically. Apart from clinical examination, a variety of other methods are available for assessing oral mucosal alterations such as exfoliative cytology. Even though for a definite diagnosis, incisional biopsy/excisional biopsy is the most reliable method but as far as tongue lesions are concerned incisional or excisional biopsy cannot be performed for a certain conditions such as geographic tongue, burning sensation, altered taste sensation, etc. [7]. Thus, exfoliative cytology, a simple and non-invasive diagnostic approach, may be regarded as a more realistic tool for evaluating the oral mucosa in diabetes.

Exfoliative cytology is applied to analyze mucosal changes in patients with diabetes and has revealed that the illness may result in identifiable alterations in oral exfoliated epithelial cells and can be assessed by cytomorphometric investigation as reported in many studies [8, 9].

The present study is a comparison of quantitative and qualitative analysis of the cellular area, nuclear area, and the cytoplasmic nuclear ratio of exfoliated epithelial cells of the tongue along with the assessment of clinical changes in the tongue with uncontrolled and controlled type 2 diabetes and assess the presence of candida organisms in smears obtained from uncontrolled and controlled type 2 diabetes that of the healthy controls.

Materials and methods

Study design and patients

The study was conducted in the Department of Oral and Maxillofacial Pathology and Oral Microbiology, A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore. The study groups were divided into three groups: group I – 125 biochemically diagnosed with uncontrolled T2DM, group II – 125 biochemically diagnosed with controlled T2DM and group III – 30 healthy controls were selected without a previous history of diabetes or any other systemic condition. The study included subjects who did not have any adverse oral habits, such as smoking, chewing tobacco, or drinking alcohol, and who were willingly interested in participating. Ethical clearance was obtained from the Intuitional Ethics Committee A B Shetty Memorial Institute of Dental Sciences, certificate number: ABSM/EC 36/2019.

Laboratory, anthropometric and clinical data collection

Diabetic patients with random blood sugar levels above 140 mg/dl were subjected to HbA_{1c} estimation. This was done to categorize controlled and uncontrolled type 2 diabetic patients, with a reference value below 8% in controlled and 8% or above in uncontrolled type 2 diabetic patients.

The tongue was examined for ulcers, median rhomboid glossitis, benign migratory glossitis, burning mouth syndrome, fissuring of the dorsum of the tongue, and any other deformity. After clinical examination, the tongue was wiped with a sterile cotton swab to remove excess saliva and debris. A pre-moistened wooden spatula was used to scrape the tongue, and the exfoliated cells were transferred to a dry glass slide and fixed immediately by immersing in 95% methyl alcohol for an hour and stained with PAP and PAS stain. Each smear was observed under Digital Motic Image Plus 2.0 version (Motic China Group co. ltd). Data for only 50 clearly defined cells per smear were recorded and PAS smears were observed for candida organisms.

Cell area (CA) measurements were obtained by drawing around the nuclear and cell boundary using a digitizer cursor and the software automatically calculates the cell area. Each field with a clear outline was selected. Cytoplasmic ratio (C/N) is the average of CA and NA. The measurements were in microns (1 microns = 3.260 pixel).

Statistical analysis

In this descriptive observational study, a sample of 125 in group I and group II and a sample of 30 in the control study group were included. The collected data were subjected to descriptive and interferential statistics to generate mean and standard deviation. For comparison of the mean, the ANOVA test was used with a 5% confidence interval and intergroup comparison was performed by Bonferroni post hoc test.

Results

The distribution of the study population based on age group and sex showed a majority of the study subjects were in the age group of 40–50 years (48.2%) followed by 51–60 years (44.6%) and 61–70 years (7.1%). The comparison of age among the three groups presented the highest mean age in group I (51.84±6.11 years) followed by group II (50.51±6.56 years) and group III (49.43±6.29 years). However, there was no statistically significant difference seen among the three groups. The mean age of the study population was 50.99±6.35 years (Table 3 and Graph 4). The comparison of gender among groups I and II showed no statistically significant association found among the gender. A total of 51.2% of male participants and 48.8% of female participants were included in the study in which 25.6% of females and 24.4% of males were subjected to the uncontrolled diabetes group and 56.0% of females and 23.2% of males

Table 1: Comparison of mean nuclear area among the three groups.

Groups	Mean nuclear area (Mean ± SD)	F	p-Value
Group I - Uncontrolled diabetic group (n=125)	79.74±7.05		
Group II - Controlled diabetic group (n=125)	73.86±6.98	137.984	<0.01*
Group - III Healthy control group (n=30)	55.82±7.86		

Table 2: Intergroup comparison of mean nuclear area.

Groups		Mean difference	p-Value
Group I - Uncontrolled diabetic group (n=125)	Group II - Controlled diabetic group (n=125)	5.88	<0.01*
	Group - III Healthy control group (n=30)	23.92	<0.01*
Group II - Controlled diabetic group (n=125)	Group - III Healthy control group (n=30)	18.03	<0.01*

Table 3: Comparison of mean cytoplasmic area among the three groups.

Groups	Mean cytoplasmic area (Mean ± SD)	F	p-Value
Group I - Uncontrolled diabetic group (n=125)	2664.48±260.64		
Group II - Controlled diabetic group (n=125)	2643.61±288.26	22.842	<0.01*
Group- III Healthy control group (n=30)	2996.04±122.18		

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were subjected to the controlled diabetes group. The comparison of the gender among group I and group II revealed an almost equal proportion of males and females in the study.

The mean nuclear area was highest in group I (79.74±7.05) followed by group II (73.86±6.98) and group III (55.82±7.86) (Table 1). There was a statistically significant difference found between all the three groups and in intergroup comparison (Table 2).

The comparison of mean cytoplasmic area among the three groups showed the highest mean cytoplasmic area in group III (2996.04±122.18) followed by group I (2664.48±260.64) and group II (2643.61±288.26) (Table 3). There was a statistically significant difference found between all the three groups. However, there was no statistically significant difference between groups I and II.

The comparison of the cytoplasmic area to nuclear area ratio among the three groups showed the highest cytoplasmic area to nuclear area ratio in group III (54.54±6.71) followed by group II (36.13±5.44) and group I (33.63±4.22) (Table 4). There was a statistically significant difference found between all the three groups and in intergroup comparison (Table 5).

The distribution of the study population based on clinical conditions for groups I and II illustrated that majority of the study subjects had no clinical conditions. In group I, 26 study subjects had fissured tongue, 17 study subjects had coated tongue, 11 subjects had Candidiasis and 7 study subjects had a geographic tongue. In group II, 10 study subjects had coated tongue, 7 study subjects had fissured tongue, 3 study subjects had candidiasis and 2 study subjects had a geographic tongue (Table 6). There was no statistically significant association found between the uncontrolled/controlled diabetic group and clinical outcomes.

The comparison of the cytoplasmic area to nuclear area ratio based on clinical conditions for groups I and II showed no significant difference seen in the cytoplasmic area to nuclear area ratio for the different clinical conditions.

Discussion

Diabetic mellitus (DM) is a metabolic disorder characterized by unusually high blood glucose levels. Diabetic mellitus is classified into various subtypes such as type 1, type 2, maturity-onset diabetes of the young, gestational diabetes, neonatal diabetes and other secondary causes associated with endocrinopathies and steroid usage [10].

Table 4: Comparison of cytoplasmic area to nuclear area ratio among the three groups.

Groups	Cytoplasmic area to nuclear area ratio (Mean ± SD)	F	p-Value
Group I - Uncontrolled diabetic group (n=125)	33.63±4.22	207.268	<0.01*
Group II - Controlled diabetic group (n=125)	36.13±5.44		
Group - III Healthy control group (n=30)	54.54±6.71		

Table 5: Intergroup comparison of cytoplasmic area to nuclear area ratio.

Groups		Mean difference	p-Value
Group I - Uncontrolled diabetic group (n=125)	Group II - Controlled diabetic group (n=125)	−2.50	<0.01*
	Group - III Healthy control group (n=30)	−20.90	<0.01*
Group II - Controlled diabetic group (n=125)	Group - III Healthy control group (n=30)	−18.41	<0.01*

Table 6: Distribution of study population based on clinical conditions for groups I and II.

Clinical conditions	Group I – Uncontrolled diabetic group (n=125)		Group II – Controlled diabetic group (n=125)		p-Value
	Number	Percentage	Number	Percentage	
Geographic tongue	7	5.6	2	1.6	0.54 NS
Candidiasis	11	8.8	3	2.4	
Fissured tongue	26	20.8	7	5.6	
Coated tongue	17	13.6	10	8	
No clinical conditions	64	51.2	103	82.4	
Total	125	100.0	125	100.0	

Type 1 diabetic mellitus (T1DM) and T2DM are the two major subtypes of DM, which are often caused by impaired insulin secretion T1DM and/or action T2DM. T1DM is considered to manifest in children or teenagers, whereas T2DM is thought to manifest in middle-aged and older people who have persistent hyperglycemia various factors including sedentary lifestyle and food habits [11].

Diabetes, like many other diseases, can exhibit manifestation in the oral cavity by inducing cellular alterations. Various oral manifestations of diabetics have been studied extensively in the past and are well documented in the literature [12]. The diabetic tongue is a well-recognized phenomenon that is documented in the literature, which represents various clinical conditions that can range from glossitis, glossodynia, glasopyrosis, etc. Burning mouth syndrome and alterations in taste sensation are also common among diabetics [13]. These clinical alterations of the oral cavity can be manifested along the course of the disease and can be related to the level of hyperglycemia.

Clinical alterations in the oral cavity secondary to diabetes can be easily recognized by a physician or a dentist during routine clinical examination, but the evaluation of subclinical cellular changes requires the aid of microscopy. The application of exfoliative cytology can serve as a useful tool in evaluating the oral cytological changes that can facilitate a better understating of the changes occurring in the oral tissues.

The present study was undertaken to evaluate and compare the exfoliative smears of tongue epithelial cells with the clinical findings in uncontrolled and controlled T2DM. Our study included a total of 280 subjects with an almost equal gender distribution of 50.4% females and 49.6% males. One hundred twenty-five subjects with uncontrolled T2DM were included in group I, 125 subjects with controlled T2DM were included in group II and the remaining 30 normal healthy controls were in group III.

The mean age of the subjects in group I was 51.54±6.11 years and the mean age of subjects in group II was 50.51±6.56 years. Our finding is similar to the study Fiagbe J. et al., who reported a mean age of 52.15±9.65 years for uncontrolled diabetics and 51.95±9.85 for controlled diabetes among their study population [14]. Our findings are also similar to the observations of Bhavesh et al., who reported 51–60 years as the common age group in which type 2 diabetic patients were seen [15].

In the present study, the exfoliative smears from the tongue were collected from the study subjects and the cytomorphometric analysis was carried out. We found a statistically significant difference in the mean nuclear area among the three groups (p-value <0.001). The mean nuclear area was highest with a value of 79±7.05 μm^2 in the uncontrolled diabetes group followed by the controlled diabetes group (73.86±6.98 μm^2) and the healthy control group (55.82±7.86 μm^2) which was the least among all. This is in accordance with the study by Nandita et al. who reported increased nuclear area of the exfoliated buccal and tongue cells in type 2 diabetes [16]. Another study by Oz et al. also reported a higher

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mean nuclear area in the exfoliated tongue cells of type 1 diabetics [17]. Our finding is also compatible with several other studies including the study by Alberti et al. [2] and Jajarm et al. [7] who had reported high nuclear area in diabetics. In our study, we observed a higher mean cytoplasmic area in the control group than in groups I and II, which was statistically significant. The mean cytoplasmic area in the uncontrolled diabetes group was 2664.48±260.64 μm^2 and in the controlled diabetic group 2643±288.6 μm^2 but we did not find any statistically significant difference between groups I and II (p-value 0.76). Our findings are consistent with Nandita et al. [16] Jajarm et al. [7] and Prasad et al. [18] which have reported decreased cytoplasmic area among diabetics.

In our present study, we found a considerable decrease in the mean cytoplasmic area to nuclear area (C:N) among the study groups. The ratio was highest in the healthy control groups. The controlled and uncontrolled diabetes group had a comparatively lesser ratio than the healthy controls, which was statistically significant (p-value <0.001). The ratio was least in the uncontrolled diabetes group (33.63±4.22 μm^2) followed by the controlled diabetic group (36.13±5.44 μm^2) which showed a statistically significant difference (p-value <0.001). The cytoplasmic area to nuclear area ratio (C:N) is the relative proportion of the area of the nucleus to the cytoplasm of the cell. The reduced C:N among diabetes may be attributed to the increased nuclear area with a relatively decreased cytoplasmic area than the normal healthy cells. Our findings are in accordance with the studies by Alberti et al. [2] who reported decreased C:N ratio among T2DM and Jajarm et al. [7] among T2DM.

Tongue smears of groups I and II were subjected to the evaluation of the presence of candida organisms using PAS staining. We observed positivity to candida hyphae in 53.6% of subjects in group I and 44.0% of subjects in group II. But we did not get a statistically significant difference in candida among the study groups (p-value of 0.14). Candida species especially *C. albicans* exist in the oral cavity as a commensal, but they tend to cause opportunistic infections in immunocompromised states including diabetes. There is sufficient evidence in the literature proving the increased infectivity and carriage of Candida in diabetes [13, 19, 20]. Shenoy et al. reported the glycemic status to have a direct effect on the candida colonization among diabetics [20]. Chouhan et al. reported an increased CFU of Candida in the uncontrolled diabetics when compared to individuals with controlled diabetes [21]. Gupta et al. also reported increased detection of candida in the oral smears of uncontrolled diabetics than the controlled diabetics [22].

Yonezawa et al. [23] reported that effective tongue and oral mucosal cleaning, especially after food to help in preventing Candida colonization and reduce the oral candida positivity rates in elderly individuals. The varied oral hygiene practice followed by our study population could have a direct role in tongue hygiene that may prevent Candida colonization. This could be the reason for not obtaining a significant difference in the candida positivity among controlled and uncontrolled diabetes, despite various solid evidence reported in the literature.

Clinical examination of the tongue can serve as a mirror for reflecting the systemic health status of the body. Various diseases including endocrinopathies such as diabetes can produce alterations in the tongue and oral mucosa. We performed a thorough clinical examination of the tongue among the subjects in groups I and II. Various tongue diseases such as fissured tongue (20.8%), coated tongue (13.6%), candidiasis (8.8%) and geographic tongue (5.6%) were present among the uncontrolled diabetes group (group I). We observed coated tongue (8%), fissured tongue (5.6%), candidiasis (2.4%) and geographic tongue (1.6%). The frequency of tongue disorders noted in group II (controlled diabetes) was comparatively lesser than that of group I, but we did not obtain a statistically significant difference between the groups (p-value of 0.54).

Bhattacharjee et al. [24] also observed a significantly increased prevalence of oral mucosal alterations in diabetic patients, coated tongue, fissured tongue, leukoedema, fungal infections such as candidiasis, median rhomboid glossitis, which is more similar to our findings. Our observation of the increased prevalence of fissured tongue among uncontrolled diabetics is similar to the findings of the study by Hamrah et al. who

reported an association of fissured tongue with diabetes [25]. On the correlation of the clinical findings of the tongue with the cytomorphometric findings, we did not get any statistically significant association of the cytomorphometry with tongue findings (p-value of 0.43).

Oral exfoliative cytology is a non-invasive investigation modality that has the potential to become a standard examination for the detection of diabetes mellitus. It can be used in conjunction with a chair-side assessment during standard dental examinations. The numerous changes in the cytomorphology of the oral mucosa associated with diabetes, as well as the characterization of these changes, provide physicians with a more realistic picture of what occurs during diabetes [26]. Though several studies evaluating the oral cytomorphometric features in diabetics available in the literature. Studies focusing on the exfoliated tongue cells in relation to the glycemic control and clinical presentation of the tongue are sparsely reported. Hence our study was an attempt to compare the cytomorphometric and clinical features of the tongue in controlled and uncontrolled T2DM. The higher prevalence of candida positive status in the tongue smears of diabetics marks the clinical importance of exfoliating cytology in the screening for candida infections and initiation of active tongue hygiene.

Conclusion

The cytomorphometric findings demonstrated in the present study confirm the microscopic changes found in the tongue cells of uncontrolled diabetes and controlled diabetes which showed an increased nuclear area and cytoplasmic area to nuclear area ratio.

Our results also suggest that uncontrolled diabetes has a high tendency for clinical alterations of the tongue. The encouraging results obtained from our study have the potential to direct future studies to explore the association between cytomorphometric changes and clinical changes of tongue in T2DM.

The results obtained from our study emphasize the role of exfoliative cytology in the early detection and management of tongue changes and other oral conditions in uncontrolled and controlled diabetes patients. However, our study is bound to certain limitations such as a smaller sample size and lack of consideration of the duration of the disease. Further large scale, multi centric prospective studies aiming to investigate the cytomorphometrical changes of the tongue throughout the course of diabetes mellitus have to be undertaken.

In conclusion, based on the current study and the previous literature, microscopical studies of the oral exfoliated cells emphasize the importance of cytological examination of the oral cavity in the diagnosis and monitoring of diabetes mellitus.

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Conflict of Interest

The authors declare no conflict of interest.

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