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Original Research Article

AN EXFOLIATIVE CYTOLOGICAL STUDY OF QUALITATIVE CHANGES IN BUCCAL MUCOSA CELLS OF TYPE 2 DIABETES PATIENTS IN SOUTH GUJARAT REGION

Neeraj T. Master *[1], Nisha Parmar [2].

[1, 2] Tutor, Department of Anatomy, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat-395010, Gujarat, India.

ABSTRACT

Background: Diabetes mellitus is a metabolic disease and affects many organs of the body including oral mucosa. Exfoliative cytology can be used to detect the effects of diabetes on buccal mucosa cells.

Aim: The present study will evaluate the qualitative changes (cytomorphology) of buccal mucosal cells in type 2 diabetic patients and compare that with the non-diabetic individuals of South Gujarat region.

Materials and Method: Present study was done on 50 type 2 diabetic patients (case) and 50 healthy individuals (control) selected as per exclusion and inclusion criteria. Procedure was explained to the participants and informed written consent was taken. Buccal mucosa smears were taken and stained with Pap's stain. 100 cells of each Pap stained smear was examined under a research microscope for various cytomorphological changes. Smears were examined for cell morphology like binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplsmic vacuoles and micronuclei. All data were noted and subjected to statistical analysis.

Results: Mean values of binucleated cells, pyknotic cells, perinuclear halo, cytoplasmic granules, karyolytic cells, karyorrhectic cells, cytoplasmic vacuoles and micronuclei were noted in controls and cases. Significant differences in between non-diabetic control and diabetic cases for mean values of binucleation ($p<0.001$), pyknosis ($p<0.001$), perinuclear halo ($p<0.001$), cytoplasmic granules ($p<0.001$), karyolysis ($p=0.026$) and karyorrhexis ($p<0.001$) was observed. But no significant differences in the mean cells for cytoplasmic vacuoles ($p=0.109$) and micronuclei ($p=0.176$) were found between diabetics cases and non-diabetics control.

Conclusion: The results of present study showed that buccal mucosa of diabetic case group is associated with significant differences in the mean values of qualitative changes like binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis and karyorrhexis when compare to non-diabetic control group. Diabetes produces significant qualitative (cytomorphologic) changes in the buccal mucosa cells that can be documented by exfoliative cytology.

KEY WORDS: type 2 diabetes, buccal mucosa, exfoliative cytology, qualitative (cytomorphologic) changes.

Corresponding Author: Dr. Neeraj Master, Tutor, Department of Anatomy, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat-395010, Gujarat, India.
E-Mail: dr.neerajmaster@yahoo.com

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INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin, associated with long-term damage, dysfunction, and failure of various organs [1]. The diabetes epidemic is more pronounced in developing countries like India than anywhere in the world

as per the World Health Organization (WHO) reports, which shows that 31.7 million people had diabetes in the year 2000 [2].

Many newly diagnosed type 2 diabetic subjects already have developed all the microangiopathic complications and suffer from so called "late complications of diabetes" at the time of diagnosis [3] and it indicates that the diagnosis may have been delayed.

Prevalence of oral mucosal lesions is significantly higher in uncontrolled diabetic patients [4]. Recording the prevalence and characteristics of oral lesions among diabetics is useful for prevention, management and reducing the incidence of these lesions.Studies done by Alberti S et al [5], Jajaram HH et al [6],Shareef BT [7] and others [8,9] have shown that diabetes mellitus produces detectable cytomorphologic changes in oral epithelial cells which can be determined by exfoliative cytology.

As with the rising prevalence of diabetes cases in general population with associated risk of micro vascular and oral complications, there is a need to develop a supplementary tool that will detect early changes of complications in type 2 diabetes patients approaching the dental clinic/ opd. So, the study of qualitative changes in buccal mucosal cells of type 2 diabetic patients is taken up to assess the usefulness of this procedure.

The present study will evaluate and compare the qualitative changes (cytomorphology) of buccal mucosal cells in type 2 diabetic patients and that with the non-diabetic individuals.

AIMS AND OBJECTIVES:

1. To study the qualitative (cytomorphologic) changes in buccal mucosa cells using pap stain among type 2 diabetic patients and compare with non-diabetic individuals of South Gujarat region.

2. To compare the results of this study with the previous studies.

MATERIALS AND METHODS

This study was carried out on 50 randomly selected type 2 diabetic patients(as case) admitted in medicine department of tertiary care hospital of South Gujarat region with history of type 2 diabetes for a minimum period of one year and 50 non-diabetic individuals (as control) selected according to inclusion and exclusion criteria. Study was done during July 2015 to October 2016. Permission from the institutional ethics committee was taken before starting the study.

Inclusion criteria: Non-diabetic Control Group was consisting of 50 healthy individuals without any history/risk factors of diabetes mellitus (to be assessed by random blood sugar) [11] and anaemia (to be ruled out by assessment of haemoglobin. Hb </= 10mg %) [12].

Diabetic group/Patients aged 25 years or above with clinically healthy oral mucosa, with type 2 diabetes mellitus for more than 1 year irrespective of type of treatment were included in the study.

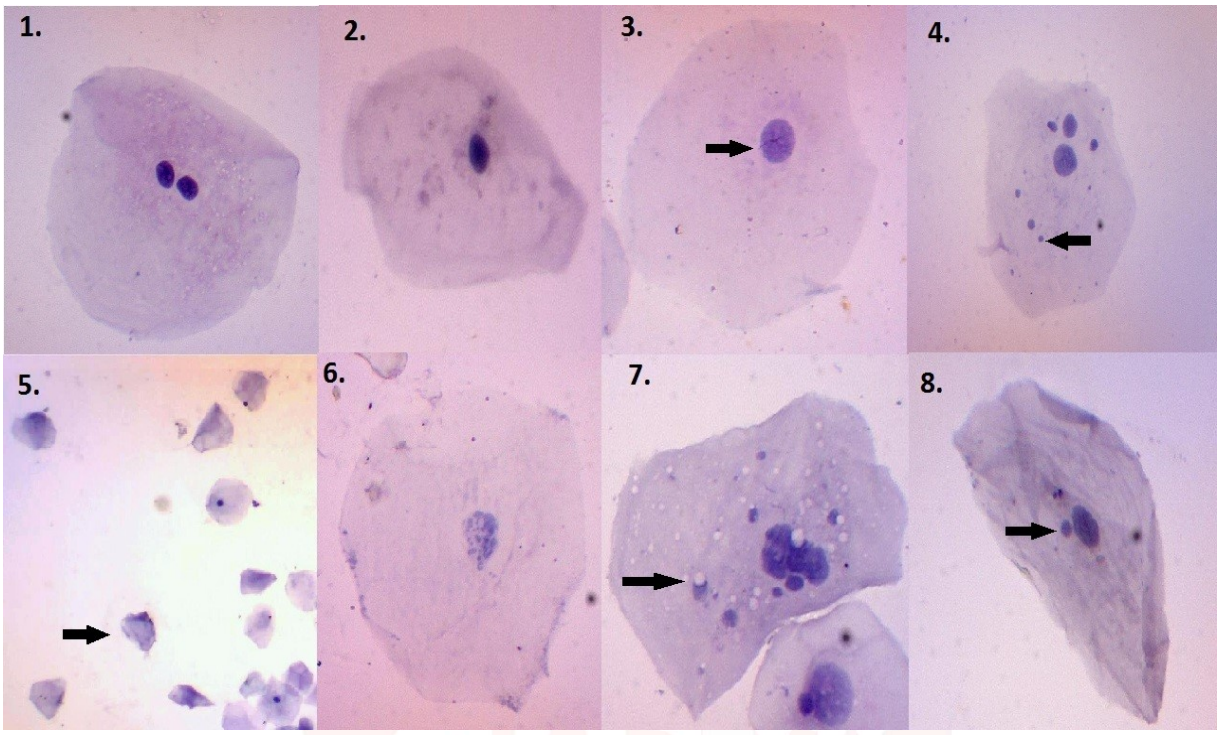
Exclusion criteria: Patients/individuals having habit of smoking or betel nut chewing, alcoholic, with poor oral hygiene, candidiasis, denture, any other systemic disease, anemia, known malignancy, on radiotherapy/chemotherapy and females [10] were excluded from this study.

Informed consent was taken after proper explanation of procedure. Personal Data for age, history of diabetes, medicine, oral hygiene, reports, any other illness etc. were noted. Confidentiality regarding identification was maintained.

Sample collection: The oral mucosa was cleaned and dried with sterile gauze swab to remove surface debris and excess saliva. Smears were obtained from buccal mucosa of both the side using pre-moistened wooden spatula and transferred to pre-coded clean dry glass slide evenly and fixed with alcohol spray. The slides were stained by using Rapid PAP (papanicolaou) stain method in the histology section of Department of Anatomy by the standard technique [13] and were visualized under low power and high power of research microscope and Photomicrography unit.

Qualitative analysis of smears: The cytomorphological (qualitative) changes were observed respectively in each case using the Labomed Lx 400 Research Microscope. Pap stained smears were examined under a microscope for various cytomorphological changes. For Cytomorpho-

Fig. 1: Shows cytomorphology of buccal mucosa cells of diabetic cases.
1.Binucleated Cell, 2.Pyknosis, 3.Perinuclear Halo, 4. Cytoplasmic granulation, 5.Karyolysis, 6.Karyorrhexis, 7.Cytoplasmic vacuoles and 8.Cells with Micronuclei



logical features, 100 cells were scanned under 10 X and 40 X objective lenses and findings were noted and photographed. The qualitative (cytomorphological) changes observed were in the form of Binucleated Cell, Pyknosis, Perinuclear Halo, Cytoplasmic granulation, Karyolysis, Karyorrhexis, Cytoplasmic vacuoles and Cells with Micronuclei [**Figure 1**]. Data collected were analysed for mean, standard deviation and student's t test in both the group.

RESULTS

Results of the present study were analyzed and tabulated. **Table 1** shows that the mean age of non-diabetic control was 46.44 ± 10.97 years and mean age of diabetic case was 49.56 ± 10.99 years. P value between both groups was 0.159 which was > 0.05 and not significant indicating non- bias nature of control and case selection.

Diabetic patients were either on oral hypoglycemic agents with/without insulin in combination. The average blood sugar in non-diabetic control was 106 ± 8.74 mg% and that in diabetic case group was 203.22 ± 67.87 mg%.

Statistical analysis of Qualitative (Cytomorphological) Data amongst diabetic cases and non-diabetic control was done. **Table 2** shows that mean of binucleated cells in controls were 0.88 ± 0.85 while in cases mean of binucleated cells were 2.54 ± 1.63. Mean of pyknotic cells in control and cases were 0.98 ± 1.39 and 2.34 ± 1.48 respectively. Mean of cells with perinuclear halo in control and cases were 0.36 ± 0.83 and 2.18 ± 1.71 respectively. Mean of cells with cytoplasmic granules in controls and cases were 0.44 ± 0.86 and 2.20 ± 1.73 respectively. Significant differences in between non-diabetic control and diabetic cases for mean values of binucleation, pyknosis, perinuclear halo and cytoplasmic granules was observed.

Table 3 shows that mean of karyolytic cells in control were 0.04 ± 0.20 while in cases mean of karyolytic cells were 0.38 ± 1.05. Mean of karyorrhectic cells in control and cases were 0.02 ± 0.14 and 0.42 ± 0.81 respectively. Mean of cells with cytoplasmic vacuoles in control and cases were 0.04 ± 0.20 and 0.20 ± 0.67 respectively. Mean of cells with micronuclei in controls and cases were 0.66 ± 1.10 and 0.40 ± 0.78 respectively.

There were significant differences between non-diabetic control and diabetic cases for the mean values of karyolysis and karyorrhexis. But for cytoplasmic vacuoles and micronuclei no significant differences in the mean cells were found between diabetics and non-diabetics.

Table 1: Distribution of subjects in study groups & mean age.

t-test applied

Sr. No.	Groups	No. of Subjects	Mean Age	Std. Deviation	P-value
1	Non-diabetic group (control)	50	46.44	10.97	0.159
2	Diabetic group (case)	50	49.56	10.99	

Table 2: Comparison of Binucleation, Pyknosis, Perinuclear Halo and Cytoplasmic granules amongst non-diabetic and diabetic group.

Unpaired t-test applied

Variable	Non-diabetic Control (n=50)	Diabetic Case (n=50)	P value (Significant/Non significant)
	Mean± SD	Mean± SD	
Binucleation	0.88 ± 0.85	2.54 ± 1.63	<0.001 (Significant)
Pyknosis	0.98 ± 1.39	2.34 ± 1.48	<0.001 (Significant)
Perinuclear Halo	0.36 ± 0.83	2.18 ± 1.71	< 0.001 (Significant)
Cytoplasmic Granule	0.44 ± 0.86	2.20 ± 1.73	< 0.001 (Significant)

Table 3: Comparison of Karyolysis, Karyorrhexis, Cytoplasmic vacuoles and Micronuclei amongst non-diabetics and diabetic group.

Unpaired t-test applied

Variable	Non-diabetic Control (n=50)	Diabetic Case (n=50)	P value (Significant/ Non significant)
	Mean ± SD	Mean ± SD	
Karyolysis	0.04 ± 0.20	0.38 ± 1.05	0.026 (Significant)
Karyorrhexis	0.02 ± 0.14	0.42 ± 0.81	< 0.001 (Significant)
Cytoplasmic Vacuoles	0.04 ± 0.20	0.20 ± 0.67	0.109 (Non-Significant)
Micronuclei	0.66 ± 1.1	0.40 ± 0.78	0.176 (Non- Significant)

Table 4: Comparison of Qualitative (cytomorphological) data of various studies in buccal mucosa of diabetic cases:

Authors	Alberti S et al [5] (2003)	Shareef et al [7] (2008)	Jajaram et al [6] (2008)	Prasad et al [10] (2010)	Mohamad t.et al [17] # (2012)	Safoura Seifi et al [18] (2014)	Present study
Variables	N= 10	N=10	N=30	N=50	N=20	N=30	N=50
	N1= 50 cells	N1 = 20 cells	N1 = 50 cells	N1= 50 cells	N1= 100 cells	N1= 50 cells	N1=100 cells
Binucleation	Present*	Present*	23.3%*	Present*	5.9%**	76%**	2.54± 1.63**
Pyknosis	-	-	-	-	-	-	2.34 ± 1.48**
Perinuclear Halo	-	-	-	Present*	-	-	2.18± 1.71**
Cytoplasmic Granules (inclusions)	-	-	-	Present*	15.3%**	-	2.20± 1.73**
Karyolysis	-	-	-	-	-	-	0.38± 1.05**
Karyorrhexis	Present*	Present*	40%**	Present*	7.3%**	92%**	0.42± 0.81**
Cytoplasmic Vacuoles	-	-	Present*	Present*	-	95.6%**	0.20± 0.67
Micronuclei	-	-	-	Present*	-	-	0.4± 0.78

N= number of diabetic cases, N1= number of buccal cells examined
* = statistically insignificant or values not mention in study
** = values statistically significant
# = values of newly diagnosed 20 cases mention
- = parameter not mention

DISCUSSION

Diabetes Mellitus - a metabolic disease is associated with many cardiovascular, renal, retinal and neural complications. Many of its symptoms though seem harmless, lead to many associated complications including oral pathology. Oral manifestations of diabetes like dry mouth, periodontitis, periapical abscess, burning mouth syndrome etc. have been shown by various studies [6, 14]. Diabetes is diagnosed

by measurement of blood glucose either fasting (FBS) or 2 hours post-prandial (PP_2BS). Strict blood sugar monitoring required in management of diabetes demands frequent venepuncture and is not accepted by many patients. Exfoliative cytology is a moderate, straight forward and non-invasive technique compared to conventional examination [5].

Qualitative changes observed in present study were, binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic vacuoles and micronuclei amongst both diabetic cases and non-diabetic controls. Out of this binucleation, pyknosis, peinuclear halo, cytoplasmic granules, karyolysis and karyorrhexis were significantly more in diabetic group compare to non-diabetic group. We have compared them with previous studies.

Results were compared with other studies and tabulated. **(Table 4)**

Binucleation: It is presence of two nuclei in cell. Binucleated cells are formed as a consequence of cytokinetic disturbance and lead to an imbalance of the cellular DNA content. This phenomenon is possibly associated with aneuploidy [15].

In present study mean of binucleated cells per 100 buccal cells examined in 50 diabetic cases were 2.54 ± 1.63 in compare to 50 non-diabetic controls which showed mean number of binucleated cells 0.88 ± 0.85 and values for binucleated cells in between diabetic cases and controls showed significant difference.

Alberti S et al (2003) had done exfoliative cytology study in 10 type 2 diabetics and 10 control individuals. They showed the morphologic changes like binucleation more in diabetic [5].

B.T. Shariff et al (2008) had done study in10 diabetic and 10 controls and studied 20 buccal cells. They found prominent binucleation in study amongst diabetics but statistically not evaluated [7].

H. Jajaram et al (2008) had studied 30 diabetic and 30 control patients and evaluated 50 cells from buccal and tongue mucosa. They found binucleation in buccal mucosa of 23.3% diabetic cases and tongue mucosa of 13.3% diabetic cases. Results were higher than control but not statistically significant [6].

Prasad et al (2010) have also mention the presence of binucleation in their study of 50 diabetic patients but were not subjected to statistical analysis [8].

Mohamad T et al (2012) had found binucleation in 5.9 % of newly diagnosed 20 diabetic cases and values were significant compare to healthy subjects in which it was 1.6% [9].

Seifi et al (2014) had found statistically significant 76% binucleation in buccal mucosa of 24 diabetic individuals compare to 21.4% in control [16].

So, all above studies indicated increased numbers of binucleated cells in diabetic cases compare to control group with significant difference. The findings of present study are in accordance with results of previous studies.

Pyknosis: These are the cells with small shrunken nucleus having high density of nuclear material which is intense stained all over. They may represent an alternative mechanism of nuclear disintegration different than process of karyorrhectic cell death stages [17,18].

In present study mean pyknotic cells were 2.34 ± 1.48 in diabetic cases and 0.98 ± 1.39 in non-diabetic control. Their values were significant indicating pyknosis observation was significantly higher in diabetics.

No other study in diabetes was found which has mentioned about pyknosis.

Perinuclear Halo: These are the cells where nucleus is surrounded by a clear rim of cytoplasm.

In present study mean of cells with Perinuclear Halo in diabetic cases were 2.18 ±1.71 and in control they were 0.36 ± 0.83. The values were statistically significant.

Prasad et al (2010) had mention the presence of more number of perinuclear halo in their study of 50 diabetic patients but were not subjected to statistical analysis [8].

Cytoplasmic Granules (inclusions): These are intra cytoplasmic granular inclusion bodies. In present study mean of cells with cytoplasmic granules in diabetic cases and in non-diabetic control were 2.20 ± 1.73 and 0.44 ± 0.86 respectively and were statistically significant.

Prasad et al (2010) had mentioned the presence

of more number of cytoplasmic granules in 50 diabetic patients but were not subjected to statistical analysis [8].

Mohamad T et al (2012) had found cytoplasmic granules in 15.3% cases of 20 newly diagnosed diabetics values were more compare to healthy subjects but statistically not significant [9].

The findings of present study are in accordance with results of other studies.

Karyolysis: These are the cells in which the nucleus is completely depleted of DNA and is apparent as a ghost like image.

In present study mean number of karyolitic cells in diabetic cases were 0.38 ± 1.05 while in control it was 0.04 ± 0.19. Mean values were significant.

No other study in diabetes was found which has mentioned about karyolysis.

Karyorrhexis: These are the cells with nuclear disintegration seen as nuclear fragmentation leading to the eventual loss of integrity of the nucleus. They have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to condensed chromatin cells.

In present study, mean number of karryorectic cells in diabetics were 0.42 ± 0.81, while in control it was 0.02 ± 0.14. Mean values were significant.

Alberti S et al (2003) had done exfoliative cytology study 10 type 2 diabetics and 10 control individuals and showed the occasional karyorrhexis in diabetic cases compare to control [5].

B.T. Shariff et al (2008) had done study in10 diabetic and 10 controls and studied 20 buccal cells. They found prominent karyorrhexis in study but statistically not evaluated [7].

H Jajaram et al (2008) had studied 30 diabetic and 30 control patients and evaluated 50 cells from buccal and tongue mucosa. They found Karyorrhexis in buccal mucosa of 40 % diabetic cases and tongue mucosa of 30 % diabetic cases. Results were statistically significant and higher than control in buccal mucosa cells [6].

Prasad et al (2010) had also mention the presence of higher number of karyorrhexis in 50 diabetic patients but were not subjected to statistical analysis [8].

Mohamad T et al (2012) had found karyorrhexis in 7.3 % of 20 newly diagnosed diabetic cases compare to 2.5% in healthy controls and values were significant [9].

Seifi et al (2014) had found statistically significant 92% karyorrhexis in 24 diabetic individuals compare to 31.6% in control [16].

So, all above studies indicated increased numbers of karyorrhectic cells in diabetic cases compare to control group with significant difference. The findings of present study are in accordance with results of previous studies.

Cytoplasmic Vacuoles: These are multiple clear spherical vacuolization in the cytoplasm of variable size. They are due to partial or temporary disturbances in the cell membrane permeability [19].

In present study mean number of cytoplasmic vacuoles was 0.2 ± 0.67 in diabetic, while in case of control it was 0.04 ± 0.198 which was insignificant.

H Jajaram et al (2008) had studied 30 diabetic and 30 control patients and evaluated 50 cells from buccal and tongue mucosa. They had found cytoplasmic vacuoles in cells in diabetic cases but statistics of results were not mentioned [6].

Prasad et al (2010) have also mention the cytoplasmic vacuoles in 50 diabetic patients but were not subjected to statistical analysis [8].

Seifi et al (2014) had found statistically significant cytoplasmic vacuoles in 95.6 % of 24 diabetic individuals examined [16].

The findings of present study are in accordance with results of other studies.

Micronuclei: These cells have both a main nucleus and one or more round or oval shaped small structures called micronuclei with diameter between 1 /3 and 1 /1 6 of the main nucleus. Their staining intensity and texture are similar to main nucleus. The number of micronuclei within cells can be one, two or more with morphology similar to nuclei of normal cells and they are confined within the cytoplasm of the cells.

In present study mean number of micronuclei was 0.40 ± 0.78 in diabetic, while in case of control it was 0.66 ± 1.09, which was not

significant

Prasad et al (2010) have also mention the cytoplasmic vacuoles in 50 diabetic patients but were not subjected to statistical analysis [8].

Cellular changes seen in any cell injury can be degenerative, inflammatory, repair or neoplastic. Degenerative changes can be swelling and enlargement of cell, wrinkled nuclear margin, pyknosis, karyorrhexis or karyolysis. Inflammatory changes can be in the form of nuclear enlargement, margination of chromatin, binucleation or mulitinucleation, perinuclear halo or cytoplasmic vacuoles. Damage repair of cell can be seen as enlarged nucleus, multinucleation, prominent nuclei and fibroblastic changes. Neoplastic changes can be hyperchromatic irregular nucleus [20]. Cells containing round cytoplasmic eosinophilic inclusions (granules) are probably corresponding to keratinisation [9]. So, the metabolic disease like diabetes by activating any of above mechanism may show the varied cytomorphological changes observed in present study.

Cellular ageing produces various morphologic alterations and genotoxic damages in cells in the form of pleomorphism, bilobed nuclei, nuclear budding, karyorrhexis which were also observed in the smears from diabetic patients in the present study [21].

Thus, present study shows that diabetic case group is associated with significant differences in the buccal mucosa cells for the mean values of binucleation, pyknosis, perinuclear halo, cytoplasmic granules karyolysis and karyorrhexis when compare to non-diabetic control group. But for cytoplasmic vacuoles and micronuclei no significant differences in the mean cells were found. Whether these changes are related to glycemic control (HbA1C level) and duration of the diabetes is the area of further research. Knowledge of which can enable this procedure to use as an alternative tool for assessment of diabetes mellitus.

CONCLUSION

From the present study we conclude that:
1. Buccal mucosa cells of Type 2 diabetes mellitus patients of South Gujarat region shows significant qualitative changes when compared to the buccal mucosa cells of non-diabetic individuals.

2. Amongst qualitative changes, buccal mucosa of diabetic case group shows significant differences in the mean values of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis and karyorrhexis when compared to non-diabetic control group.

3. Diabetes produces significant qualitative (cytomorphologic) changes in the buccal mucosa cells that can be documented by exfoliative cytology.

ABBREVIATIONS

OPD - outdoor patient department,
HbA1C - glycosylated heamoglobin,
DNA - deoxyribose nucleic acid.

Conflicts of Interests: None

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