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Cytological Features of Oral Cytobrush Smears in Type II Diabetes Mellitus Patients

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Key words
type II diabetes mellitus, cytology, gingiva.

Abstract
Oral cytology is a renewed field that aids in diagnosis and observation of possible epithelial changes associated with oral mucosal diseases. Aim; to study the main cytomorphological alteration in gingival and buccal smears from type II diabetics in relation to their hyperglycemic status.
The study includes 40 type II diabetic patients **(**20 new-diagnosed and 20 treated diabetics patients) and 20 healthy persons of both sex. Papanicolaou stained smear were prepared from their cheek and gingiva. The morphological features of 100 unfolded epithelial cells were evaluated under light microscope.
Results of this study show that diabetics' oral mucosa cells characterized by large nuclei with frequent evidence of binucleation, granular chromatin, prominent nucleoli. However, there was frequent small blue cytoplasm and buccal smears showed altered keratinization.
As conclusion oral cytology from type II diabetics is associated with detectable cytomorphological changes that is site specific and indicate epithelial cell regeneration and degeneration with altered keratinization especially in buccal mucosa.

Introduction

Oral cytology is a relatively inexpensive, simple, noninvasive, and risk-free technique that is well accepted by the patient with no contraindications [(1,2)]. With the application of advance technology and immuno-or genetic- cytochemistry, there is much improve in the potential accuracy of oral cytology [(3-6)].Cytomorphological features include nuclear (nuclear size, shape, chromatin pattern, nuclear membrane and nucleoli), cytoplasmic qualities (degree of differentiation) and the slide background. Some characteristic are unique to certain disease processes, aiding greatly in diagnosis [(7)]. Accordingly, the cytology of the oral cavity in the absence of disease is simple. Basal cells appear as small, round to oval in shape with low cell area to nuclear area ratio (CA/NA ratio). While prickle cells appear round, their cytoplasm is thick the nucleus is centrally located with higher CA/NA ratio when compared to basal cells. On the other hand, mature granular cells have thin transparent cytoplasm, polygonal in shape and central round nuclei with granular chromatin. Finally, keratinized cells are similar to those of the granular cell layer but with absence of nucleus [(7)].However several oral mucosal changes are reported in certain disease conditions. Oral cytology has been used for early detection, monitor and follow up of premalignant and malignant oral lesions [(8)], in microbial diseases [(9,10)], in vesiculo–bullous lesions and dermatological lesions [(11,12)], in heavy

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metal precipitation in the oral cavity [13], in assessment of nutritional status-Fe deficiency [14] and in forensic dentistry [15]. It has been also used to study cell proliferation in the smoker's oral mucosal cells [16] and evaluating genetic changes in patients with oral leukoplakia [17]. Concerning cytomorpho--logy of oral mucosa in type II diabetics, recently few published literatures were available [18-20] that give an idea about buccal mucosal changes by using different methods of smear collection and measurements without specification of patient's hyperglycemic status. Until 2010, Prasad etal [21] partially declared the last point; however they neglected treatment and site variation. later on in 2011, Hallikerimath etal studied cytomorphological changes and glycogen content in exfoliated cells buccal mucosa[22].Therefore, the goal of our study was to identify the morphological changes of oral epithelial cells using the most reliable tool in oral cytology (cytobrush) from different sites (buccal and attached-gingiva) in different type II diabetic patients (newly-diagnosed and treated cases: well-/ poorly-controlled) to evaluate its significance since dentist has a major role in 1) identifying undiagnosed patients, 2) proper dental management for their oral manifestations and 3) prevention of systemic and local complications [22-25].

Materials and Methods

This study included 40 type II diabetic patients, 20 of them were newly-diagnosed cases (8 male, 12 female) and another 20 DM were treated with metformin, 500mg (tid) for not less than 1 year, 10 of them (7 male, 3 female) were well-controlled treated and 10 (3 male, 7 female) were poorly controlled, from Ali Naji Dispensary Clinic in Sulaimani city from Feb. to Aug. 2009, after estimation of their HbA1c and fasting serum glucose (FSG) level. The control group included 20 non-diabetic healthy volunteers (6 male,14 female) with no risk factor for diabetes and their fasting glucose level was <126 mg/dl (7.0 mmol/L) and/or their HbA1c< 6.5%. All participants ranged from 40-50 years of age and had clinically healthy oral mucosa. The exclusion criteria were: 1) smoker [26,27] or alcoholic [28] patients, 2) systemic diseases or other medications that affect the assay [14], and 3) ladies who were pregnant or during menstrual period or taking contraceptives[29].The study was approved by the local ethical committee and all patients signed a written consent form. Patient's name, age, sex, medical history were recorded.The participants were asked to gargle with tap water. The oral mucosa was dried with gauze to remove surface debris and excess saliva. Two smears were collected, one from the buccal mucosa and the other from upper anterior attached gingiva of each individual using oral cytobrush (Rover Orcellex/ Netherlands) and transferred to labeled, clean, dry glass slides. They were then fixed at once by soaking in 95% ethanol and stained using the Papanicolaou technique.From each individual, 100 unfolded, clearly outlined, separated cells (50 from buccal and 50 from attach gingiva) were selected manually by moving the slide in a stepwise manner (from upper left corner to the right and then downwards and going back in reverse direction in order to avoid measuring the same cells again). Subjective morphological features for both nucleus and cytoplasm of epithelial cells, regarding color and texture were recorded beside slide background findings. Data were analyzed by chi-square test using SPSS software. The level of significance was set at $P \leq 0.05$.

Results

Patients in each studied group were distributed according to sex and glycemic status (Table-1). The distribution of cytomorphological features were shown in table-2 and figure-1. Cells from newly-diagnosed diabetics showed irregular shaped nuclei especially in gingiva (Figure 1 a-d). All DM groups had more significant evidence of bi- or multi nucleation especially in newly-diagnosed cases [Figure 1e-g]. The chromatin was fine/coarse granular randomly distributed (Figure 1e,f); however, it seems to be

evenly distributed in gingival smear (Figure -1 a). There were significant prominent nucleoli [Figure-1g], few karyorrhexis (Figure-1h,i) and evidence of nuclear vaculation (Figure 1j,k). Although the color changes in Papanicolous stained smear of DM patients indicated different oral mucosa keratinization stages still the cytoplasm of gingival mucosa was predominately ($p<0.05$) blue stained and unexpectedly the buccal smear showed keratinization (Figure 1 g) ($p<0.05$) (table-3) with intra-cytoplasmic eosinophilic granular inclusions of different size (Figure 2L-n). The small yellow cells that lack nucleus were observed in gingiva. Leukocytes and bacteria were seen especially in gingival smear ($p<0.05$) (Figure-2o). Peri-nulclear hallo were also evident (Figure-2o,p). On the other hand, cells from healthy control subjects showed small and compact nuclei with even distributed chromatin. There was no keratinization in buccal smear. These morphological changes not corrected after therapy except for the reduction in inflammatory cells and bacteria (Table-3).

Discussion

The subjective morphological changes that observed in this study are in line with what had been published [18,22]. However there is not known explanation for the significance of observing multinucleation, nuclear creases or grooves in epithelial cells [30]. Nevertheless, a bilobed nucleus was suggested of ageing cells [31]. On the other hand, minor nuclear abnormalities such as slight-to-moderate nuclear enlargement, slight irregularities of the nuclear contour, and increase in granularity of the chromatin indicate reactive or regenerative state [30] in the presence of inflammatory process which was more evident in gingiva especially of untreated cases. Other features that referred to cell degeneration including karyorrhexis, chromatin clumping and margination, nuclear vaculation and evidence of perinuclear haloes, were also part of our finding. We cannot identify cytoplasmic vacuolization. Cells containing round cytoplasmic eosinophilic inclusions are probably corresponding to keratinization. Full keratinization of gingival smear occurred primarily in the absence of inflammation and became infrequent when inflammation was present, as inflammation leads to decrease in the amount and degree of keratinization [32]. However, the evidence of keratinization in buccal mucosa of DM needs explanation. The hypoglycemic status of our patient did not alter the subjective morphological finding except for the reduction in inflammatory cells and of bacterial in the back ground of the slide in well-controlled group.Previous studies that concern with cytology of oral smears in DM patients remark to morphometric alterations, with intraoral site variation unrelated to sex variation (18,21, mouhammed). The possible explanations for these changes were related to the reduction in epithelial proliferation and turnover secondarily to metabolic disorders [33], reduction in the stimulatory effect of insulin and IGF-I [34] and reduction in cellular nourishment associated with microvascular disorders related to DM [4]. Furthermore diabetics are suffering from xerostomia and atrophic oral mucosa with possible increase in the frequency of intraoral minor trauma due to sensory defects [35].The sub classification of diabetic patients according to their glycemic state and treatment revealed different morphometric results (Mouhmeed) since metformin had side effect that produce lacto acidosis [36], cellular swollen and coarsen of the nuclear chromatin [37] and alter nuclear size [mouhammed].

Conclusion

Oral cytomorphologic changes that observed in type II DM patients are site specific and indicate epithelial cell regeneration and degeneration with altered keratinization especially in buccal mucosa.

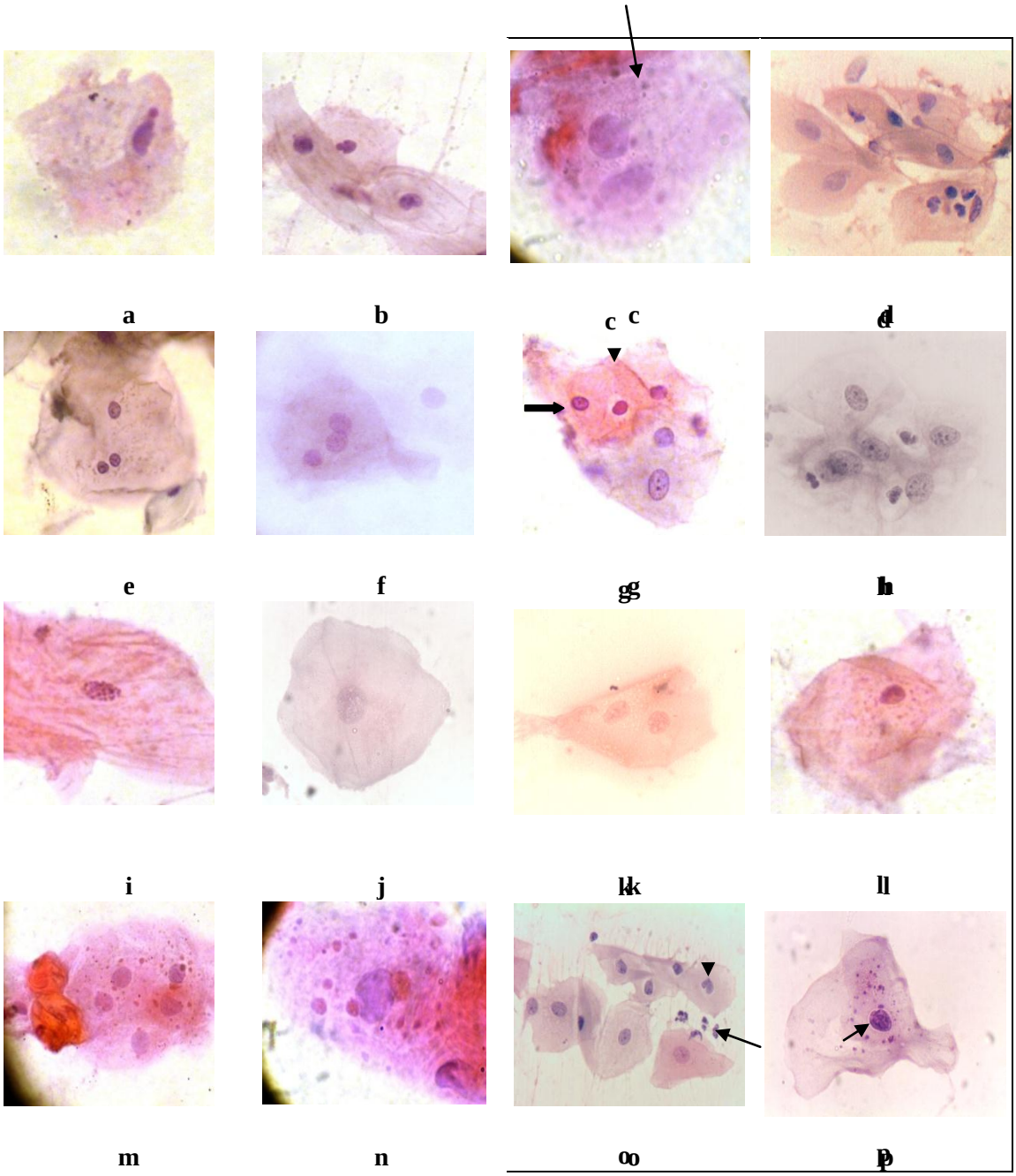

Fig.(1):-Different cytomorphological features from the buccal and gingival smears of diabetic patients. Abnormal nuclear shape in gingiva and buccal mucosal with hemogenous chromatin (a- c). Nuclear fragmentation and apoptotic bodies (d). Bi- and tri- nucleation in the gingiva (e ,f), with evidence of keratinization (g-arrow head) and prominent bi-nucleoil (g-bold arrow) in buccal mucosa. Karyorrhexis in buccal (h) and gingival smears (i). Nuclear vacuoles (j,k). Cytoplasmic eosinophillic granules in buccal mucosa (l). Keratinization and cytoplasmic eosinophillic granules of variable size with bi-nuleation (m) / nuclear groove (n) in gingival epithelial cell. Perinuclear halos with abnormal nuclear shape (o-arrow head). Bacteria are seen in the background (o-arrow). Perinuclear halos with cytoplasmic granules (p-arrow). (Pap, ×40)

Table (1):- Distribution of studied sample according to sex and glycemic status.

Group	Total	Male	Female
Healthy subjects	20	6	14
Newly-diagnosed	20	8	12
Well-controlled	10	7	3
Poorly-controlled	10	3	7

Table (2):- The percentage distribution of the main subjective morphological alteration of gingival and buccal smears of healthy subjects and all studied diabetic groups.

Site	Group	Bi or multi-nucleated	Karyorrhexis	Prominent nucleoli	Granular cytoplasm	inflammation	bacteria
Buccal	Healthy subjects	1.6	2.5	1.8	9.4	1.4	1.6
	Newly-diagnosed	5.9*	7.3*	5.5	15.3	3.8*	2.8
	Well-controlled	5.2*	6.6*	3.8	10.4	1.2*	1
	Poorly-controlled	5.2*	4.6*	6.2	10.6	4*	3.6
Gingiva	Healthy subjects	2.8	1.9	3.2	10	0.5	1
	Newly-diagnosed	5.2	8.8*	9.4	9.9	4.8*	3.9*
	Well-controlled	5.8	4.8*	5	8.6	1.8*	2.4*
	Poorly-controlled	6.2	6*	8.4	9.6	3.6*	4.8*

* P<0.05

Table (3):- The percentage distribution for the features of Papanicolaou stained smears from gingival and buccal mucosa of healthy subjects and all studied diabetic groups.

Group	Gingiva				Buccal			
	Blue	mix	orange	keratin	Blue	mix	orange	keratin
Healthy subjects	37.4	11.5	37.1	14	55.7	12	32.3	0
Newly-diagnosed	48.9*	9	29.4	12.7	64.8	10.7	22.6	1.9*
Well-controlled	36.6*	8.6	41.8	13	58.6	9.4	30.2	1.8*
Poorly-controlled	44.4*	9.4	33	13.2	63.4	9.8	24.6	2.2*

* P<0.05

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