ejpmr, 2016,3(10), 203-206.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

COMPARISON AND CORELATION OF THE PERIODIC ACID SCHIFF STAINING INTENSITY OF ORAL EXFOLIATIVE CELLS WITH FASTING BLOOD SUGAR IN TYPE 2 DIABETIC PATIENTS

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Article Received on 04/08/2016 **Article Revised on 24/08/2016** **Article Accepted on 13/09/2016**

ABSTRACT

Objectives: Type-2 Diabetes Mellitus (DM) has considerable prevalence in India. A non-invasive diagnostic tool will be more appropriate in conditions like DM. In present study we are intend to find glycogen accumulation (if any) in buccal mucosa exfoliated cells of type-2 DM patients when compare to non-diabetic healthy individuals, and establish its diagnostic role. **Methods:** In present study 36 known DM patients with at least 1 year history (Case group) and 36 healthy, age and gender matched subjects (control group) were included. Patients with any other systemic disease were excluded from the study. Buccal mucosa exfoliate cytology smears were prepared from all 72 subjects and stained with PAS stains. Number of PAS positive cells per 50 cells in the smear was calculated. The data was tabulated and statistically analysed using chi square test, student't' test, Karl pearson's correlation coefficient and significance (2tailed) test. **Results:** Statistically significant difference was found in number of PAS positive cells in exfoliative cytology of DM patient group when compared with control group. **Conclusion:** glycogen accumulation in exfoliated buccal mucosa cells of type-2 DM patients can have diagnostic value which needs to be explored further.

KEYWORDS: Exfoliative cytology, diabetes mellitus, glycogen.

INTRODUCTION

Diabetes Mellitus (DM) affects many populations. An estimated 100 million people are affected by diabetes mellitus worldwide, of whom 7 million are in the Asian region.[1] Diabetes mellitus is one of the most common endocrine metabolic disorders and its prevalence has been increasing worldwide.[2]

Hyperglycemia caused by complete or relative insulin deficiency,[3,4] leading to impaired metabolism of carbohydrates, lipids and proteins.[5,6] These problems can cause structural changes in tissues and increase the risk of infection and vascular defects.[7]

Functional changes such as hyperplasia and increased glycogen are seen in diabetes. These changes may be due to glucose homeostasis disruptive factors such as impaired insulin secretion, insulin resistance in muscle, liver and adipocytes.

Periodic Acid Schiff reaction (PAS) is typically used in histochemistry and cytochemistry in order to check the glycogen. Periodic acid is a powerful oxidizing agent that react with the aldehyde groups of carbohydrates (without doing too much oxidation) then the Schiff reagent is involved with new product and a red or red-purple color develops. It is indication of PAS positive reaction (PAS+).[8]

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. Thus, glycogen content in exfoliated cells could be a non-invasive diagnostic marker for DM. Hence the purpose of this study was to demonstrate glycogen content in the exfoliated oral mucosal cells of type 2 diabetic patients, to establish its role in diagnostic criteria.

MATERIALS AND METHODS

The present study was conducted in the Faculty of Dental Sciences, IMS, BHU, Varanasi over a period of 12 months. Subjects for the study were obtained from the Out Patient Department. Case group, consisting of 36 subjects with history of DM for a minimum period of one year & control group consisting of 36 age and sex matched healthy subjects without any history of diabetes

were included in the study. Subjects suffering from any other systemic disease, with any tobacco/ alcohol associated habits, or with any obvious oral mucosal lesions were excluded from the study.

After obtaining a written informed consent, the detailed information about the history of DM including duration, type, medication etc was recorded. Fasting blood sugar (FBS) levels was estimated & recorded for all the subjects from case group and control group.

People were excluded from the study with the following factors.
Patients with clinical lesions of oral Candidiasis
Patients wearing dentures
Edentulous patients
Patients with harmful oral habits
Recent history of antibiotic therapy
Acute and chronic diseases
Endocrine disorders
Immunodeficiency diseases
Nutritional deficiency diseases

Patients were asked to rinse the oral cavity with water. Scrapings were obtained by using a wooden spatula moistened with normal saline. Using a gentle scraping motion exerting little pressure, cells were scraped from apparently normal buccal mucosa of both case and control groups and smears were prepared. Prepared smears were fixed immediately with 95% ethyl alcohol.

Fixed smears were stained with PAS (Periodic Acid Schiff) reagent.[9] This method is described as follows.
1. Progressively hydrate the cytological smear with decreasing concentration of alcohol to distilled water
2. Oxidize in periodic acid for 5 minutes
3. Rinse well in distilled water
4. Place in Schiff reagent for 15 minutes
5. Wash in running tap water for 10 minutes to allow pink colour to develop.
6. Counterstain with haematoxylin for a few seconds
7. Dehydrate in 95% alcohol and absolute alcohol
8. Clear in xylene
9. Mount with DPX

Within each slide, 50 cells with normal appearance, unfolded and unrepeated cells were evaluated by two expert observers who were blind about the groups.

RESULTS

In cases group, out of 36 subjects, the mean age was 52.56±8.06 years with maximum subjects i.e. 14 (38.9%) were more than 55 year of age. Where as in control group, out of 36 subjects (mean age= 49.14±7.78 years) the maximum number i.e. 13 (36.1%) were from 35 to 45 year of age range. In cases group, out of 36 subjects the majority i.e. 23 (63.9%) were males. Where as in control group, 26 (72.2%) were males. Out of 36 case subjects the maximum number i.e. 20 (55.6%) had history of diabetes mellitus less than five years, and the majority ie 35(97.2%) of subjects were on oral hypoglycemic drugs where as only 1 (2.8%) patient was on insulin. 17 cases(47.2%) had fasting blood sugar levels in range of 126 to 135 mg / dl.

Out of 36 cases, 27 subjects (75%) were having 11 or more PAS positive cells per 50 cell in the cytology slide, while 33 (91.7%) control group subjects were having less than 10 PAS positive cells. Chi square value for PAS +ve cells was found to be 36.109 and P value was < .001 showing strong significant association. (Table 1).

Table-1: Distribution of Subjects according to demographics, history of disease and investigation variables in case & control groups.

		No. of subjects in Cases Group	No. of subjects in Control Group	chi square value χ^2	p value
Age (years)	35-45	9(25%)	13 (36.1%)	1.127	.569
	46-55	13 (36.1%)	12 (33.3%)		
	>55	14 (38.9%)	11 (30.6%)		
Gender	Male	23 (63.9%)	26 (72.2%)	.575	.448
	Female	13 (36.1%)	10 (27.8%)		
Duration/ history of Diabetes (years)	1-5	20 (55.6%)	-	-	-
	6-10	14 (38.9%)	-		
	>10	2 (5.6%)	-		
Method of Glycemic control	Oral	35 (97.2%)	-	-	-
	Insulin	1 (2.8%)	-		
FBS (mg/dL)	126-135	17 (47.2%)	-	-	-
	136-145	7 (19.4%)	-		
	146-155	5 (13.9%)	-		
	>155	7 (19.4%)	-		
No. of PAS +ve cells	1-5	2 (5.6%)	18 (50%)	36.109	<.001 significant
	6-10	7 (19.4%)	15 (41.7%)		
	11-15	15 (41.7%)	0		
	16-25	12 (33.3%)	3 (8.3%)		

Table No. 2: Mean ± SD of variables in Cases and control group.

Variables	Case Group	Control Group	p value
Age	52.56±8.062	49.14±7.787	.072
PAS +ve cells	13.42±4.71	6.31±3.91	.000 significant

Table No. 3: Karl Pearson's Correlation coefficient between FBS levels and NA, CA, CNR & number of PAS positive cells in Cases group.

Variables	Karl Pearson's Correlation coefficient (r)	p value
FBS& PAS +ve cells	0.191	.264

DISCUSSION

Present study showed that there was an increase in staining intensity of oral exfoliative cells of diabetic patients compared to healthy controls. In line with our results Hallikerimath et al. also showed that the number of PAS positive cells (PAS+) were significantly higher in oral mucosa of diabetic patients compared to controls.[10]

Ziskin (1942)[5], in a study on oral epithelium of diabetic patients reported that in diabetics some important changes of beneficial nature take place which are associated with hyperplasia and glycogen deposits. Kronman et al., in a histochemical study on gingiva of diabetics reported that the intensely reactive PAS positive material in the epithelium of diabetics was more widely distributed and more strongly stained than in the non diabetic controls. It was also proposed that this finding may provide an early diagnostic test for diabetes.[11]

The accumulation of glycogen may be a functional change in the metabolic activity of epithelial cells. Saoussen B et al. in their study on tubular epithelial cells of kidney explained the mechanism of accumulation of glycogen. Glycogen synthase kinase (GSK-3), which is expressed in mammalian tissue was identified as an enzyme that regulates the glycogen synthesis. It phosphorylates and inactivates glycogen synthase (GS), the final enzyme in glycogen biosynthesis. The GS is regulated by phosphorylation and dephosphorylation. When the active form GS(I) is phosphorylated by protein kinase such as GSK-3, GS(I) is converted into the less active form GS(D). This form is modulated allosterically and becomes active when stimulated by its allosteric modulator glucose 6- phosphate.[12]

Khandelwal et al. have demonstrated that in diabetic rats, the increase in kidney glycogen is associated with increase of the D forms of GS and its allosteric activator, the glucose 6- phosphate.[13] It is speculated that decrease in GSK-3 phosporylation may contribute to glycogen accumulation. Thus it can be assumed that this mechanism works in oral epithelial cells also.

In the present study PAS stained smears were analyzed for glycogen in exfoliated cells of the case and control groups. Values were given as number of PAS positive cells per 50 cells. In case group the values ranged from 5 to 24 PAS positive cells with a mean value of 13.42±4.71 cells. Where as in control group the number of PAS positive cells ranged from 1 to18 cells with a mean value of 6.31±3.91 PAS positive cells (Table-2). On statistical analysis the difference in mean value of PAS positive cells was found to be significant.

In the case group the numbers of PAS positive cells were correlated with the FBS levels (Table-3). The test of correlation was found to be statistically non- significant (r= 0.191, p= 0.264). This has been reported in previous studies also.

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

REFERENCES

1. Shareef BT, Ang KT, Naik VR. Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients. Oral Med Oral Pathol Oral Cir Bucal., 2008:1; 13(11): E693-696.
2. Jajarm HH, Mohtasham N, Rangiani A. Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method. J Oral Sci., 2008; 50(3): 335-340.
3. Hallikerimath S, Sapra G, Kale A, Malur PR. Cytomorphometric analysis and assessment of periodic acid Schiff positivity of exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients. Acta cytol., 2011; 55(2): 197-202.
4. Al-Maskari AY, Al-Maskari MY, Al-Sudairy S. Oral Manifestations and Complications of Diabetes Mellitus. SQU Med J., 2011; 11(2): 179-86.
5. Prasad H, Ramesh V, Balamurali P. Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J cytol., 2010; 27(4): 113-7.
6. Ship JA. Diabetes and oral health. JADA., 2003; 134: 4-10
7. Bastos AdS, Leite ARP, Spin-Neto R, Nassar PcO, Massucato EMS, Orrico SRP. Diabetes mellitus and oral mucosa alterations: Prevalence and risk factors. Diabetes research and clinical practice., 2011; 9: 100-5.
8. Noori-Mughahi SMH, Mahmoodzadeh-Sagheb HR, Heidari Z. Applied method and terminology of

histotechnique, stereology & morphometry. Third Edition ed. Tehran: Tehran University of medical sciences, 1388.
9. Bancroft JD: Theory and practice of histological techniques. In microorganisms. Sixth edition. Elsevier: London., 2008; 321 – 322.
10. Daniel E. Ziskin, Eli H. Siegel, Winifred C. Loughlin. Diabetes in relation to certain oral and Systemic problems - Clinical study of dental caries, tooth eruption, gingival Changes, growth phenomena and related observations in juveniles. J. D. Res., 1942; 21: 296.
11. Kronman JH, Cohen MM, Colte D, Waitzken L: Histologic and histochemical study of human diabetic gingiva. J Dent Res., 1970; 49: 177.
12. Saoussen Bamri-Ezzine, Zhu Jun Ao, Irene Londono, Diane Gingras and Moïse Bendayan. Apoptosis of tubular epithelial cells in glycogen nephrosis during diabetes. Lab Invest., 2003; 83: 1069–1080.
13. Khandelwal RL, Zinman SM, Knull HR: The effect of streptozotocin induced diabetes on glycogen metabolism in rat kidney and its relationship to the liver system. Arch Biochem Biophys, 1979; 197: 310–316.