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Original Research

Evaluation of Glycemic Control in Type 2 Diabetes Mellitus using Cytomorphometry of Buccal Cells and Correlation with Glycosylated Hemoglobin

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Abstract:

Background: To study cytological alterations in the exfoliated buccal cells of diabetic patients. To analyze the cytomorphometric findings in the smears of uncontrolled and controlled diabetic patients and compare it with that of normal healthy controls. To establish a correlation between cytomorphometric changes and glycosylated hemoglobin (HbA_{1c}) in diabetics and normal controls, for evaluation of glycemic control.

Materials and Methods: The study was carried out in 40 confirmed diabetic patients from a hospital out-patient diabetic ward and 20 healthy individuals as controls (Group A: *n* = 20), in Chennai. Specific exclusion criteria were used to select the study group from a larger group of subjects. Based on HbA_{1c} values, the diabetic patients were categorized into Group B = Controlled diabetics (*n* = 20) (HbA_{1c} <7%) and Group C = Uncontrolled diabetics (*n* = 20) (HbA_{1c} >9%). After informed consent, buccal smear was collected from clinically normal appearing mucosa and stained with papanicoloau (PAP) stain. Cytomorphometric analysis of selective PAP stained cells was done using image analysis software, Image Pro Plus 5.5 (Olympus) and parameters determined were average cytoplasmic area (CA), average nuclear area (NA) and cytoplasmic:nuclear (C: N) ratio for an average of 50 cells/patient.

Results: Comparing the average NA among three groups, an increase through Group A, B, C, with a maximum significance between Group C and A was seen. The average C: N ratio showed a statistically significant difference between all three groups. Significant correlation existed between the HbA_{1c} values and both the C: N ratio and average NA in all the three groups.

Conclusions: Cytomorphometric analysis of buccal smears using the C: N ratio alteration as a reliable criteria, may serve as yet another non-invasive tool for screening programs for diabetic detection. And the technique may possibly be used also for evaluation of glycemic control in known diabetics.

Key Words: Cytomorphometry, diabetes mellitus, exfoliative cytology, glycemic control

Introduction

Diabetes mellitus is a syndrome of disordered metabolism with inappropriate hyperglycemia due to either an absolute or relative deficiency of insulin secretion or a reduction in the biologic effectiveness of insulin (or both) and is associated with disturbances in the metabolism of carbohydrates, protein, and lipids. Diabetes mellitus is one of the most important non-communicable diseases and a major global health problem of great concern today. It is more prevalent in developing countries like India and has the potential to affect all classes of people irrespective of their age, sex, and socioeconomic status. Its impact can be seen not only by the number of individuals affected per year but also by its ability to cause damage virtually to every part of the body.

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.[1] It has attained the dubious distinction of being the diabetic capital of the world. 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.[2]

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.[3] 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.[4]

Owing to paucity of information regarding oral epithelial cell alterations in individuals with uncontrolled and controlled diabetes mellitus, this study was undertaken to assess the role

of oral exfoliative cytology of the clinically normal mucosa in the evaluation of glycemic control.

Thus, the aims of the study were:

- To analyze the cytomorphometric changes in the smears of uncontrolled and controlled diabetic patients and to compare it with that of normal healthy controls
- To correlate the cytomorphometric changes in uncontrolled and controlled diabetics with glycosylated hemoglobin (HbA_{1c}) and comparing it with normal controls, for possible evaluation of glycemic control.

Materials and Methods

Patient selection

All diabetic patients under treatment and regular follow-up attending the diabetic outpatient department of Sri Ramachandra Medical College and Hospital, Chennai and MV Diabetic Specialties and Research Center, Gopalpuram, Chennai were included in the study group ($n = 40$). Age and sex matched staff of Sri Ramachandra Dental College who volunteered for the study (as a part of their annual diabetic screening) and tested negative for diabetes were included as control ($n = 20$). Informed written consent was obtained for oral cytology.

Hospital records of all the subjects attending the outpatient department were serially screened, and intra oral examination was performed for each patient, following informed written consent. Specific exclusion criteria were used to select the study group from a larger group of subjects, such as; individuals with smoking and tobacco chewing habits, habitual alcohol intake, presence of oral sepsis, presence of other systemic diseases (vesiculo-bullous lesions, infections, autoimmune conditions, and endocrine disorders), presence of clinically evident nutritional deficiencies (especially anemia).

All the 60 subjects underwent routine hematological investigations including HbA_{1c} and were grouped as follows,

Group A: Normal healthy subjects (20 samples).

Group B: Controlled diabetic patients those with $HbA_{1c} < 7\%$ (20 samples).

Group C: Uncontrolled diabetic patients those with $HbA_{1c} > 9\%$ (20 samples).

Cytopreparatory technique

All smears were made using a sterile metal spatula with a gentle scraping motion, exerting little pressure; cells were scrapped from the clinically normal appearing buccal mucosa behind the commissural area in all individuals. The collected sample was evenly smeared onto the center of the glass slides. The slides were immediately immersed in 95% isopropyl alcohol to ensure proper fixation, for one hour. Air-drying of the smears was strictly avoided as it leads to alterations in the cellular morphology. Smears were stained as per the papanicolaou technique (PAP).[5]

Cytomorphometric analysis

Prior to image acquisition, a general consensus was reached among the observers that only cells that were fully included in the field of vision and with clearly defined cellular and nuclear outlines were to be selected. And cells that were clumped, overlapped, or folded were not considered for the analysis. PAP stained smears were manually screened by one operator and the cells were selected from the field of vision beginning at one end of the smear, moving horizontally, and at the other end, moving downward to the next level and again moving horizontally in the opposite direction. This systematic sampling avoids repeating the cell count.

- An average of 50 clearly defined cells belonging to all levels of differentiation were selected and photographed using the charged couple device camera, (Evolution LC camera) in the microscope
- The cells were projected onto the monitor via the camera at ×40 magnification and images were captured onto a compact disc
- The images of individual cells were subjected to morphometric analysis using IMAGE PRO (version 5.5) image analysis system which is a semi-automatic system
- Using the measurement mode and the polygon tool, the region of interest (ROI) was selected
- The measurements of nuclear area (NA) and cytoplasmic areas (CA) were obtained by utilizing the "auto trace" function in the software. The number of pixels in the ROI was used to derive the "area" in square microns. The values (NA and CA) that were automatically displayed were recorded and the cytoplasmic:nuclear ratio (C:N) was calculated
- All the above parameters were calculated by the Image analysis software thereby considerably reducing the subjective error.

Results

The patients in the study ranged from 22 to 74 years of age. The mean age of the subjects in Group A, B, C was 40, 54.3, and 53.4 years, respectively, and showed no statistically significant difference among the different groups with $P > 0.13$ (one-way ANOVA was used to calculate the P value set at $P < 0.05$).

For every subject belonging to three groups, the HbA_{1c} was determined and the mean HbA_{1c} was tabulated along with the average cytomorphometric values of the selected parameters, which include average CA, average NA and C:N ratio as shown in Table 1.

Student's t-test was used to calculate the P value, where $P < 0.05$ was considered significant. On comparing the average NA among

the different study groups, it was found that the average area increased from the healthy individuals to controlled diabetics to uncontrolled diabetics (Figure 1). The results of our study showed that the mean NA was significantly higher in the diabetic study Groups B and C (NA = 351.97 μm² and 447.15 μm²), respectively, than in the control group (NA = 338.5 μm²). Statistically significant differences existed among the three groups. And the maximum significance was between Groups C and A with a $P = 0.00001$. The mean CA showed no statistical difference between the study and control groups (CA = 13682.01, 13503.17, 13686.61 for Group A, B, and C, respectively).

The mean C:N ratio between the groups showed a statistically significant reduction in the diabetic study groups compared to the control group. (C:N ratio = 41.38, 39.07, and 30.95 for Groups A, B, and C, respectively), with the maximum significant difference between Groups C and B (Table 2).

An attempt was made to assess the correlation between average HbA_{1c} value and the average of the three-cytomorphometric parameters (NA, CA, and C:N ratio) in each group (Table 3). Pearson's coefficient test was used to calculate the *R* value, where *R* value between ± 0.8 and 1.0 is the maximum correlation. It was found that a significant correlation existed between HbA_{1c} and C:N ratio and also between HbA_{1c} and average NA, the former being highly significant within all three groups. Whereas no significant correlation existed between HbA_{1c} values and average cytoplasmic area (CA).

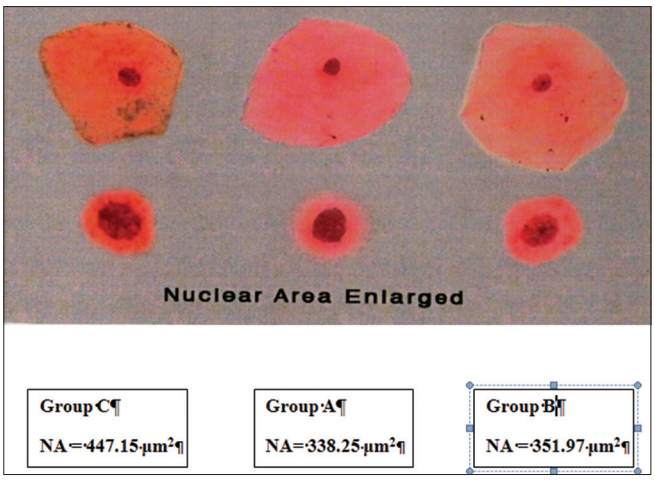

Figure 1: Differences in the nuclear area among the three groups. Papanicoloau stain (×40) shows nuclear area of 447.15 μm² for Group C, 338.25 μm² for Group A and 351.97 μm² for Group B.

Discussion

The exfoliative cytology demonstrates its importance in the field of diagnosis, on the principle that any change in the superficial

Table 2: Comparison of average NA, average CA, C: N ratio among the three different groups.

Variable	Groups	*N*	Mean	Standard deviation	*t*-value	*P* value*
Average N area (μm²)	A	20	338.25	13.32	17.14	0.00001* (significant)
	C	20	447.15	25.10		
	A	20	338.25	13.32	2.59	0.017* (significant)
	B	20	351.97	19.59		
	C	20	447.15	25.10	13.37	0.0001* (significant)
	B	20	351.97	19.59		
Average C area (μm²)	A	20	13682	512.14	0.02	0.99 (not significant)
	C	20	13687	964.44		
	A	20	13682	512.14	1.93	0.06 (not significant)
	B	20	13503	721.42		
	C	20	13687	964.44	1.44	0.16 (not significant)
	B	20	13503	721.42		
Average C:N ratio	A	20	41.38	1.67	19.23	0.00002* (significant)
	C	20	30.95	1.76		
	A	20	41.38	1.67	3.65	0.000023* (significant)
	B	20	39.07	2.29		
	C	20	30.95	1.76	12.57	0.00000* (significant)
	B	20	39.07	2.29		

*Student's *t*-test was used to calculate the *P* value ($P<0.05$ is significant). NA: Nuclear area, C:N: Cytoplasmic:nuclear ratio, CA: Cytoplasmic area

Table 3: Correlation of HbA1c values with the different cytomorphometric parameters (average NA, average CA, and average C: N ratio) among the different groups.

Groups	Variables	*N*	*R* value#	SE (*r*)	*t*-value	*P* value*
A (normal)	HbA_{1c} average C	20	−0.11	0.23	0.48	0.65
	HBA_{1c} average N	20	0.76	0.23	0.52	0.01*
	HbA_{1c} C:N ratio	20	−0.85#	0.23	0.78	0.007*
B (controlled)	HbA_{1c} average C	20	0.05	0.24	0.21	0.7
	HbA_{1c} average N	20	0.89#	0.22	1.64	0.005*
	HbA_{1c} C: N ratio	20	−0.95#	0.22	1.24	0.0009*
C (uncontrolled)	HbA_{1c} average C	20	−0.09	0.19	3.11	0.47
	HbA_{1c} average N	20	0.75	0.69	0.26	0.01*
	HbA_{1c} C:N ratio	20	−0.79	0.43	1.82	0.009*

*Student's *t*-test was used to calculate the *P* value ($P<0.05$ is significant), #Pearson's coefficient test was used to calculate the *R*-value; (*R*-value between ±0.8 and 1.0 is maximum correlation), NA: Nuclear area, C:N: Cytoplasmic:nuclear ratio, CA: Cytoplasmic area, HbA_{1c}: Glycosylated hemoglobin

Table 1: HbA_{1c} and average CA, NA, and C:N ratio values in Group A, B, C.

Groups	Mean age (in years)	HbA_{1c} (average) (%)	Average CA (μm²)	Standard deviation	Average N A (μm²)	Standard deviation	Average C:N ratio
A	40	5.9	13682.01	512.14	338.25	13.32	41.38
B	54.3	6.7	13503.13	721.42	351.97	19.59	39.07
C	53.4	9.9	13686.61	964.44	447.15	25.10	30.95

HbA_{1c}: Glycosylated hemoglobin, NA: Nuclear area, C:N: Cytoplasmic:nuclear ratio, CA: Cytoplasmic area

cells can be the reflection of the change in the immediate underlying tissue. Oral exfoliative cytology interpretation usually suffers from the subjective nature of observation and hence results in ambiguous interpretation. These limitations are overcome by the introduction of quantitative methods such as image analysis systems especially in the assessment of cytomorphometric cellular alterations.[6] Cytomorphometric evaluation of buccal epithelial cells was done in this study to determine the cellular alterations in diabetes mellitus.

Alberti *et al*. studied the oral smears of buccal mucosa, floor of the mouth, and dorsum of the tongue in Type 2 diabetic patients and reported that the mean NA was increased in diabetics than in healthy controls and no significant difference was found in the mean CA. As a consequence, the C:N ratio was considerably reduced in the diabetic individuals. They also attributed qualitative or morphologic changes to inflammation of the oral mucosa of diabetic patients, possibly due to decreased salivary flow.[4]

Similar results were observed in the present study in all the buccal epithelial cells belonging to different stages of differentiation. It was found there is a significant increase in mean NA in diabetic study Groups B and C (NA =351.97 μm^2 and 447.15 μm^2), respectively, than in the control group (NA = 338.5 μm^2). Interestingly, the mean CA showed no statistical difference between the study and control groups. Consequently, significant reduction in C:N ratio was observed among the diabetic study groups when compared to control group (C:N ratio = 41.38, 39.07, and 30.95 for Groups A, B, and C). It was also demonstrated that in uncontrolled diabetics, the NA was significantly higher and C:N ratio was significantly lower when compared with controlled diabetics.

Previous age-related surveys showed statistically insignificant quantitative alterations with respect to CA in the oral epithelial cells of normal healthy subjects. Gender-related studies found that gender factor has no influence on either CA or NA of oral epithelial cells.[7,8]

Quantitative cellular alterations in oral smears of subjects with tobacco smoking and betel chewing with tobacco habits revealed cytomorphometric alterations similar to the present diabetic study. An increase in NA in smokers was observed when compared with controls and it was suggested that the cellular modifications found in the oral smears in these subjects could have resulted from chronic irritation of the oral mucosa caused by the tobacco habits.[9] It is also possible that smoking habits cause delay in epithelial differentiation leading to a predominance of PAP stained blue cells belonging to basal cell layer.[10] On the other hand in the present study, cytological alterations were observed in the cells of varying differentiation stages.

Other variables such as nutritional deficiencies like megaloblastic anemia (deficiency of vitamin B12 and folic acid) and in iron deficiency, NA changes similar to those found in oral smears of diabetic patients are observed. In these patients, there was an increase in NA and CA. This is possibly due to a disturbance in DNA synthesis with a consequent increase in both CA and NA.[11]

Strict exclusion criteria were followed in including individuals in all three groups to avoid all the other possible causes that give rise to increase in NA. The possible hypothesis for explaining the increase in the mean NA is as follows: One factor that causes an increase in NA in diabetic patients is the increased susceptibility to trauma of the oral mucosa in diabetic patients, possibly aggravated by xerostomia as well as increase in the background inflammatory component (proneness to oral infections) in the mucosa. The activity of basal cells is enhanced to replenish the loss of cells (atrophy) which results in increased proportion of actively dividing cells characterized by prominent nuclei (yet to be confirmed by proliferative indices based studies).

With an increase in the NA, there should be a concomitant increase in the CA in actively proliferating cells. Interestingly, this does not happen as shown in the current study and possibly is due to the relative insufficiency of insulin that prevents glucose uptake by epithelial cells, required for cell growth. Hence, the amount of cytoplasm the cell makes decreases relative to the amount of nucleoplasm. Furthermore, inflammation is one of the factors that can increase NA and lead to a poorly preserved cytoplasm.

It appears that cytomorphometric changes in buccal cells of Type 2 diabetic patients may be independent of factors such as age, sex, smoking, and nutritional deficiencies and can be attributed to the diabetic manifestations.

Conclusion

Early diagnosis of diabetes coupled with efforts to create awareness in the public about the implications and complications is the need of the hour. It is important to know the current prevalence and future trends of diabetes as this information is of help in planning healthcare and public health interventions. Reliable diagnostic criteria and simple diagnostic procedures will be highly valuable in screening the target group of individuals (risk prone category) and in evaluating the glycemic control in previously diagnosed diabetic patients.

Oral exfoliative cytology can be used as a simple chair side investigation that is quick, simple, less technically demanding, painless, non-invasive procedure for microscopic investigation of the mucosa.

In evaluating the glycemic control in diabetic patients, HbA_{1c} has been established as a prognostic indicator. In the present study, we found that statistically significant correlation of C:N ratio to HbA_{1c} values as shown in Table 3. Based on the results

obtained, we are encouraged to venture with a suggestion that estimation of C:N ratio by image analysis can be used as an effective adjunct to HbA_{1c} estimation in the control of diabetes.

Further prospective studies have to be conducted with a larger sample size using fully automatic image analyzer. Including such criteria in the samples will be very helpful in assessing the usefulness and reliability of C:N ratio in glycemic control of diabetes mellitus.

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References

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J 2014;7(1):45-8.
2. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 2007;125(3):217-30.
3. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92(3):281-91.
4. Alberti S, Spadella CT, Francischone TR, Assis GF, Cestari TM, Taveira LA. Exfoliative cytology of the oral mucosa in type II diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 2003;32(9):538-43.
5. Papanicolaou GN. A new procedure for staining vaginal smears. Science 1942;95:438-9.
6. Koss LG. The future of cytology. The Wachtel lecture for 1988. Acta Cytol 1990;34(1):1-9.
7. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: An age, site and sex-related survey. J R Soc Med 1985;78(12):995-1004.
8. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma. J Oral Pathol Med 1998;27(2):83-6.
9. 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;28(9):385-8.
10. Zimmermann ER, Zimmermann AL. Effects of race, age, smoking habits, oral and systemic disease on oral exfoliative cytology. J Dent Res 1965;44:627-31.
11. Koss LG. The oral cavity, larynx, trachea, nasopharynx and paranasal sinuses. In: Koss LG, (Editor). Daignostic Cytology, 4th ed. Philadelphia: J.B.Lippincott; 1992. p. 865-89.