THE RELATIONSHIP BETWEEN THE STAINING INTENSITY OF ORAL EXFOLIATIVE CELLS WITH PERIODIC ACID SCHIFF AND CYTOMORPHOMETRIC INDICES WITH FASTING BLOOD SUGAR IN TYPE 2 DIABETIC PATIENTS

Azam Asemi-Rad
Genetic of Non-communicable Diseases Research Center, and Department of histology, Zahedan University of Medical Sciences, Zahedan, Iran.
PO Box: 98155-878, Zahedan, Iran

Zahra Heidari (Corresponding author)
Genetic of Non-communicable Diseases Research Center, and Department of histology, Zahedan University of Medical Sciences, Zahedan, Iran.

Hamidreza Mahmoudzadeh-Sagheb
Genetic of Non-communicable Diseases Research Center, and Department of histology, Zahedan University of Medical Sciences, Zahedan, Iran.

Abstract
In this case-control study, 50 patients with type 2 diabetes and 50 healthy controls participated. After measuring blood glucose level, smear was prepared form their buccal mucosa. Then oral smears stained by the periodic acid Schiff (PAS) reagent. The results indicated higher staining intensity in oral mucosal exfoliative cells of diabetic patients compared to controls (p <0.05). There was a significant correlation between staining intensity and fasting blood sugar (FBS), and also between the percentage of PAS+ cells and FBS. The relationship between the nuclear area (NA) and the cytoplasm to the nuclear ratio (CNR) with the staining intensity of cell was also significant (p <0.05). The correlation between cytoplasmic area and staining intensity was not significant (p >0.05). The result showed that diabetes may cause changes in glycogen content of oral mucosal cells and could change morphometry of these cells. It seems that diabetes affects oral mucosa and may relate to other oral manifestations of this disease. This noninvasive method can be used as a complementary method in diagnosis for diabetes and oral complications.

Keywords: Diabetes, Cytomorphometry, periodic acid Schiff

1. Introduction:
Diabetes mellitus is a common endocrine and metabolic disease (1, 2) which is due to deficiency of insulin (type 1 diabetes) or resistance of the cells of target tissues to the effects of insulin (diabetes type 2) (3). The most common form of diabetes is type 2 diabetes. About 95 percent of people with diabetes have type 2 and also it is the sixth leading cause of death in elderly (4). It has been estimated that 7.7 percent of adults aged 25 - 64 years are affected by this disease (5).

Hyperglycemia caused by complete or relative insulin deficiency (1, 6), leading to impaired metabolism of carbohydrates, lipids and proteins (7, 8). These problems can cause structural changes in tissues and increase the risk of infection and vascular defects (9).

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+) (10).

Hallicerimat et al evaluated PAS positive exfoliative cells (PAS+) with periodic acid schiff cytochemistry techniques in order to achieve a fast and easy method for screening of diabetes and the results showed the number of PAS+ exfoliative cells in the oral mucosa of diabetics were higher than the controls (1). But they didn't examine the relationship between cytological indices and number and severity of PAS positivity of exfoliative cells. Despite of this fact that they were looking for a tool for detection and screening of diabetes, they didn't achieve this tool.

Due to these shortcomings, the purpose of this study was using the PAS cytochemistry technique for estimation of the number of PAS+ cells and exfoliative cells staining intensity, as well as the relationship between these variables and cytomorphometric parameters with fasting blood sugar in type 2 diabetic patients compared with healthy controls to achieve a contemporary tool for screening and monitoring the diabetes.

2. Material and method:

In this case-control study, the case group consisted of 50 type 2 diabetic patients (22 men and 28 women) and the control group consisted of 50 healthy subjects (23 males and 27 females). The sampling was done by simple convenience probability method. The members of the case group were selected from the patients who referred to the diabetes clinic of Zahedan Ali-Ashgar hospital consisted of patients with a history of disease between 2-8 years. All patients were receiving medications for control of diabetes.

and the control group were selected from the patients' fellows who were in healthy status. Duration of diabetes was 2 to 8 years. Two groups were matched according to age and gender.

Inclusion criteria for the study population were to have type 2 diabetes at least for 6 months from diagnosis. Exclusion criteria included consuming tobacco, alcohol, drugs other than drugs for diabetes, anemia, infectious diseases, severe infections of any type in the mouth, oral cavity cancer, malignant diseases, and systemic diseases such as kidney failure, liver disease, rheumatologic diseases, pregnancy and the menopause.

Written informed consent in accordance with the ethical codes adopted by the National Committee for Medical Research Ethics, completed by all participants. All the procedures had received prior approval of the ethics committee of Zahedan University of Medical Sciences according to the agreement No: 1761-90. After confirmation of the disease by an expert endocrinologist, smears of the oral mucosa exfoliative cells were prepared and then stained by PAS. For this instance, first mouth washed and cleaned with tap water and then a piece of gauze gently pulled on the area to ensure that there were no food particles, then a smear was prepared from buccal mucosa using a disposable cytobrush. The cytobrush was rotated 10 times in the selected region. Each smear was spread on a prepared, clean and dry microscopic slide which had been previously labeled and then immediately fixed by fixation spray.

Smear stained with periodic acid Schiff (PAS) technique (7). Cytomorphometrical assessment was started from one side of the slide to another side in a systematic manner. Within each slide, 50 cells with normal appearance, unfolded and unrepeated cells were evaluated by two expert observers who were blind about the groups.

Exfoliated cells based on staining intensity divided in 4 groups: no staining (0), mild staining (1), moderate (2) or severe (3).

Non-parametric Mann-Whitney U test was used for comparison of intensity of staining between the two groups.

Nuclear Area (NA) and the Cytoplasmic Area (CA) in the cells were calculated using the Cavalieri’s point counting technique.

To investigate the relationship between the NA, CA, cytoplasm nuclear ratio (CNR) and fasting blood sugar (FBS) with the staining intensity, the Spearman correlation coefficient test was used. The significant level was set at P<0.05.

3. Result:

In this study, 45% were men and 55% were women. The age of participants was 37-65 years. The mean age of patients was 49.26 ± 6.39 and mean age in control group was 49.23 ± 5.35. The cases and control groups were matched for age and gender, there was no significant difference in this regard (P>0.05).

Cytochemical study showed that the staining intensity of oral mucosa cells of diabetic patients significantly increased compared to healthy subjects (P< 0/05) (Table 1) (Fig. 1).
The relationship between the FBS and staining intensity of exfoliative cells analyzed with Spearman nonparametric test and results showed that there was a significant correlation between these two variables ($r=0.745$, $p<0.001$) (Table 2).

Also, the relationship between FBS and the number of PAS-positive cells was positively and significantly correlated ($r = 0.714$, $p<0.001$) (Table 2).

The relationship between NA and CNR with the intensity of staining of cells was correlated using spearman correlation coefficients (NA, $r = 0.365$, $p< 0.001$) and (CNR, $r = -0.231$, $p = 0.02$).

There was no significant relationship between CA and intensity of staining ($P>0.05$).

4. Discussion:

Present study showed that there was an increase in staining intensity, NA and CNR in 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 (1). Kronman et al. investigated the gingival epithelium of diabetic patients with PAS histochemistry techniques and showed an increase in staining intensity of these cells. These changes were associated with glycogen distribution in gingival epithelium and cell hyperplasia (11).

Study of Desoye showed that women with diabetes had an increased distribution of glycogen in the blood vessels of their placentas (12).

Pathmaperuma et al. in their study on placenta showed that hyperglycemia could increase intracellular accumulation of glycogen in trophoblast cells (13).

Study of Bamri-Ezzine et al. on renal tubular cells of diabetic patients showed accumulation of glycogen in tubular cells (14).

Results of Khandelwal study also showed that the increment of glycogen in renal cells of diabetic rats was associated with increment of inactive form of glycogen synthase (15).

Increased staining intensity of oral mucosal cells with PAS reaction is probably due to glycogen accumulation in these cells. Glycogen synthase kinase (GSC) is an enzyme that regulates glycogen synthesis in mammalian tissues. This enzyme, phosphorylate and inactive the glycogen synthase, which is the last enzyme in the process of glycogen biosynthesis. When the active form of glycogen synthase phosphorylated by protein kinase GSK-3, it converted into low-activity form and activated when excited by glucose 6-phosphate. Decrease in GSK-3 phosphorylation caused to accumulation of glycogen in the cells (14).

Also after sustained hyperglycemia, glycation of proteins, lipids and nucleic acids increased which caused to accumulation of end products of this process in the wall of large vessels and basement membrane of small vessels (16). The mentioned factors can progressively reduce the size of the lumen of blood vessels and decrease spread to target tissues resulting delay in keratinization of epithelial tissue and enlarged cells (1, 16).

The cases reviewed in this study to determine the relationship between FBS and the staining intensity in exfoliative cells. Results showed a significant relationship between these two variables and it is not consistent with the results obtained in the study by Hallikerimath (1). The reason for the difference in results could be due to differences in FBS of patients in these two studies. The mean FBS in diabetic patients in Hallikerimath study was 174/18 ± 86/143 (1), while the mean FBS in diabetic patients in our study was 203/71 ± 98/208. So it can be said increasing FBS caused to increment of glycogen accumulation and higher staining intensity of oral mucosa cells. This study showed that with the increment of staining intensity of exfoliative cells, cytoplasm to the nuclear ratio reduced in diabetic patients. This is due to increasing nuclei area in the oral exfoliated cells of diabetic patients without any changes in sizes of cytoplasm in both groups. This has been reported in previous studies (1, 2, 17 and 18).

On the other hand, with this increment, the cytoplasmic area in oral mucosal cells of diabetic patients does not change. Preserved cytoplasmic area is a compensatory mechanism for maintain cell size in terms of stress (1).
5. Conclusion
The result showed that diabetes may cause changes in glycogen content of oral mucosal cells and could change morphometry and morphology of these cells. It seems that diabetes affects oral mucosa and may relate to other oral manifestations of this disease. This noninvasive method can be used as a complementary method in diagnosis for diabetes and oral complications.

6. Acknowledgment
Hereby, the authors are very thankful to the deputy of research and technology of Zahedan University of Medical Sciences for financial support of this study. We are also very thankful to the respected personnals of the Histology Department and the Diabetes Clinic of Zahedan Ali-Asghar hospital and all of the participants who engaged in this study.
References


[8] SHip JA. Diabetes and oral health.JADA. 2003;134:4-10


Table 1. Comparison of intensity of staining in oral mucosa cells of diabetic patients and normal subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diabetes N=50</th>
<th>Control N=50</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS Positivity</td>
<td>1.54±0.4</td>
<td>0.37±0.29</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

NS: not significant, * P<0.05, ** P<0.0001

Table 2. Comparison of intensity of cell staining with NA, CA, CNR and FBS in diabetic patients

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS Positivity and NA</td>
<td>0.365</td>
<td>0.0001**</td>
<td></td>
</tr>
<tr>
<td>PAS Positivity and CA</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PAS Positivity and CNR</td>
<td>-0.231</td>
<td>0.021*</td>
<td></td>
</tr>
<tr>
<td>PAS Positivity and FBS</td>
<td>0.745</td>
<td>0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant, * P<0.05, ** P<0.0001.

Figure 1. Oral mucosal cell of normal subject(A) compared with PAS* cell in oral mucosa of diabetic patients(B)