Research Article

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Screening of Exfoliative Cytology in Patients with Diabetes Using Pas-D Stain

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ABSTRACT

Background: Diabetes mellitus is one of the major endocrinal disorder characterized by hyperglycemia. Purpose of this study was to analyze the glycogen accumulation in the exfoliated cells of oral mucosa as a diagnostic adjunct in diabetes mellitus.

Materials and Methods: Twenty diabetic patients (study group) and twenty control patients were selected. Three smears were prepared from each patient, stained with hematoxylin and eosin stain, periodic acid Schiff (PAS) stain and PAS-Diastase (PAS-D) stain in both groups. Because of digestion of glycogen by diastase enzyme, magenta colored PAS positive cells were stained lighter with PAS- D stain.

Result: Results were statistically analyzed and showed that mean and standard deviation values of RBS were found to be higher in study group than the control group and the data was statistically significant. Also the total number of PAS positive cells were more in study group. The study group showed nuclear changes such as enucleation and binucleation in H & E stained slides.

Conclusion: Microscopic examination shows presence of glycogen in buccal exfoliated cells in diabetic patients that can be used as diagnostic tool but need further extensive study.

Keywords: Diabetes mellitus, PAS- D stain, exfoliated cells.

INTRODUCTION

Diabetes mellitus is one of the fast growing major endocrine metabolic disorder with an estimate of 100 million people affected worldwide.[1] It is characterized by increase glucose caused by absolute or relative lack of insulin, resulting in reduced use of glucose by body cells.[2] This can result in tissue structure changes such as hyperplasia and increased glycogen content in the cells in diabetes patient. Periodic Acid Schiff (PAS) stain is routinely used in histochemistry and cytochemistry to check the glycogen content within the cells of the body. Analysis of glycogen content in exfoliated oral mucosal cell could be a non-invasive diagnostic or minimal invasive monitoring technique for diabetic patient.[3-4] The study is carried out with the aim of glycogen content demonstration as well as assessing the number of PAS positive glycogen containing cells in oral exfoliative cytology and correlate the staining quality with the random blood glucose level to exhibit exfoliative cytology as diagnostic method in type II diabetic patients using Hematoxylin and Eosin (H & E) stain, Periodic Acid Schiff (PAS) stain and Periodic Acid Schiff- Diastase (PAS – D) stain.

MATERIAL AND METHODS

Study population

The present study consisted of total 40 subjects of which study group consisted of 20 randomly

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selected cases of known type II diabetes mellitus patients with history of diabetes mellitus for at least one year, and the control group consisted of 20 healthy, age and gender matched subjects without any history of diabetes.

Exclusion criteria

Patients with habits (tobacco chewing and smoking), other systemic diseases /malignancies /taking medications other than the diabetes medications were excluded.

Sample collection

An informed consent was taken from each patient. After obtaining a written consent, detailed case histories with duration and medication for the disease were recorded. Patient's venous blood (2ml) was drawn for random blood sugar level estimation using oxidase method.[5] Diagnostic criteria for diabetes mellitus is as follows: Symptoms of diabetes plus RBS greater than or equal to 180 mg/dl.

Smear preparation

Before cytosmear preparation, subjects were instructed to rinse their mouth thoroughly with water & debris was removed gently with the help of gauze piece. Oral scrapings were obtained by using a cytology brush moistened with normal saline. The scraping was then transferred on to clean glass slide over the large area preventing the clumping of the cells and the prepared smear fixed immediately with 95% ethyl alcohol. Fixed smears were stained with Hematoxylin & Eosin (H & E) stain, Periodic Acid Schiff (PAS) stain and Periodic Acid Schiff-Diastase (PAS – D) stain.[6] Within each slide 50 unfolded oral exfoliated epithelial cells were evaluated for the number of PAS positive cells and staining quality of the cell in both the groups. For staining quality, the cells were analyzed as mild PAS positive, moderately PAS positive and intensely PAS positive cells [FIGURES- 1,2,3].

Statistical analysis

Data obtained was statistically analyzed.

RESULTS

In our study, mean age of the subjects from the study group and control group was 57.25 years and 54.3 years respectively. In both the groups, Male: Female ratio was 1:1.

Mean RBS level values were 233.75±56.25 and 102.15±22.44 in the study and control group respectively [Table 1]. So, on comparing we found that high RBS levels were seen in the study group as compared to control group and data was statistically significant ($p<0.01$) [Table 1]

All subjects from study group and 16 cases from control group showed PAS positive cells. Our result showed that number subjects with PAS positive cells in cytosmear was higher in study group than the control group [Table 2].

Our study also showed that as the range of RBS increases, the staining intensity of PAS positive cells also increases [Table 3, Graph 1].

PAS stained smear when analyzed for glycogen content in the oral exfoliated cells of study and control group, the result showed that the mean value of number of PAS positive cells in the study group was 23±10.15 cells whereas in control group it was 8.45±7.59. The statistical analysis revealed the significant difference. [Table 4].

In the study group the number of PAS positive cells were correlated with RBS level of the diabetic patients [Table 5]. However, the correlation was found to be statistically non-significant.

Cytology smear of the study group, stained with H and E stain exhibited nuclear changes like binucleation and enucleation. Inflammatory cells were seen in both the study as well as control group. [FIGURE – 4, 5].

Table 1: Correlation of mean value of random blood glucose level in the study and control group.

Groups	RBS mg/dl Mean ± SD	't' value	'p' value	significance
Study group (*n*=20)	233.75±56.25	9.71	<0.01	Highly significant
Control group (*n*=10)	102.15±22.44			

Table 2: Number of subjects with PAS positive cells in both the groups.

	No. of subjects	
No. of PAS +ve cells	Study group	Control group
1-10	3	9
11-20	4	5
21-30	7	2
>30	6	0
Total	20	16

Table 3: Correlation between RBS and staining intensity of PAS positive cells in the study group.

Range of RBS mg/dL	Mean of RBS	No. of cases	Staining intensity
176-185	180.50	2	Mild PAS positive cell
178-280	207.62	8	Moderately PAS positive cell
190-352	265.30	10	Intensely PAS positive cell

Table 4: Correlation of number of PAS +ve cells in the study and control group.

	Study group Mean ± SD	Control group Mean ± SD	'p' value
PAS +ve cells	23±10.15	8.45±7.59	Significant

Table 5: Karl Pearson's correlation coefficient between RBS levels and number of PAS +ve cells in study group.

Variable	Karl Pearson's correlation coefficient (r)	'p' value
RBS & PAS +ve cells	0.09	0.706

DISCUSSION

Even with the advancements in the modern diagnostic techniques, the monitoring the blood sugar levels in diabetes mellitus still requires an invasive, painful procedure for blood collection which is expensive and time consuming. So minimally invasive or non-invasive monitoring techniques are recently gaining more interest in the current scenario. One such technique is exfoliative cytology, which is a simple, rapid method that does not require specific technician and armamentarium. Apart from that, it is also less expensive, bloodless and painless method. Ziskin (1942)[7], in his study reported some important changes in oral epithelial cells associated with hyperplasia and glycogen deposits in diabetic patients. In histochemical study conducted by Kronman et al.(1970)[8], on gingiva of diabetic patients reported strongly stained, intensely reactive PAS positive material in the epithelium than in the non-diabetic controls .

The aim of our study was to use the non-invasive cytology procedure to detect the glycogen content in exfoliated cells of oral mucosa and to correlate the obtained results with random blood sugar levels to utilize exfoliative cytology as a diagnostic marker in diabetes mellitus.

Glycogen accumulation in the epithelial cells may be regarded as a functional disturbance in the metabolic activity of epithelial cells. Glycogen synthase kinase (GSK-3) regulates the glycogen synthesis by phosphorylation and inactivation of the final enzyme in glycogen biosynthesis, glycogen synthase (GS). GS(I), active form is converted into the less active form GS(D) by phosphorylation from GSK-3 protein kinase. It is contemplated that the decrease in GSK-3 phosphorylation leads to accumulation of glycogen.[9] It can be presumed that the same mechanism also works in oral epithelial cells leading to glycogen accumulation.

In the present study PAS and PAS-D stained smears were analyzed for glycogen content in oral exfoliated cells of both the groups. Periodic acid is a powerful oxidizing agent which reacts with aldehyde group of carbohydrates. Then the Schiff reagent forms a new product giving red or magenta color indicating PAS positivity (presence of glucose).

The fundament behind the use of PAS-D stain was that diastase depolymerizes glycogen to smaller sugar subunits, maltose and glucose. Glycogen takes up magenta color on PAS stained slide whereas

color is absent on PAS-D stained slide as diastase enzyme digests the glycogen. So the detection of glucose in the cells can be used in diagnosis of diabetes. On comparing total number of PAS positive cells in all the cytosmears of both the groups, study group revealed significant increase in number of PAS positive cells(p=0.001) which was similar to the study result of Hallikerimath et al.,[10] Asemi-Rad et al.,[11] Yasmin satpathy et al.,[12] Raty Ravindran et al.,[13] and Latti et al.[14]

In our study, number of PAS positive cells were correlated with the RBS levels [Table-3]. The correlation test was found to be statistically non-significant (r= 0.191, p= 0.264). Similarly, this has been reported in previous study conducted by Agrawal et al. (2016).[15] In the present study while comparing the slides stained with H & E in both the groups, we found that epithelial cells in the study group exhibited binucleation, enucleation and nuclear enlargement as well as more inflammatory cells than the control group.

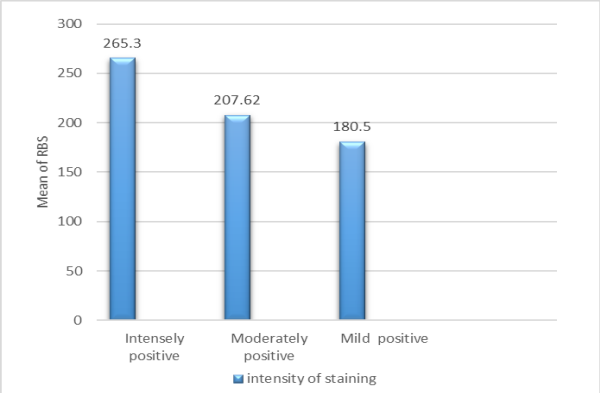
Observed nuclear changes in the diabetic group may be related to increased cellular age and decreased cellular turnover as a result of ischemia. Increased number of inflammatory cells were also observed which may be due to decreased salivary flow resulting from hypofunction of the salivary glands secondary to adverse hormonal, microvascular and neuronal changes in diabetes.

Thus, from our study, it can be said that exfoliative cytology can be used as an additional tool for diagnosis as well as for screening the patients for diabetes using PAS stain. However, larger sample size studies needed to be performed for a definite conclusion.

CONCLUSION

From this study we can say that, exfoliative cytology can be used as an adjunct in diagnosis of the diabetes and can be used in screening on a large scale population using PAS stain. Still study on large scale is needed. On the other hand, we can know only presence or absence of diabetes, but not the amount of blood sugar.

Graph 1: correlation between mean of RBS and intensity of staining in study group



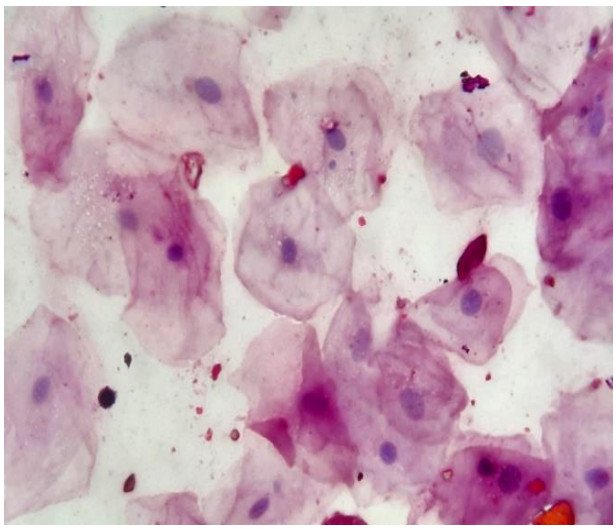


Figure 1: Photomicrograph showing mild PAS positive cells (40x magnification).

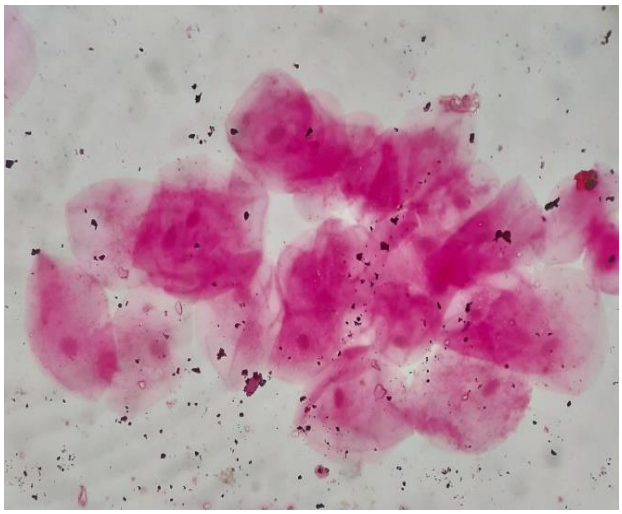


Figure 2: Photomicrograph showing moderately PAS positive cells(40x magnification).

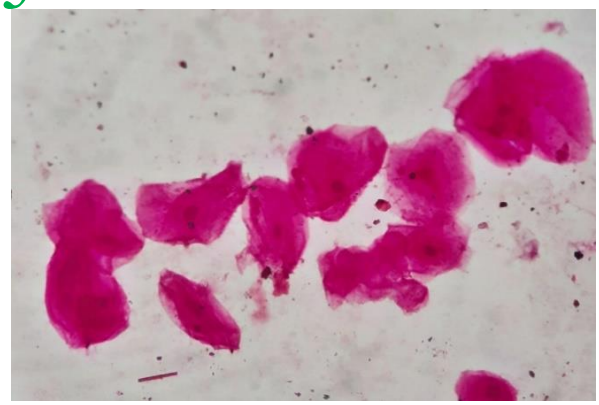


Figure 3: Photomicrograph showing intensely PAS positive cells (40x magnification).

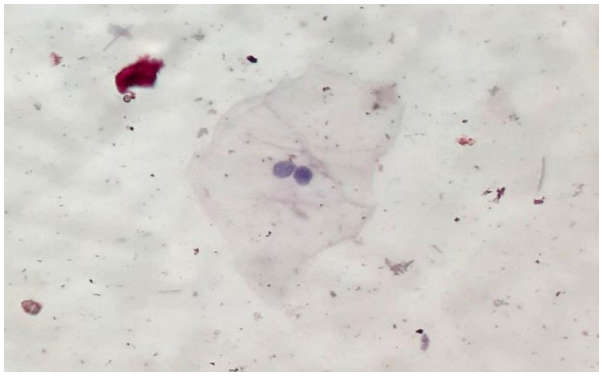


Figure 4: Photomicrograph showing binucleation in exfoliated cells.(H & E Stain) (40x magnification).

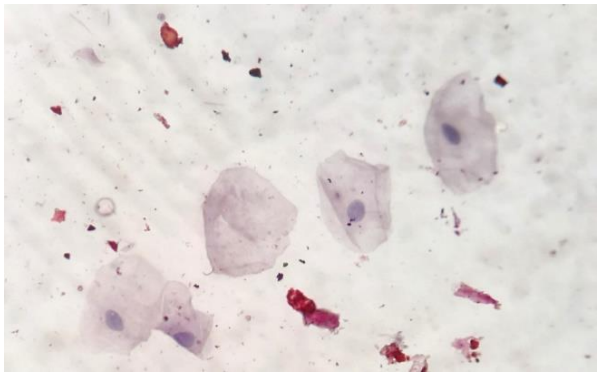


Figure 5: Photomicrograph showing enucleation in exfoliated cells. (H & E Stain) (40x magnification).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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