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Cytomorphometric Alteration of Oral Mucosa in type 1 Diabetes Mellitus Children.

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KEYWORDS

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ABSTRACT:

Type 1 Diabetes Mellitus (DM) is a chronic disorder characterized by hyperglycemia due to deficiency of insulin secretion. Oral complications of diabetes is devastating. The oxidative stress caused by hyperglycemia alters cellular and molecular mechanisms which induce tissue injury. The mitochondria and nucleus are two major targets of oxidative stress due to hyperglycemia, which contain a variety of DNA repair enzymes to repair oxidant DNA modifications. The reduced salivary flow and atrophy of the oral mucosal cells could impact cytomorphometry of oral mucosal cells. The viability of invasive techniques for the evaluation of oral mucosal changes is diminished in diabetics. Alternatively oral exfoliative cytology combined with quantitative methods such as image analysis system has a potent diagnostic tool that allows a quick and fairly accurate assessment of cellular alteration by cytomorphometric analysis. The detection of these qualitative and quantitative cellular alterations by exfoliative cytology may aid in the diagnosis of diabetes Mellitus

1. INTRODUCTION

Type 1 diabetes (T1D) is a chronic disease characterized primarily by a deficiency of insulin secretion. It remains a widespread public health concern and mainly occurs in the first decades of life.[1] The incidence of type 1 diabetes mellitus(T1DM) has increased in children and teenagers during past 30 years.[2,3] . The chronic metabolic complications are generally more severe in the person with type 1 diabetes. The oral complications of uncontrolled diabetes mellitus are devastating, which may include, but are not necessarily limited to, gingivitis and periodontal disease; xerostomia and salivary gland dysfunction; increased susceptibility to bacterial, viral and fungal (oral candidiasis) infections; caries; periapical abscesses; loss of teeth; impaired ability to wear dental prostheses; taste impairment; lichen planus; and Burning mouth syndrome.[4] Providing an appropriate level of screening, diagnosis and effective care is critically important to reduce mortality and disability from the disease.[5]

Chronic hyperglycemia has been reported to be a hallmark in diabetic patients and can result in various complications. The mitochondria and nucleus are the two major targets of oxidative stress due to hyperglycemia, which contain a variety of DNA repair enzymes to repair oxidant-induced DNA modifications. Oxidative stress caused by hyperglycemia alters cellular and molecular mechanisms which induce tissue injury. This along with reduced salivary flow and atrophy of the oral mucosal cells could impact the cytomorphometry of oral mucosal cells .[6-8]

The most accepted clinical technique for the diagnosis of lesions in the oral mucosa is incisional or excisional biopsy. [5] However, in specific clinical conditions, such as diabetes mellitus, a great many invasive techniques like multiple daily insulin injections, frequent self-monitoring of blood glucose by multiple finger pricking and amount of insulin administered , lose viability as a result of variations in blood glucose, infection, poor healing and the disease itself in these cases exfoliative cytology may be more appropriate .[2]

Oral exfoliative cytology is a relatively simple and non invasive technique defined as the obtention and characterization of cells from the oral mucosa. Cytology is a method of description, measurement, and evaluation of cell characteristics, such as cell size, nucleus size, cytoplasmic-nuclear ratio, and aneuploid or diploid

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nuclei . [9] Hence the present study was conducted to analyse cytomorphometric alteration in T1DM children .

2. OBJECTIVES

The study's objective was to analyse qualitative quantitative alteration of buccal mucosal cells in type 1 DM children.

3. METHODS

The present study will be conducted on 40 children in the Age range between 4-16 years out of which 20 children diagnosed with T1DM atleast 6 months before the study and who were on regular follow up and undergoing treatment at RajaRajeswari Medical College Hospital, Bengaluru and 20 diabetes free children. An informed consent was obtained . Patients were asked to rinse their mouth to remove any debris. Following this, with a gentle scraping motion, cells were scraped from clinically normal appearing buccal mucosa. Scrapings were then evenly smeared onto the glass slide and immersed in 95% ethyl alcohol. Smears from each individual stained by papanicolaou method. Morphometric analyses were performed with Papanicolaou-stained smears. A microscope was connected to a video camera and computer. After transferring microscopic images to a computer, morphometric parameters were automatically measured by image analysis program.

In each slide, twenty clearly defined cells with predominant staining were selected manually in a random fashion from different fields, and in order to avoid measuring and counting the same cells again, we moved the microscope stage from left to right, and then down and across in a step-wise manner. A total of 1200 cells from type 1 diabetic patients and 1200 cells from control subjects were analyzed. The morphometric parameters studied were as follows: Nuclear area, nuclear perimeter, cellular area, cellular perimeter and cytoplasmic nuclear ratio. Area is the area enclosed inside the contour; the perimeter is the contour perimeter,. All measurements were made under 400X magnification and expressed in square microns.

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses .Descriptive analysis of all the explanatory and outcome parameters will be done using frequency and proportions for categorical variables, whereas in Mean & SD for continuous variables.

Independent Student t Test was used to compare the mean HbA1c levels between diabetic and non-diabetic groups.

Mann Whitney Test was used to compare the mean age and Cellular morphometric parameters between Diabetics and Non-diabetics groups.

Chi Square Test was used to compare the gender distribution between 2 groups.

The level of significance was set at $P<0.05$.

4. RESULTS

The comparison between diabetic and non-diabetic groups based on various parameters revealed several notable differences. While there were no significant differences in age and gender distribution, suggesting similar demographic profiles for both groups, the diabetic group exhibited significantly higher mean HbA1c levels, reflecting poorer blood sugar control. Additionally, diabetic children had significantly larger nuclear perimeter with a mean difference of 191.73, nuclear areas with a mean difference of 13501.77, cellular perimeters with a mean difference of 653.49, and cellular areas with a mean difference of 112500.56 compared to non-diabetic children. Furthermore, the Nuclear/Cytoplasmic (N/C) ratio was significantly lower in the diabetic group than non-diabetic group with a mean difference of 17.12. These findings highlight important distinctions in blood sugar control and cellular characteristics between diabetic and non-diabetic children, which may provide insights into the biological changes associated with diabetes.

5. DISCUSSION

DM is a syndrome characterised by abnormalities in metabolism of carbohydrate, lipids and protein which results either form a profound or an absolute deficiency of insulin .[4]

Our study concluded that children who are diagnosed with T1DM showed increase in nuclear perimeter, nuclear area, cellular area and cellular perimeter but decrease in cytoplasmic nuclear ratio. In the qualitative changes we found 240 micronuclei, 150 degenerated cells and 60 cells with microorganisms out of 1200 cells.

Alberti *et al*., Jajarm *et al*.,and Shareef *et al*,examined the morphological changes of the oral epithelial cells and reported a binucleation, karyorrhexis, cytoplasmic vacuolization, and nuclear enlargement in type 2 diabetic patients. In addition to these findings, Shareef also reported polymorphonuclear leukocytes infiltration in

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buccal cell. Also, Prasad *et al*. noticed a binucleation, intracytoplasmic inclusion, perinuclear halo, and keratinized squamous cells in smears from uncontrolled diabetics. Tozoglu and Bilge performed cytomorphometric analysis of the oral epithelium in type 1 diabetic patient, and they found that there is an increase in the nuclear and cytoplasmic volume. Our cytologic findings are in agreement with the study by Alberti *et al*., Jajarm *et al*., Shareef *et al*., and Prasad *et al*.[3,4]

In diabetics due to sustained hyperglycemia resulting in increased glycation end products of proteins, lipids and nucleic acids, which accumulate in the walls of large vessels causing progressive arrowing decreased tissue perfusion and decreased cell turn over this delays keratinization and the cell differentiation process leading to an increase in the number of cells with a large nucleus. [1.10]Other reasons could be xerostomia related to hyperglycemia, dehydration, medicaments and membranopathy of ducts which cause increased oral mucosal trauma leading to high cell loss,which further enhances basal cells to replenish the lost cells by increasing the proportion of actively dividing compartment with a prominent and large nucleus. including initiation of DNA synthesis in certain cell. These may account for the increase in NA seen in diabetic patients .[5,11] .

The finding that the CA did not show any significant change in diabetic individuals corroborates with other studies.[12,13] The gender factor was not assessed in this study as previous studies had reported no influence of gender on the CA and NA of oral epithelial cells.[14,15] The role of nutritional deficiencies in oral mucosal changes similar to those seen in diabetic patients has been reported. Alterations in the size of the nucleus and cytoplasm are caused by an impeded DNA synthesis due to a deficiency of folic acid and vitamin B_{12}.[16] .

In DM patients Glucose does not diffuse easily through the pores of the cell membrane causing an increase in pressure. This increase in osmotic pressure in ECF causes osmotic transfer of water out of the cells explaining the reduction in CA. [1,2]

When the cytomorphometric changes were correlated with HbA1c levels, a significant correlation existed only between CNR and HbA1c level among both the T1D and healthy control groups. A similar finding was reported in a study on type 2 diabetics by Karthik *et al*. (2015).[1] This suggests that the CNR by image analysis could be used as an effective adjunct to HbA1c level estimation in the control of diabetes.

6. CONCLUSION

From our study we conclude that diabetes produces significant alterations in the cytomorphometry and cytomorphology of buccal epithelial cells.

1. That there is a clear increase in nuclear area, nuclear perimeter, cellular area, cellular perimeter and decrease in cytoplasmic nuclear ratio. In addition to these morphometric changes morphological changes in the form of cell degeneration, micronuclei and cells with microorganisms is observed.
2. It showed that exfoliative cytology can be used to evaluate morphological parameters of type 1 diabetes.

7. CLINICAL SIGNIFICANCE

- Oral exfoliative cytology is helpful in diabetic patients who have aversion and fear to needle pricks as it is painless and can be carried out regularly.
- Though it may not be used as a diagnostic tool, they can aid in monitoring of DM throughout the lifetime of the patient. Thereby decreasing the morbidity and preventing long term complications.

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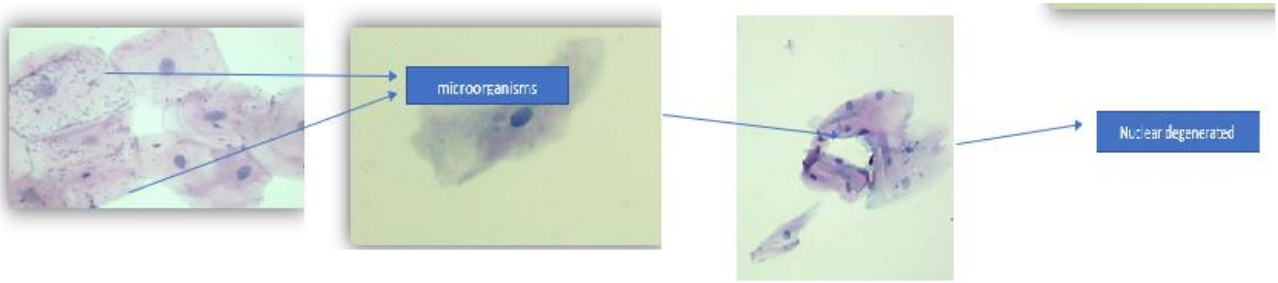

Comparison between 2 groups using Mann Whitney Test						
Parameter	Groups	N	Mean	SD	Mean Diff	p-values
Nuclear Perimeter	Diabetic	20	357.23	61.63	191.73	<0.001*
	Non-diabetic	20	165.5	25.51		
Nuclear Area	Diabetic	20	15619.03	20181.31	13501.77	<0.001*
	Non-diabetic	20	2117.26	1087.52		
Cellular perimeter	Diabetic	20	1944.35	305.06	653.49	<0.001*
	Non-diabetic	20	1290.86	177.58		
	Diabetic	20	209160.06	92398.45	112500.06	<0.001*

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Cellular Area	Non-diabetic	20	96660	40982.59		
Nuclear / Cytoplasmic Ratio	Diabetic	20	19.35	7.97	-17.12	<0.001*
	Non-diabetic	20	36.47	6.28		