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ORIGINAL ARTICLE

A COMPARATIVE STUDY ON CYTOMORPHOLOGICAL CHANGES IN BUCCALMUCOSAL CELLS OF TYPE 2 DIABETICS AND NON-DIABETIC SUBJECTS

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ABSTRACT

AIM: To evaluate cytomorphological changes in exfoliated buccal mucosal cells of type 2 diabetic patients in comparison with healthy non-diabetic individuals.

MATERIALS AND METHODS: This comparative study included 20 outpatients from Priyadarshini Dental College and Hospitals, Tiruvallur district. Of these, 10 participants were diagnosed with type 2 diabetes mellitus, while 10 were healthy non-diabetic controls. Smears were collected by scraping the buccal mucosa and vestibule of each participant. The specimens were fixed in ethyl alcohol and stained with Papanicolaou stain. Quantitative parameters such as nuclear diameter, cytoplasmic diameter, and nuclear–cytoplasmic (N/C) ratio were measured and analysed.

RESULTS: The cytological evaluation demonstrated significant differences between diabetic and non- diabetic individuals. Diabetic patients showed an increase in nuclear diameter, a decrease in cytoplasmic diameter, and a higher nuclear–cytoplasmic ratio compared to healthy controls.

CONCLUSION: The study highlights distinct cytomorphological alterations in buccal mucosal cells of type 2 diabetic patients, suggesting that exfoliative cytology can serve as a simple, non-invasive, and valuable tool in assessing cellular changes associated with diabetes.

KEYWORDS: Type 2 diabetes, Exfoliative cytology, Papanicolaou stain, Cytomorphological changes

INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disease in which blood glucose levels are not adequately controlled. The primary kinds of diabetes mellitus are type 1 and type 2; each has a unique pathogenesis, presentation, and treatment approach, but all can result in hyperglycemia. Type 2DM has a more insidious beginning, with a functional deficit of insulin resulting from an imbalance between insulin levels and insulin sensitivity. Although there are several contributing factors, obesity and ageing are the main causes of insulin resistance [1]. An estimated 77 million adults over the age of 18 in India have type 2 diabetes, and almost 25 million are prediabetics, meaning they have a higher chance of getting the disease in the near future [2].

Diabetes can cause the nucleus to enlarge and the nuclear cytoplasmic ratio to decrease, which can make cells more vulnerable to cancerous alterations [3]. In the normal physiological process, the epithelial cells move from basal layer to the surface and ultimately shed or exfoliated. The exfoliative oral cytology can be defined as the obtention and characterization of cells from the surface of the oral mucosa [4]. Cytomorphological features includes changes in both nucleus and cytoplasm like nuclear shape, size, chromatin pattern, nucleoli and nuclear membrane, cytoplasmic qualities and overall shape of the cell [5]. The Papanicolaou (PAP) staining method used to study the nuclear features such as chromatin arrangement, intranuclear invaginations, and membrane abnormalities are revealed by this stain and are crucial for making a cancer diagnosis. It contains three cytoplasmic dyes: light blue, eosin Y, and orange G. It can stain cells in ambiguous (gray-blue), pink (eosinophilia), orange (orangeophilia), or blue (basophilia). The cytoplasm of squamous cells changes from blue- green to pale orange to vividly orange as they keratinise. Compared to a basic H&E stain, this stain is significantly more sensitive and more selective for keratin staining in cytology [6]. A couple of investigations have looked at alterations in diabetic patients' oral mucosa and documented the replacement shift in epithelium cytology method. Thus, using exfoliative cytology, the current study aims to investigate the quantitative and qualitative features of the buccal mucosa epithelium in patients with type I diabetes and compare them to those in healthy individuals.

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MATERIALS AND METHODS:

The study was commenced after obtaining clearance from the Institutional Ethical Committee. Twenty outpatients from the department of Oral Medicine in Thiruvallur participated in a comparative study. The inclusion criteria include Ten of these individuals had type 2 diabetes, while the remaining ten were in the control group. Type 1 diabetes group was named as group 1 and healthy group was named as group 2. Patients who were older than eighteen were included in the research. Participants who were younger than eighteen were not allowed to participate. Furthermore, to guarantee the validity and correctness of the study results, patients with weakened immune systems were not included, as they may have an impact on the course of the disease or undermine the quality of cytomorphological evaluation. Demographic details like name, age, sex and relevant medical history were collected. Written informed consent was obtained from all the participants. The participants were instructed to rinse their oral cavity with water to remove any debris, after which smears were obtained from the buccal mucosa using a wooden spatula. The collected material was transferred onto clean, dry, and sterile glass slides that had been pre-labelled with the patient's reference number. Each smear was evenly spread across the slide in a single, one- directional motion from one end to the other. The prepared smears were immediately fixed in 95% ethanol and subsequently stained using the Papanicolaou (PAP). The slides were mounted in the DPX (Di-N-butyl phthalate in xylene).

PAP-stained smears were examined systematically, moving vertically downwards from left to right to prevent repetition in cell counting. An average of 20 well-defined epithelial cells from each sample were selected and projected onto a monitor via a camera at 40× magnification, where images were captured. Morphometric analysis of individual cell images was performed using Image Analysis Software (AP view software A delta Optec.India) under a Binocular Research Microscope (DELTAPLAN) at 40× magnification. The unit of measurement for the cell area was square micrometres (µm². The cytoplasmic-to-nuclear ratio (CNR) was computed after the nuclear area (NA) and cytoplasmic area (CA) were evaluated. The statistical software program SPSS [IBM Corp. IBM SPSS Statistics for Windows, version 22.0, IBM Corp. USA] was used. Student's t-test and one- way ANOVA were used to compare the mean values between the type 2 diabetes (group 1) and healthy subjects (group 2). P values less than 0.05 were considered significant.

RESULTS

This study involved 20 participants in total, including 12 male and 8 female participants. The mean values of the cytoplasmic area (CA), nuclear area (NA), and cytoplasmic-to-nuclear ratio (CNR) for both the Type 2 diabetes and healthy control groups are presented in **Table 1**. The mean NA values was found to be higher in the type 2 diabetes group compared with the healthy controls, whereas the mean CNR was greater in the healthy group. The mean CA values were same in both healthy and type
2 diabetes group. The mean nuclear area was significantly higher in Type 2 diabetic subjects (75.30 ± 3.96 µm²) compared with healthy individuals (60.47 ± 3.60 µm²; $p < 0.001$). Conversely, the cytoplasmic area showed no statistically significant difference between the two groups ($p = 0.896$).

Table 1: Comparison of cytomorphometric parameters between Type 2 diabetes and healthy groups

Parameter	Group 1 (Mean±SD)	Group 2 (Mean±SD)	t-value	p-value
Nuclear area (NA) µm²)	75.30 ± 3.96	60.47 ± 3.60	8.759	**0.001***
Cytoplasmic Area (µm²)	355.33 ± 28.70	353.89 ± 18.89	0.132	0.896
Cytoplasmic-to-Nuclear Ratio (CNR)	4.64 ± 0.56	7.02 ± 0.42	−10.628	**0.000***

The P value ≤ 0.005* is statistically significant, Group 1- Type 2 diabetes, Group 2- Healthy subjects cytoplasmic-to-nuclear ratio (CNR) was markedly reduced in diabetic samples (4.64 ± 0.56) compared to controls (7.02 ± 0.42; $p < 0.001$). These findings indicate prominent nuclear enlargement and altered similar to the studies done by Sravani et al [8], Joy s et al [9] and Rivera et al [10]. The increase in nuclear area (NA) observed in diabetic patients may be attributed to prolonged hyperglycemia, which promotes the formation of advanced glycation end products involving proteins, lipids, and nucleic acids. These products accumulate in the walls of large and small blood vessels, leading to progressive narrowing, reduced tissue perfusion, and impaired cellular metabolism. As a result, epithelial cell turnover slows down, delaying keratinization and normal differentiation processes. This disruption in cellular maturation contributes to the presence of cells with enlarged nuclei and altered nuclear morphology [9-11]. In contrast, the cytoplasmic area

showed no statistically significant difference between the two groups, suggesting that the cytoplasmic volume remains relatively unaffected in the early or moderate stages of Type 2 diabetes. The stability of cytoplasmic dimensions, despite nuclear enlargement, points to the possibility of cellular adaptation rather than overt cytoplasmic degeneration [8]. The results were contradictory, where few studies showed increased Cytoplasmic area [12, 13] and few studies showed decreased cytoplasmic area. This can be the result of dehydration-induced cell shrinking [14] (Figure 1). Numerous factors contribute to the reduction in cytoplasmic size in diabetes. The primary cause of the reduction in cytoplasmic size in diabetes patients is the relative insulin deficit, which impedes the uptake of glucose by growth-promoting epithelial cells. In addition to this in diabetic individuals, vascular lesions cause oral epithelial cells to age prematurely, which lowers the number of cellular organelles and reduces the production of proteins and nucleic acids. The size of the cytoplasm eventually decreases as a result of these modifications [15]. The cytoplasmic-to-nuclear ratio (CNR) was markedly lower in diabetic samples (4.64 ± 0.56) than in controls (7.02 ± 0.42; $p < 0.001$). A reduced CNR is indicative of an increase in nuclear size relative to the cytoplasmic area, which may represent early cytological manifestations of cellular stress, oxidative damage, or metabolic imbalance in diabetic individuals. Such morphometric alterations are consistent with previously reported findings that hyperglycemia and advanced glycation end-products can induce nuclear changes and impair cellular homeostasis in oral epithelial tissues [16]. These findings were similar to the studies by Shareef et al [17, 18]. It is thought that the inflammation causes the oral mucosa cells' nuclear size to increase and their cytoplasmic size to decrease. However, this only applies to the immature cells. Due to hormonal defects and/or abnormalities in their function, diabetic individuals' epithelial cells age more quickly [10]. Overall, the differences seen between various studies are likely due to many factors. These include how long the person has had diabetes, whether their diabetes is well controlled or not, their level of glucose control, their age, the number of people studied, and how long it has been since they were first diagnosed. Differences in the software used to measure the cells, the microscope magnification, and the lack of a standard method for examining the smears can also lead to different results. The limitations of the present study include a small sample size.

In addition, important factors such as blood glucose levels, obesity, and salivary flow rates were not assessed, which may have influenced the findings.

CONCLUSION

The present study demonstrates that people with Type 2 diabetes have notable cytomorphometric changes in their buccal epithelial cells. Diabetes causes both quantitative and qualitative alterations in oral mucosa cytomorphometry. Significant nuclear enlargement and disruption of normal cellular proportions in diabetic people are indicated by a notable increase in nuclear area and a significant decrease in the cytoplasmic-to-nuclear ratio. On the other hand, there was no discernible difference between diabetics and healthy people in the cytoplasmic area. These findings imply that cytomorphometric examination of exfoliated oral epithelial cells could be a helpful, non-invasive way to detect cellular alterations linked to Type 2 diabetes. To support these findings and determine their diagnostic utility, more research with bigger sample sizes and the addition of more clinical factors is advised.

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