Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients

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Abstract

Objective: The main purpose of this study is to emphasize the relevance of exfoliative cytology as an additional tool to aid in the diagnosis of diabetes mellitus. Materials & Methods: This is a comparative cross-sectional study. Oral smears were obtained from 10 diabetic patients and 10 healthy individuals. These smears were stained with Papanicolaou stain. The nuclear (NA) and cytoplasmic (CA) areas of 20 integral cells predominant in the buccal mucosa were measured using the Leica Qwin Version 2.1 image analysis system (LEICA GMBH GERMANY).The cytoplasmic/nuclear ratio (C/N) was then calculated. For comparing cytomorphometric parameters (NA, CA & C/N ratio) the Mann-Whitney test was used. Significance was set at $P \leq 0.05$.   Results: The morphologic alterations seen in buccal mucosal epithelial cells of the diabetic group were nuclear enlargement, karyorrhexis, binucleation and infiltration of polymorphonuclear leukocytes. The NA was significantly higher ($P < 0.05$) in the diabetic group. The CA between these two groups were not significantly different ($P > 0.05$). The C/N mean was significantly lower ($P < 0.05$) in the diabetic group. Conclusion: Exfoliative cytology is useful as an additional tool to aid in the diagnosis of diabetes mellitus.

Key words: Diabetes mellitus, exfoliative cytology, morphology, cytomorphometry.

Introduction

Diabetes mellitus affects many populations. An estimated 100 million people are affected by diabetes mellitus worldwide, of whom 7 million are in the ASEAN region. A significant observation is that the incidence of this chronic disease is on the increase. In Malaysia, the prevalence in 1960 was 0.65% and had increased to 2.1% in 1982. A limited population-based study in 1984 showed a prevalence of nearly 4 % (1).
Diabetes mellitus is associated with increased mortality and morbidity. Death is usually related to cerebrovascular diseases or chronic renal failure (1, 2). In addition, diabetics are more prone to stroke (1, 3) and chronic infections such as tuberculosis (1). Diabetics are at higher risk of hypertension and hyperlipidaemia (1, 3). Other complications associated with diabetes mellitus are diabetic retinopathy, diabetic nephropathy and diabetic neuropathy (2, 3). In general, diabetics have shorter life span and a reduced quality of life compared to a healthy general population (1).
The majority of diabetics are of type 2 or non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes occurs usually in patients over 40 years and is strongly fa-

milial, though the genetic markers are yet to be identified. It is usually associated with obesity (1, 2).

Alberti et al. have investigated the effect of type2 diabetic mellitus on oral epithelial cells; they found nuclear enlargement, karyorrhexis, binucleation and polymorphonuclear leukocyte infiltration with a significant increase in the nuclear area (NA). However, there was no significant difference in cytoplasmic area (CA) between type 2 diabetics and control group. There was also a decrease in the cytoplasmic/nuclear ratio in type 2 diabetics (4). The authors attributed the qualitative/morphologic changes to inflammation of the oral mucosa which was found in majority of the subjects in diabetic group.

Previous age-related surveys showed insignificant qualitative or quantitative alterations in the buccal mucosa of normal healthy subjects (5) particularly in the older age group 30-40 and above (6). Regarding gender, Cowpe et al. found that gender factor has no influence on either cytoplasmic area or nuclear area of oral epithelial cell (6).

Early diagnosis of the Diabetes mellitus is an important aspect of health care. In 1982, it was estimated that there were 310,800 diabetics in Malaysia. However, based on drug prescriptions, only 123,800 could be accounted for. More than 60% either refused to seek treatment or were otherwise not aware that they had diabetes. Most diabetics were diagnosed during the routine survey based on population studies (1). Exfoliative cytology is a relatively easy, simple and non-invasive clinical technique which has the potential to be developed as a routine investigation for screening of diabetes mellitus. It can be done chair-side during routine dental examination. The objectives of this study were to investigate alterations in the morphology and cytomorphometry of buccal mucosal cells of type 2 diabetics using exfoliative cytology technique. The parameters used to measure cytomorphometric changes were nuclear area (NA), cytoplasmic area (CA) and cytoplasmic/nuclear ratio (C/N). Comparisons were made between type 2 diabetic group and normal healthy subjects.

Materials and Methods
- Subjects
Subjects were randomly selected into two groups; type 2 diabetic group and healthy control group. Diabetic patients were selected from the Diabetic Outpatient Clinic of Hospital Universiti Sains Malaysia (HUSM). The control group was selected from normal healthy patients attending the Outpatient Dental Clinic of HUSM. Subjects of both groups should have clinically healthy oral mucosa (7). The age range of study subjects were 30-60 years. Smokers or betel nut chewers were excluded. Diabetic subjects who have other systemic diseases or taking medications other than the diabetic medications were also excluded. Written informed consent was obtained and the pro forma inventory was completed detailing name, age, sex and relevant medical history.

Glycosylated hemoglobin (HbA1c) levels were reviewed from patients' medical records. The (HbA1c) was considered valid when the record was within the past 4 months. Random blood sugar was measured for each patient attending diabetic clinic.

- Specimen preparation
Subjects were instructed to gargle with normal saline. The oral mucosa was dried with gauze swab to remove surface debris and excess saliva. Smears were obtained using endocervical sampling brush and transferred onto clean dry glass slides. They were immediately fixed by soaking in a container containing 95% ethyl alcohol and stained using the Papanicolaou technique.

- Assessment
The stained smears were placed onto a motor-driven stage attached to a LEICA DMRE microscope (LEICA GMBH GERMANY, Germany) and the cells projected onto the monitor via 3-CCD color video camera KY-F55B (JVC, Japan) at 100X magnification.

The morphology of epithelial cells was observed. The cytomorphometric measurements were determined by the use of the Leica Qwin Version 2.1 image analysis system (LEICA GMBH GERMANY, Germany). Twenty clearly defined cells with good staining were selected by systematic sampling. The stage was moved in a stepwise manner, from left to right and then down and across in order to avoid measuring the same cells again. The nuclear (NA) and cytoplasmic (CA) areas were obtained by drawing around the nuclear and cell boundaries using the digitizer cursor. Subsequently, the cytoplasmic/nuclear (C/N) ratio was calculated.

- Statistical analysis
Study variables include NA, CA and C/N ratio of both groups were analyzed by using SPSS version 12.0. As the sample size was < 30 in each group and the normality assumption and equal variance assumption were not met; a non-parametric test was used (Mann-Whitney). The level of significance was set at $P \leq 0.05$. Data were reported as median and inter-quartile range.

Results
The age of participants in both groups ranged from 31 to 58 years. The number of subjects according to study group and sex are shown in Table 1.

Table 1. Number of subjects according to study group and sex.

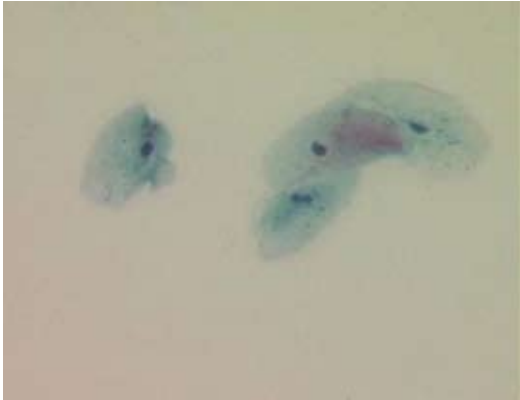| Study group	Male	Female	Total
Controls	4	6	10
Type 2 diabetics	1	9	10
Total	5	15	20

Fig. 1. Oral epithelial cells of the healthy individual showing normal size nuclei.
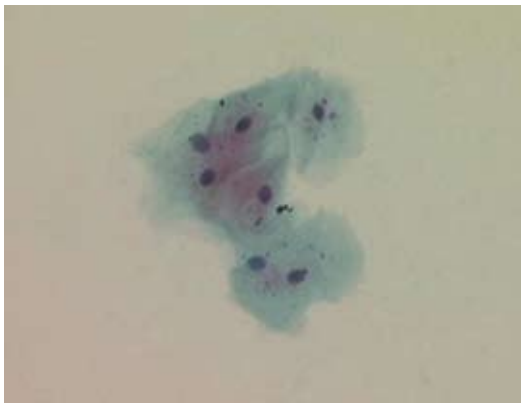

Fig. 2. Oral epithelial cells of diabetic patient showing enlarged nuclei.

Fig. 3. Binucleation in oral epithelial cells of diabetic patient.

The diabetic patients were either taking oral hypoglycemic agents only or combined with insulin. The duration of the disease ranged from 1 to 8 years; their mean level of random blood sugar (RBS) and glycosylated hemoglobin (HbA1c) were 11.03 ± 4.43 mg/dl and 9.69 ± 2.21 % respectively.

The stained oral epithelial cells were classified morphologically into three types based on the size and color of cell components. In fact, each of these cellular types represents cells from different layers of oral epithelium which has different stages of keratinization. The most keratinized pink cells were present in the superficial layer of the oral epithelium whereas the intermediate keratinized large green cells were present in the intermediate layer and non-keratinized small green cells were present in the basal layer of the epithelium.

The nuclei of epithelial cells of healthy subjects (Figure 1) were small and compact. Whereas the nuclei of oral epithelial cells of diabetic patients were found to be larger and more porous (Figure 2). The epithelial cells of the diabetic group showed binucleation (Figure 3) and karyorrhexis. In addition, polymorphonuclear leukocytic infiltration was noted. In contrast, no morphological changes were observed in the epithelial cells of control subjects.

This study revealed a significant increase in the NA ($P < 0.05$) of diabetic subjects. However, the CA showed no significant difference between the study groups ($P > 0.05$). There was a significant decrease in C/N ratio ($P < 0.05$) of diabetic subjects. Results of the cytomorphometric analysis are shown in Table 2.

Discussion

In this study, the oral mucosal cells of type 2 diabetics showed qualitative and quantitative changes compared to that of normal healthy individuals. These findings were similarly found by Alberti et al. (4). The morphologic changes in the oral epithelial cells of diabetic subjects in our study were nuclei enlargement, binucleation, karyorrhexis and polymorphonuclear leukocyte infiltration. In this study, cytomorphometric findings revealed an increase in NA of diabetic subjects. Meanwhile, there was no significant difference in CA between two groups. C/N ratio was found to be lower in diabetic group. These cellular changes

Table 2. Cytomorphometric analysis in type 2 diabetic and control groups.

Variable	Control group (n=10) Median(IQR)	Diabetic group (n=10) Median(IQR)	Z statistic	P value
Nuclear area (NA)	142.83(34.15)	170.18(60.15)	-2.46	0.014
Cytoplasmic area (CA)	5623.50(1224.98)	5386.33(3407.1)	-0.38	0.705
Cytoplasmic/nuclear ratio (C/N)	40.1(9.68)	29.38(8.88)	-3.1	0.002

Mann-Whitney test

involved all the layers of the oral squamous epithelium. Zimmermann and Zimmermann (5) demonstrated cellular changes in the buccal mucosa of patients with systemic diseases such as endocrine disorders and respiratory illnesses. They suggested that there is a difference in recovery rate of keratinizing cells of this area following systemic illnesses.

Ogden et al. revealed cytomorphometric changes in the buccal mucosal cells of cigarette smokers similar to those noted in diabetics (8). Their findings were supported by Ramaesh et al. (9). Quantitative changes found in the buccal mucosa of smokers (5) were attributed to the presence of larger numbers of non-keratinized cells of parabasal layer which are relatively smaller in cell size but have a larger nuclei; this will give an impression of nuclei enlargement and decreased C/N ratio similar to the changes seen in type 2 diabetic patients.

Nutritional deficiencies have been associated with changes in oral mucosa, similar to those noted in type 2 diabetic patients. Deficiencies of vitamin B12 and folic acid retard the synthesis of DNA, which is the core substance of cell nuclei, and hence may alter the size of nucleus and cytoplasm (10, 11).

Although the qualitative and 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 (12).

A conclusion that the morphology and cytomorphometric changes in buccal mucosa of type 2 diabetic group can not be attributed to factors such as age, sex, smoking habit and systemic diseases but are due to type 2 diabetes mellitus itself can be drawn from this study. Therefore, detection of these qualitative and quantitative cellular changes by exfoliative cytology can help in the diagnosis of diabetes mellitus.

Exfoliative cytology is a simple, non-invasive clinical technique which causes only mild discomfort as reported by a small number of patients.

We experienced some difficulties in using a cervical sampling brush to obtain the buccal smear due to its size and design. A special designed brush for oral mucosa (Rovers® Orcellex® Brush) is recommended; It was proven to be well accepted by the volunteers and was easy to use (13). In addition the technique of slide preparation can be enhanced by the use of liquid-based technology. Liquid-based cytology demonstrated a statistically significant overall improvement in smear thickness and in cell distribution and a reduction in cell overlapping and presence of blood (14).

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