Original Research Article

Study of quantitative changes in buccal mucosa cells among type 2 diabetes mellitus patients of Gujarat

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Abstract

Background: Oral mucosal cells of type 2 diabetes patients produces distinct cytomorphometric changes which can be determined by exfoliative cytology. **Aim:** The aim of this study was to study the quantitative changes of buccal mucosal cells in type 2 diabetic patients with the non-diabetic individuals and also in relation to glycosylated haemoglobin (HbA1C) level and duration of diabetes in the patients. **Materials and Method:** This study was done on 50 type 2 diabetes patients and 50 healthy controls selected as per exclusion and inclusion criteria. After explaining the procedure and written consent buccal mucosal smears were taken and stained with pap's stain. Slides were examined under research microscope and photographed. 50 cells from each slide were measured for nuclear area (NA) and cellular area (CA) via image J 1.48 software. The cellular area to nuclear area(C: N) ratio was calculated manually. The data of the diabetic group were further analyzed for subgroups based on glycosylated haemoglobin (HbA1C) level and duration of diabetes. The result data were noted and analysed with statistical tests. **Results:** Mean nuclear area (µm2) in non-diabetic control was 47.16 ± 6.01 and in diabetic case was 68.49 ± 13.73 ($p < 0.001$). The mean cellular area (µm2) in non-diabetic control was 2332.38 ± 348.19 and in diabetic case was 2425.66 ± 331.01($p = 0.173$). Cellular area to nuclear area ratio in non-diabetic and diabetic were 47.51 ± 6.30 and 36.44 ± 7.01 respectively ($p < 0.001$). Sub-groups based on glycosylated haemoglobin showed significant increase in mean nuclear area (µm2) values amongst group 1, 2 1nd 3 (51.34 ± 6.23, 66.10 ± 10.38 and 80.94 ± 9.28). Significant decrease in mean cellular to nuclear area ratios was observed in same subgroups. Sub-groups based on duration of diabetes also showed significant increase in the mean value of NA and significant decrease in the mean C: N ratios. **Conclusion:** Buccal mucosa of type 2 diabetes patients shows significant quantitative changes when compared to non-diabetic individuals. The subgroups based on glycosylated haemoglobin level and duration of diabetes also shows significant difference in the mean values of quantitative changes like nuclear area (NA) and C: N ratio.

Key Words: Diabetes Mellitus, glycosylated haemoglobin, quantitative changes (cytomorphometry), Nuclear area (NA), Cellular to nuclear area ratio(C: N ratio)

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INTRODUCTION

Diabetes Mellitus is a metabolic disease that is associated with many cardiovascular, renal, retinal and neural complications as well as oral disorders. Diabetes is said to be an iceberg disease. The prevalence and incidence of type 2 diabetes is increasing globally and are more in newly industrialized countries and developing countries [1]. India has the world's largest number of diabetic patients and earning the dubious distinction of being termed the "Diabetes Capital of the World". The International Diabetes Federation (IDF) estimates the total number of

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diabetic subjects to be around 69.2 million in India and this is further set to rise to 123.5million by the year 2040[2]. Microvascular as well as macrovascular complications are more common in the individuals who are unaware of their disease status are left untreated. Therefore, it is necessary to detect the large numbers of undiagnosed diabetic subjects and to start early therapy in these individuals in India [3].While HbA1C is the standard test for measuring glycemic control, it is not readily available. Poor glycemic control and delay in initiation of insulin therapy is a major problem in Indian patients with Type 2 diabetes where failure to achieve treatment targets and higher mean HbA1C levels are observed.[4] Various studies done by Alberti S *et al*[5], Jajaram HH *et al*[6], Shareef BT[7] and others[8,9] have shown that oral exfoliative cytology determines distinct quantitative (cytomorphometric) changes in oral mucosal cells of diabetes. This study was taken to evaluate and compare the cytomorphometric changes of buccal mucosal cells in type 2 diabetic patients as compared to the non-diabetic individuals and also in relation to Glycosylated haemoglobin(HbA1C) level and that with duration of diabetes in the patients of Gujarat region. The knowledge of this procedure may enable its use as alternative tool for assessment of diabetes mellitus.

AIMS AND OBJECTIVES

1. To evaluate the quantitative (cytomorphometric) changes in buccal mucosa cells using pap stain and image analysis software among type 2 diabetic patients and non-diabetic individuals.
2. To compare the quantitative (cytomorphometric) changes observed in diabetic group with the subgroups based on the glycosylated haemoglobin (HbA1C) levels and with the subgroups based on duration of diabetes in type 2 diabetic patients.
3. To compare the results of this study with the previous studies.

MATERIALS AND METHODS

The comparative study was carried out in a tertiary care hospital of South Gujarat during the year 2015-2016. After the permission from institutional ethical committee, 50 random patients(as case) admitted in medicine ward with history of type 2 diabetes for more than one year and 50 non-diabetic healthy individuals (as control) as per inclusion and exclusion criteria were selected for the study. Sample size was calculated by using OPEN EPI software, considering the prevalence of one month pilot survey of type 2 diabetic patients in the tertiary hospital in the department of medicine male ward. Where, p1 = prevalence of diabetic patients by pilot study = 5%, p2 = prevalence of control = 27.5%, Z1-β = power of the study = 80%, Z α/2 = level of significance = 5%, N= 100, n1 = 50 (diabetic case group), n2 = 50 (non-diabetic control).

Diabetic Case Group:

The diabetic subjects were grouped into three different groups according to levels of glycosylated haemoglobin levels [10].

1. Well-controlled diabetics (HbA1C ≤ 7.0%)
2. Moderately controlled diabetics (HbA1C >7.0% to 9.0%)
3. Poorly controlled diabetics (HbA1C > 9.0%)

Diabetics were also categorized according to duration of diabetes into four groups.

1. Less than or equal to 5 years
2. 5-10 years
3. 10-15 years
4. More than 15 years

Inclusion criteria

Diabetic group/patients aged 25 years or above, with type 2 diabetes mellitus for more than 1 year irrespective of type of medication for diabetes with healthy oral mucosa were included in the study. Non-diabetic Control Group was consisting of volunteers with clinically healthy oral mucosa. All the individuals were ruled out for diabetes mellitus and anaemia based on laboratory investigations.

Exclusion criteria

Patients/controls with history of alcohol intake, smoking, tobacco chewing, poor oral hygiene, systemic disease, on any chemotherapy or radiotherapy and females [11] were excluded from this study. After taking written informed consent, they were explained about the procedure to be done. Detailed information of age, history, medicine, reports, duration of diabetes etc. were collected and noted. Confidentiality regarding identification was maintained.

Sample collection

The selected subjects were instructed to gargle the mouth with saline water and allowed to dry. With pre-moistened wooden spatula smears were collected from buccal mucosa by scrapping and transferred to pre-coded clean glass slide. Smear was spread over a large area avoiding cell clumping and immediately fixed by alcohol spray. The slides were stained with Rapid PAP (papanicolaou) stain method as per standard technique [12] and were visualized under research microscope attached with photomicrography unit.

Quantitative analysis of smears

For quantitative (cytomorphometric) analysis, the observed cell images were projected on the monitor via mounted Photomicrography unit adapter provided with research microscope. A 1280 X 720 pixel digital images were taken with 10X eyepiece and 10X objective. Image

analysis was done using the Image J 1.48 image analysis software. Cytomorphometric analysis of 50 cells per slide was done. For measurement, the software was calibrated and scale setting was changed from square pixels to micrometers squared (µm2) after following the instructions given in the manual of the software. Appropriate care was taken in cell selection (clearly outlined) and measurement to avoid duplication (Figure 1). Nuclear area (NA) and cellular area (CA) were measured. The nucleus and the cell outline were traced using digital cursor on the screen and the software automatically calculated the cellular area and the nuclear area. The C: N ratio (cellular area to nuclear area ratio) was calculated manually. The cytomorphometric (quantitative) data like nuclear area, cellular area and ratio of cell to nuclear area were measured in square micrometer and mean of each parameter for individual subject was calculated in both the groups. The data of the diabetic group were further noted based on their subgroups i.e. (a) according to glycosylated haemoglobin (HbA1C) level and (b) according to duration of diabetes.

Statistical Analysis:

To assess cytomorphometric (quantitative) data between non-diabetic control group and diabetic case group as well as within diabetic subgroups the data obtained were subjected to appropriate statistical test like mean, standard deviation, student's t test and analysis of variance (ANOVA) test. Tukey-HSD post hoc test was applied were values were statistically significant for further comparison of subgroups. The data were analysed with use of SPSS 20.0 software and open EPI 3.0.3 software.

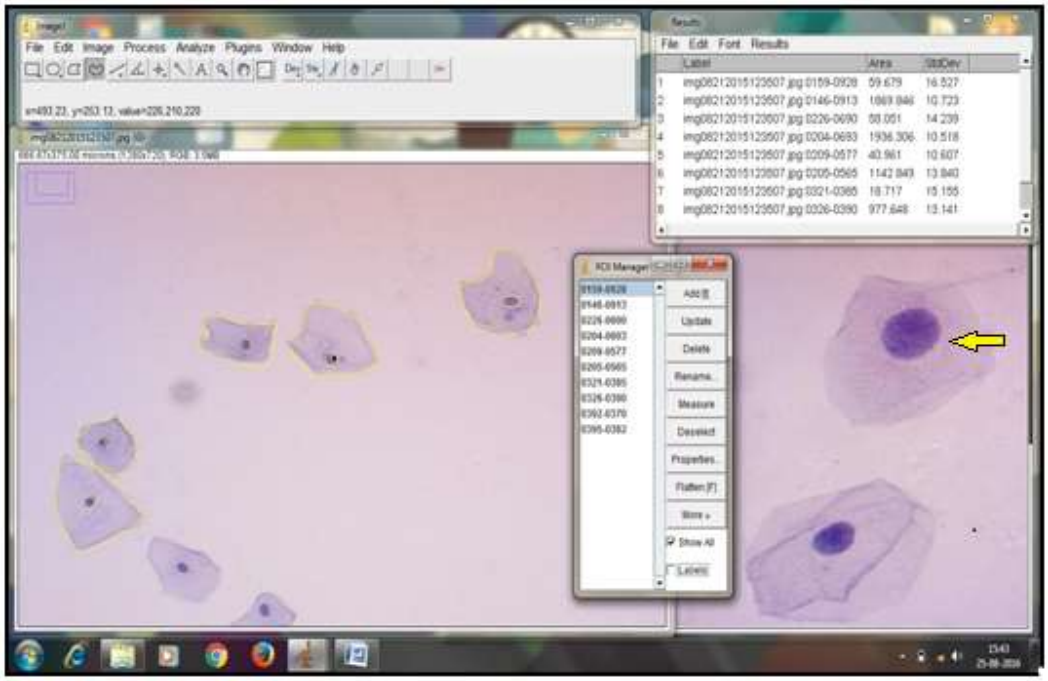

FIGURE 1: CYTOMORPHOMETRIC MEASUREMENT OF BUCCAL MUCOSAL CELLS BY IMAGE J (1.48) SOFTWARE (10X). ARROW SHOWS ENLARGED NUCLEUS IN DIABETIC CASE (40X)

RESULTS

Various quantitative (cytomorphometric) findings were observed and following results were obtained. 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%. Average HbA1C level in non-diabetic control was 5.33 ± 0.30 % and that in diabetic case group was 8.72 ± 1.74 %. The average duration of diabetes in cases was 9.96 ± 5.18 years. Table 1 shows mean nuclear area (µm2) in non-diabetic control was 47.16 ± 6.01 and in diabetic case was 68.49 ± 13.73. The mean cellular area (µm2) in non-diabetic control was 2332.38 ± 348.19 and that in diabetic case was 2425.66 ± 331.01. Cellular area to nuclear area ratio in non-diabetic and diabetic were 47.51 ± 6.30 and 36.44 ± 7.01 respectively. There was significant difference between non-diabetic control and diabetic case for mean values of nuclear area and cellular to nuclear ratio. But difference in mean cellular area was not statistically significant. Table 2 shows mean ± sd for Nuclear Area, Cellular Area and Cellular/Nuclear Ratio amongst three diabetic sub-groups based on HbA1C level. Mean Nuclear area (µm2) in group 1, group 2 and group 3 were 51.34 ± 6.23, 66.10 ± 10.38 and 80.94 ±

9.28 respectively. Mean Cellular area (µm2) in group1, 2 and 3 were 2274.19 ± 308.06, 2390.24 ± 324.63 and 2558.94 ± 323.48 respectively. While mean cellular to nuclear ratio (C: N ratio) in group1, 2 and 3 were 44.82 ± 7.95, 36.62 ± 5.22 and 36.62 ± 5.22 respectively. There was significant difference amongst well control diabetes group 1, moderately controlled diabetes group 2 and poorly controlled diabetes group 3 for increase in mean nuclear area and decrease in cellular to nuclear ratio but increase in values of cellular area (CA) were not significant. So, to assess further post hoc –Tukey HSD test was applied and findings were presented in table 3. Post hoc tukey HSD showed that significant difference of mean nuclear area (NA) was observed amongst group 1(well controlled diabetes) and 2(moderately controlled diabetes), 2(moderately controlled diabetes) and 3(poorly controlled diabetes) and group 1(well controlled diabetes) and 3(poorly controlled diabetes). In cellular to nuclear(C: N) ratio significant difference for mean was observed between group 1(well controlled diabetes) and 2(moderately controlled diabetes), 2(moderately controlled diabetes) and 3(poorly controlled diabetes) and group 1(well controlled diabetes) and 3(poorly controlled diabetes). Table 4 shows mean ± sd for Nuclear Area, Cellular Area and Cellular/Nuclear Ratio amongst four diabetic sub-groups based on known duration of diabetes in years. Mean Nuclear area (µm2) in group 1, group 2, group 3 and group 4 were 51.40 ± 4.81, 64.62 ± 7.68, 73.14 ± 10.54 and 84.85 ± 4.73 respectively. Mean Cellular area (µm2) in group1, 2, 3 and 4 were 2307.77 ± 343.76, 2376.41 ± 336.29, 2445.25 ± 329.30 and 2588.00 ± 288.58 respectively. While Mean C: N ratio in group1, 2, 3 and 4 were 45.03 ± 6.58, 36.82 ± 3.52, 33.85 ± 5.27 and 30.64 ± 4.13 respectively. There was significant difference amongst group 1: less than 5 years duration, group 2:duration 5-10 years, group 3:duration 10-15 years and group 4:duration >15 years for increase in mean nuclear area and decrease in cellular to nuclear ratio but increase in cellular area (CA) were not significant. So, to assess further post hoc –Tukey HSD test was applied and findings were presented in table 5. Post hoc tukey HSD shows that there were significant difference of mean nuclear area (NA) between group 1 (less than 5 years duration) and 2(duration 5-10 years), 1(less than 5 years duration) and 3(duration 10-15 years), group 1(less than 5 years duration) and 4(duration >15 years), group 2(duration 5-10 years) and 3(duration 10-15 years), group 2(duration 5-10 years) and 4(duration >15 years) and group 3(duration 10-15 years) and 4(duration >15 years). For cellular to nuclear (C: N) ratio there were significant difference amongst group 1(less than 5 years duration) and 2(duration 5-10 years), 1(less than 5 years duration) and 3(duration 10-15 years), group 1(less than 5 years duration) and 4(duration >15 years) and group 3(duration 10-15 years) and 4(duration >15 years).

Table 1: Comparison of Nuclear Area, Cellular Area and Cellular/Nuclear Ratio amongst non-diabetic control and diabetic case group
NA = Nuclear Area, CA = Cellular Area, C: N ratio = cellular area to nuclear area ratio

Variable	Non-diabetic Control (n=50)	Diabetic Case(n=50)	P value
	Mean ± SD	Mean ± SD	
Nuclear Area (NA) µm^2	47.16 ± 6.01	68.49 ± 13.73	< 0.001 (Significant)
Cellular Area (CA) µm^2	2332.38 ± 348.19	2425.66 ± 331.01	0.173(Non Significant)
Cellular/ Nuclear ratio (C:N Ratio)	47.51 ± 6.30	36.44 ± 7.01	< 0.001(Significant)

Unpaired t test applied

Table 2: Comparison of Nuclear Area (NA), Cellular Area (CA) and Cellular/Nuclear Ratio(C: N) amongst diabetic subgroups based on HbA1C level, **Group1:** well controlled diabetes (HbA1C ≤ 7%), Group 2: moderately controlled diabetes (HbA1C =7 to 9%) and Group3: poorly controlled diabetes (HbA1C > 9%).

Variable	Group 1 Well controlled diabetes n=8	Group 2 Moderately controlled diabetes n= 26	Group 3 Poorly controlled diabetes n=16	P value
	Mean± SD	Mean± SD	Mean± SD	
Nuclear Area (NA) µm^2	51.34 ± 6.23	66.10 ± 10.38	80.94± 9.28	<0.001 (Significant)
Cellular Area (CA) µm^2	2274.19 ± 308.06	2390.24 ± 324.63	2558.94 ± 323.48	0.100 (Non Significant)
Cellular/ Nuclear ratio (C:N Ratio)	44.82 ± 7.95	36.62 ± 5.22	31.97 ± 5.18	<0.001 (Significant)

ANOVA Applied.

Table 3: Multiple Comparisons for cytomorphometric variables between different groups: Group1: well controlled diabetes (HbA1C ≤ 7%), Group 2: moderately controlled diabetes (HbA1C =7 to 9%) and Group3: poorly controlled diabetes (HbA1C > 9%). NA = Nuclear Area, C: N ratio = cellular area to nuclear area ratio

Post Hoc Tests Tukey HSD

Variable	Groups compared	Mean Difference	P value Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Nuclear Area (NA)	Group 1 and 2	-14.76	0.001	-24.07	-5.45
	Group 2 and 3	-14.84	<0.001	-22.16	-7.52
	Group 1 and 3	-29.60	<0.001	-39.57	-19.63
Cellular/ Nuclear ratio (C:N Ratio)	Group 1 and 2	8.21	0.002	2.63	13.78
	Group 2 and 3	4.65	0.035	0.27	9.03
	Group 1 and 3	12.85	<0.001	6.88	18.83

* The mean difference is significant at the 0.05 level.

Table 4: Comparison of Nuclear Area, Cellular Area and Cellular/Nuclear Ratio amongst diabetic groups based on duration of diabetes (Group 1= < 5, Group 2= 5-10, Group 3= 10-15, Group 4= >15 years)

Variable	Group 1 Duration ≤ 5 years n=11	Group 2 Duration=5 to 10 years n=13	Group 3 Duration = 10-15 years n=16	Group 4 Duration > 15 years n=10	P value
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
Nuclear Area (NA) µm²	51.40 ±4.81	64.62 ±7.68	73.14 ±10.54	84.85 ±4.73	<0.001 (Significant)
Cellular Area (CA) µm²	2307.77 ±343.76	2376.41 ±336.29	2445.25 ±329.30	2588.00 ±288.58	0.250 (Non Significant)
Cellular/ Nuclear ratio (C:N Ratio)	45.03 ±6.58	36.82 ±3.52	33.85 ±5.27	30.64 ±4.13	<0.001 (Significant)

ANOVA Applied.

Table 5: Multiple Comparisons in different groups based on duration of diabetes: (Group 1= < 5, Group 2= 5-10, Group 3= 10-15, Group 4= >15 years) Post Hoc Tests Tukey HSD

Variable	Groups compared	Mean Difference	P value Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Nuclear Area (NA)	Group 1 and 2	-13.27	0.001	-21.75	-4.69
	Group 1 and 3	-21.74	0.0001	-29.90	-13.59
	Group 1 and 4	-33.45	0.0001	-42.55	-24.35
	Group 2 and 3	-8.53	0.027	-16.30	-0.75
	Group 2 and 4	6.19	0.026	0.58	11.80
	Group 3 and 4	-11.71	0.003	-20.09	-3.31
Cellular/ Nuclear ratio (C:N Ratio)	Group 1 and 2	8.21	0.001	2.74	13.67
	Group 1 and 3	11.18	0.0001	5.95	16.40
	Group 1 and 4	14.39	0.0001	8.56	20.22
	Group 2 and 3	2.97	0.395	-2.01	7.95
	Group 2 and 4	6.19	0.026	0.58	11.79
	Group 3 and 4	3.22	0.392	-2.16	8.59

The mean difference is significant at the 0.05 level.

Table 6: Comparison of mean nuclear area, mean cellular area and mean cellular to nuclear ratio of present study with previous studies.

Author (study year)	No. of control /case	Mean Nuclear Area(NA)(µm²)			Mean Cellular Area (CA) µm²			Mean C:N Ratio		
		Non-diabetic control	Diabetic cases	P value	Non-diabetic control	Diabetic cases	P value	Non-diabetic control	Diabetic cases	P value
Jajaram *et al*[6] (2008)	30/30	40.9± 0.68	60.48 ± 0.62	<0.0001	1659.44± 31.64	2129.85 ± 25.94	<0.0001	44.07± 0.49	39.04 ± 0.55	<0.0001
Shareef *et al*[7] (2008)	10/10	142.83 ± 34.15	170.18 ± 60.15	0.014	5623.50 ± 1224.98	5386.33 ± 3407.1	0.705	40.1 ± 9.68	29.38 ± 8.88	0.002
Sankhla B	10/10	67.49 ±	87.27 ± 8.92	<0.001	2731.43 ±	2642.89 ±	Not	40.86 ±	30.45 ±	<0.001

et al[17](2010)		6.78			239.19	243.19	significant	5.42	2.93	
Suvarna *et al*[8] (2012)	40/40	137.28 ± 3.87	169.25 ± 3.02	<0.001	5484.28 ± 165.99	5492.86 ± 181.26	0.826	40.07 ± 0.85	32.13 ± 1.03	<0.001
D Parmar *et al*[15] (2014)	50/50	55.4 ± 10.01	77.32 ± 9.31	<0.001	2929.75 ± 379.2	2573.65 ± 368.36	<0.001	0.0193 ± 0.0044*	0.0304 ± 0.0043*	<0.001
Present study (2016)	50/50	47.16 ± 6.01	68.49 ± 13.73	<0.001	2332.38 ± 348.19	2425.66 ± 331.01	0.173	47.51 ± 6.30	36.44 ± 7.01	0.0001

*= nuclear to cell ratio (N: C ratio)

Table 7: Comparison of present study with other studies based on glycemic control groups in diabetic cases

Study	Groups and HbA1C level	NA /ND (Mean ± SD)	CA/CD (Mean ± SD)	C:N ratio /N:C ratio (Mean ± SD)	P-value
Prasad *et al*[9] (2010)* N=50	Well controlled (≤ 8%)	9.17 ± 0.33	54.22 ± 2.47	0.204±0.017	Significant (Except for cell diameter)
	Moderately controlled (>8 to 10%)	9.44±0.43	54.96 ± 5.04	0.210± 0.030	
	Poorly controlled (>10 to 12%)	9.78±0.43	55.19 ± 4.32	0.217± 0.022	
	Uncontrolled (>12%)	10.01±0.60	50.96 ± 4.10	0.246± 0.023	
Karthik *et al*[20] (2014) N=20	Well controlled (<7%)	351.97±19.59	13503.13± 721.42	39.07± 2.29	Significant (except for CA)
	Uncontrolled (>9%)	447.15±25.10	13686.61± 964.44	30.95± 1.76	
Present study (2016) N=50	Well controlled ≤ 7%	51.34 ± 6.23	2274.19 ± 308.06	44.82 ± 7.95	Significant (except for CA)
	Moderately controlled (7 to 9%)	66.10 ± 10.38	2390.24 ± 324.63	36.62 ± 5.22	
	Poorly controlled (>9%)	80.94± 9.28	2558.94 ± 323.48	31.97 ± 5.18	

*= values are ND= nuclear diameter, CD= cell diameter, N: C ratio= nuclear to cell diameter ratio, NA= nuclear area (µm2), CA = cellular area (µm2), C: N ratio = cellular to nuclear ratio, N= number of diabetes cases

DISCUSSION

Diabetes Mellitus - a metabolic disease accounts for approximately 90-95% of cases and is the fifth most common chronic condition [6], which is associated with increased mortality and morbidity [7]. To reduce the microvascular complications of diabetes reasonable HbA1C goal for the control of diabetes is to lower the values below or around 7% [13]. The early diagnosis of diabetes is an important aspect of health [7] and undiagnosed diabetes is a hidden danger. Condition is also aggravated because of undiagnosed or poorly managed pre-diabetic cases. Blood sugar monitoring requires frequent venepuncture in diabetes and many patients do not like it. Exfoliative cytology is a technique that can be repeated frequently with little discomfort to the patient [14]. The subjective nature of observation in interpretation can be overcome by the introduction of quantitative methods such as image analysis systems especially in the assessment of cytomorphometric cellular alterations [15]. Computer assisted morphometry are more reliable, objective and reproducible[16].Present study has been done to access the knowledge of this procedure to enabling it to use as an alternative tool for assessment of diabetes mellitus. Data of present study were compared with other studies.(Table 6) Cytomorphometric findings in present study were in form of significant increase in mean nuclear area (NA) and significant decrease in cellular to nuclear (C: N) ratio in the diabetic group compare to non diabetic controls which was similar to studies done by studies done by Jajaram *et al*, Shareef *et al*, Sankhla B *et al*, Suvarna *et al* and D Parmar *et al*. Mean cellular area in diabetic cases compare to non-diabetic control in present study was increased but did not show significant difference. Jajaram *et al* had found significant increase in cellular area in their study. Other studies did not show significant difference in cellular area in diabetes. Study done by D Parmar *et al* showed significant decrease in mean cellular area.

Alberti *et al* (2003) had done cytomorphometric study in 10 diabetics and 10 control individuals and revealed

significant increase in nuclear area (NA) and significant decrease in C/N ratio which was 37% lower in diabetic group. No significant difference between cytoplasmic area (CA) of control and diabetic group was observed. They suggested cytomorphometry can be used for diagnosis of diabetes [5]. Our results were similar to them for nuclear area and C/N ratio. Similar results were seen in various other studies; Prasad H *et al* (2010) had found significant increase in nuclear diameter in 50 diabetic patients compare to 5 controls. They showed that glycemic control had significant effect on nuclear diameter and nuclear to cell ratio [9]. Hallikerimath Seema *et al* (2011) had studied buccal mucosa of 30 type 2 diabetes patients and 30 control case. They found significant increase in nuclear area, decrease in C/N ratio and statistically insignificant increase in cellular area (CA) in diabetes (2707.89 ± 411.47µm2) compared to control (2589.4 ± 280.79 µm2). Results of cellular area were in accordance with present study. They also showed increase count of PAS stain positive cells in diabetic group compare to control [18]. Ceser revera *et al* (2013) had studied 30 diabetic patients for cytomorphometry and salivary flow and found significant increase in nuclear area and N: C ratio while insignificant decrease in the size of cytoplasm [19]. Nandita *et al* (2014) had also done study of 10 type 2 diabetic patients and 10 controls and examined cells of buccal mucosa, dorsum of tongue and floor of mouth. They found significant increase in NA and decrease in C: N ratio in diabetic patients as compare to control. They also found significant increase in cellular area (CA) and cytoplasmic area in diabetic group similar to study done by Jajaram *et al* [14].Thus, most of the above study showed that there was significant increase in mean nuclear area (NA) and significant decrease in mean cellular to nuclear ratio(C: N) in buccal mucosa smears of diabetes case group compared to non-diabetic control group which were in accordance with present study. Analysis of quantitative data of diabetic cases as per subgroups based on HbA1C level showed that as we go from well control diabetic (group 1) to poorly control diabetic (group 3), there was increase in mean nuclear area (NA) (µm2) which was significant. But there was no significant difference found in cellular area (CA) (µm2) of all 3 groups. In case of cellular to nuclear(C: N) ratio, there were decreasing values amongst group 1, 2 and 3, which was significant.(Table 2) Further analysis with post hoc –Tukey HSD test had supported the findings results. (Table 3) Prasad *et al* (2010) [9] and Karthik *et al* (2014)[20] have also studied buccal mucosa changes based on glycosylated haemoglobin levels in diabetes patients. Findings of their studies were compared with the present study in the table 7. Prasad *et al* (2010) had found that the level of diabetic control as noted by HbA1C values, definitely influenced the nuclear diameter (p=0.0042) and nuclear to cell ratio significantly (p=0.0055). Cell diameter (CD) was influenced but values were statistically insignificant. They said that the severity of diabetes or in other words, amount of glycemic control had affected nuclear diameter and nuclear to cell ratio [9]. Karthik *et al* (2014) had also found that mean nuclear area was significantly higher in diabetic groups compared to control group and maximum significance was between control group and uncontrolled diabetes group. The mean C: N ratio showed statistically significant decrease in diabetic group compared to control group. Maximum significance was between uncontrolled diabetes and controlled diabetes groups. They further assess the correlation between the cytomorphometric (NA, CA and C: N ratio) parameters and HbA1C value in each group. They found significant correlation between HbA1C values and C: N ratio and also between HbA1C values and nuclear area (NA) [20]. Results found in these studies showed that, degree of glycemic control as represented by well controlled, moderately controlled and poorly controlled diabetic groups significantly affected the mean nuclear area (NA) and mean C: N ratio amongst diabetic group. Results were in accordance with present study. Analysis of quantitative data of diabetic cases as per subgroups based on duration of diabetes showed significant increase in the mean value of NA(µm2) for group 1, 2, 3 and 4 (less duration group to more duration group) and significant decrease in the mean C: N ratio for group 1, 2, 3and 4.Post hoc Tukey HSD test had confirmed the results. These shows that as we go from less duration group <5 years to more duration group >15 years the mean values of NA significantly increases and C: N ratio significantly decreases. Prasad *et al*[9] had mention about duration of diabetes in their study but results were not statistically analysed. No other study in diabetes was found which has compared the cytomorphometry with duration of diabetes. Studies have shown that hormonal changes [11], Nutritional deficiencies like anaemia, vitamin B12, folic acid [21] smoking [22] can alter nuclear diameter and cytoplasmic diameter of oral mucosa. In the present study, we have tried to avoid all such possible causes that can affect our results. The quantitative changes seen in present study can be attributed to the increase in cellular age in patients with diabetes. Atherosclerosis can occur in diabetes and this may lead to ischemia. This would cause secondary reaction like decrease cellular turnover and limited production of newer cells. i.e. more number of mature cells with large nuclei in smears [6]. The increase in nuclear area seen in present study in diabetic group can be explained by various hypotheses like delay in keratinization of oral epithelium, dehydration/atrophy,

and inflammatory process. Sustained hyperglycaemia causes greater accumulation of advanced glycation end products which ultimately leads to progressive narrowing of the vessel lumen and decreased cell turnover, thereby explaining the delay in the keratinization process of the epithelium. This delayed epithelial differentiation leads to an increase in the number of mature cells, which show a large nucleus as a primary characteristic [5, 14]. Diabetic patients are more likely to have dry mouth and atrophic mucosa due to dehydration caused by disease process or due to decreased salivary flow rates as reported by various study [19]. This may lead diabetic patients more prone to secondary infection and inflammatory changes leading to cell with large nuclear diameter from basal or parabasal layer [9].In present study increase in cellular area was found in diabetic group which was statistically insignificant. Similar increase in cellular area was found in studies done by Jajaram *et al*, Seema *et al* and Nandita *et al*. This could be because of the altered cell membrane integrity leading to excessive accumulation of lipid droplets in the cytoplasm, thus, creating an increased intercellular space as reported by Caldeira *et al*[23] in an experimental animal study where they examined the alterations in the histology and ultra structure of oral epithelium of diabetic mice. In diabetes there is relative insufficiency of insulin which prevents glucose uptake by epithelial cells required for cell growth. So, the amount of cytoplasm that cell makes decreases relative to the amount of nucleoplasm. Associated inflammation also increases NA further. All this leads to disproportionate changes and leads to decrease in C: N ratio [20].The significantly greater increase in nuclear area compared to insignificant changes in cellular area explains the significant decrease in cellular to nuclear ratio found in present study. Although the quantitative changes found in the oral smears of type 2 diabetic patients are features that point to malignancy, it can be differentiated from the latter by the diminished C/N ratio and uniformity in the nuclear configuration [24].The reasons for difference between the results of different studies can be due to difference in age and number of participants, type of medication used, duration of diabetes, glycemic control of case (well controlled, moderate controlled or poorly controlled),time of diagnosis, type of software used and methodology. Thus the quantitative(cytomorphometric) findings of present study showed significant increase in mean nuclear area (NA) and significant decrease in mean cellular to nuclear(C: N) ratio in the buccal mucosa smears of diabetes case group compared to non-diabetic control group. Comparison of these findings in sub-groups of diabetes based on glycemic control and duration of diabetes, shows that degree of glycemic control(HbA1C values) and duration of diabetes shows significant difference in the mean values of nuclear area (NA) and cellular to nuclear (C: N) ratio in diabetes patients. Whether such varied quantitative changes are detectable in all the cases of diabetes having associated other systemic complications can be the further research area to be addressed. This can have prognostic potential in timely prevention of diabetic complication. Present study was limited to type 2 diabetic male patients without any systemic complications. So, further study with larger sample size using standardised fully automatic image analyser system has to be conducted. This will help to assess the usefulness and reliability of quantitative changes like, nuclear area and C: N ratio for prediction of glycemic control in wider range of population.

CONCLUSION

From the present study we conclude that:

- Buccal mucosa of Type 2 diabetes patients shows quantitative changes like significant increase in mean nuclear area (NA) and significant decrease in mean cellular to nuclear ratio(C: N) when compared to non-diabetic control.
- The subgroups based on degree of glycemic control and duration of diabetes shows significant increase in the mean values of nuclear area(NA) and significant decrease mean cellular to nuclear ratio(C:N ratio) in diabetes patients.

The present study highlights the use of exfoliative cytology as an adjunct tool to evaluate the effects of diabetes on oral mucosa, especially for those diabetic patients who are attending the dental surgeon for various oral problems. Although exfoliative cytology cannot replace the currently available methods for diagnosis and control of diabetes but non-invasive nature of the test can have better patient compliance.

Abbreviations:

HbA1C= glycosylated haemoglobin A1C, NA=Nuclear area, CA= cellular area, C:N ratio=cellular area to nuclear area ratio, ADA= American Diabetes Association

REFERENCES

1. Park K, Textbook of Preventive and Social Medicine, Non-communicable diseases, Diabetes Mellitus; chapter 6; 23rd edition, BanarsidasBhanotPublicers, India, 2015; p.393.
2. The Global Picture, International Diabetes Federation, IDF Diabetes Atlas, UK, Chapter 3,Table 3.3,7th edition,2015; p.50-52.
3. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C, Epidemiology of type 2 diabetes: Indian scenario, Indian J Med Res, March 2007; 125: 217-30.
4. Moses CR, Seshiah V, Sahay BK, Kumar A, Asirvatham AJ, Balaji V *et al*. Baseline results indicate poor glycemic control and delay in initiation and optimization of insulin therapy: results from the improving management practices

and clinical outcomes in type 2 diabetes study. Indian J Endocrinol Metab. 2012; 16(Suppl 2): S4:32-3.
5. Alberti S., Spudella CT, Francischone TRCG, Assis GF, Crstari TM, Taverira LAA. Exfoliative cytology in type 2 diabetes patients- morphology and cytomorphometry. J Oral Pathol Med 2003; 32: 538-43.
6. Jajaram HH, Mohtasham N, Rangiani A. Evaluation of Oral Mucosa Epithelium in Type 2 Diabetic Patients by an Exfoliative Cytology Method. J Oral Science, 2008; 50(3):335-40.
7. Shareef BT, Ang KT, Naik VR. Qualitative and Quantitative Exfoliative Cytology of Normal Oral Mucosa in Type 2 Diabetic Patients. Oral Med Oral Pathol Oral Cir Bucal, 2008:1; 13(11):E693-6.
8. Suvarna M, Anuradha C, Kumar KK, Shekhar PC, Chandra KLP, Reddy BVR. Cytomorphometric Analysis of Exfoliative Buccal Cells in Type 2 Diabetic Patients. J Dr. NTR Univ Health sci 2012; 1(1):33-37.
9. Prasad H, Ramesh V, Balamurali PD. Morphologic and Cytomorphometric Analysis of Exfoliated Buccal Mucosal Cells in Diabetes Patients. J Cytol 2010; 27(4):113-7.
10. Teitz NW, High Performance Liquid Chromatography Method,Fundamentals of Clinical Chemistry 2nd Edition, WB Saundas Company, chapter 23, 1986; p 454.(Glycosylated hemoglobin were estimated by using D-10 HPLC based analyzer by BIO RAD, USA.).
11. Donald PM, George R, Sriram G, Kavitha B, andSivapathasundharamB , Hormonal changes in exfoliated normal buccal mucosal cells, J Cytol. 2013 Oct-Dec; 30(4): 252–56.
12. Bancroft JD, Rapid Pap Stain method, Theory and Practicle of Histological Techniques, 6th Edition, p. 127-8.
13. American Diabetic Association, Standards of Medical Care in Diabetes-2015, Chapter 6-Glycemic Targets, Diabetes Care Journal, January 2015; 38(Suppl 1) S35, S37.
14. Nandita KP, Boaz K, Srikant N, Lewis AJ, Manaktala N. Oral epithelium in diabetics: A cytomorphometric correlation. Dent Hypotheses 2014; 5:59-65.
15. Parmar D, Sawke G K, Sawke N. A cytomorphometric Evaluation of the Oral Mucosal Cells in Type II Diabetics. PJSR2014; 7(2):22-27.
16. Nayar AK, Sivapathasundharam B: Cyto-morphometric analysis of exfoliated normal buccal mucosa cells. Indian J Dent Res 2003; 14:87–93.
17. Sankhla B, Shah S, Madhusudhan AS,Bhagat P, Exfoliative cytology of oral mucosa: Cytomorphometric analysis of diabetic patients, Journal of Oral Sign, 2010; Vol 2(2),p.7-11.
18. Hallikerimath S, Sapra G, Kale A, Malur PR: Cytomorphometric analysis and assessment of periodic acid Schiff positivity of exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients,. Acta Cytol 2011; (55): 197–202.
19. César R, Camila NM, Exfoliative cytology of oral epithelial cells from patients with type 2 diabetes: cytomorphometric analysis, Int J Clin Exp Med 2013; 6(8):667-676.
20. Karthik KR, Malathi N, Poornima K, Prakash S, Kadhiresan R, Arunmozhi U. Evaluation of glycemic control in type 2 diabetes mellitus using cytomorphometry of buccal cells and correlation with glycosylated haemoglobin. J Int Oral Health 2015; 7(2):20-24.
21. Macleod RI, Hamilton PJ, Soames JV. Quantitative exfoliative oral cytology in iron-deficiency and megaloblastic anemia. Anal Quant CytolHistol. 1988 Jun; 10(3):176-80.
22. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO. The effect of tobacco smoking and of betel chewing with tobacco on the buccal mucosa: a cytomorphometric analysis. J Oral Pathol Med. 1999 Oct; 28(9):385-8.
23. Caldeira EJ, Garcia PJ, Minatel E, Camilli JA, Cagnon VH. Morphometric analysis and ultrastructure of the epithelium of the oral mucosa in diabetic autoimmune NOD mice. Braz J Morphol Sci 2004; 21:197-205.
24. Maeda MY, Di Loreto C, Shirata NK, Shih LW, Cavaliere MJ, Longatto Filho A, *et al*. Image analysis of nuclear/cytoplasmic ratio in cervical smears to discriminate three grades of cervical intraepithelial neoplasia. Acta Cytol. 1997 May-Jun; 41(3):744-8.

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