Periodontal Therapy and Gingival Health in Patients with Diabetes Mellitus

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ABSTRACT

Objective: Due to difficult identification of pathologic conditions of oral mucosa in diabetic patients, the aim of the study was to evaluate gingival epithelial cells by exfoliative cytology in patients with type 2 DM and periodontal disease before and after periodontal therapy.

Methods: The group I (100 patients with periodontal disease and type 2 DM) and the group II (100 patients with periodontal disease) participated in the study. Oral examination was done, the gingival smears taken and periodontal therapy applied. Seven days afterwards, smears were taken again and the cytomorphometric analysis was done.

Results: Cytomorphometric values were higher in the group I before and after therapy.

Conclusion: Noteworthy differences in the cytomorphology of the gingiva found in patients with DM type 2 comparing to systemically healthy patients decreased after periodontal therapy. Changes are not specific for DM and further studies are needed to determine the predictive value of cytomorphometric analysis.

Keywords: Exfoliative cytology, diabetes, periodontal disease

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INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent endocrine metabolic disorders (1). During DM aberrant hydrate, fat and protein metabolism may be present which is cause for DM complications (2). Type 2 DM accounts for approximately 95% of DM cases and is the fifth most common chronic condition nowadays. Its frequency has been increasing and it the sixth leading cause of mortality among the elderly in the world (3).

The damaging effects of DM on the oral cavity have been well investigated, and investigators have reported DM as a risk factor for periodontal disease (4). Gingival inflammation is very common during periodontal disease and shows its activity and development (5, 6). Prevention and therapy of periodontal disease in patients with DM is very important due to its potential negative effect on glycemic control and stimulation of diabetic complications.

Most of pathologic conditions of oral mucosa can be easily diagnosed, but some of them need detailed investigation and several techniques are accessible (5, 7). In DM routinely used techniques like biopsy usually cannot be performed due to glycemic variations (8). Exfoliative cytology is more practical technique because it can be applied even during inflammation. It is a simply and noninvasive diagnostic method that can be repeated frequently with little discomfort to the patient. It picks superficial desquamated cells and analyzes them microscopically. This method has been discussed over the last 40 years from aspect of value in the diagnosis and prognosis of precancerous lesions (7, 8).

Some investigators have used exfoliative cytology to evaluate alterations of the oral mucosa in DM and shown that DM can produce changes in oral epithelial cells such as enlargement of cell nuclear area (NA) and decrease of keratinization. They are of the opinion that exfoliative cytology may help in the diagnosis of DM if the causes that lead to cellular changes are understood. One of the main reasons of cytomorphometrical alterations are cellular aging and

atrophy. Cellular aging in diabetic patients is connected with a decrease in cell changing, which is the cause of nucleus and cytoplasm alterations (9). Also, decrease in salivary flow which leads to mucosal damage and atrophy is usually present in diabetic patients (10). So, it could be assumed that morphometric analysis of gingival tissue in diabetic patients could reveal the activity of DM related to the changes in whole body (7).

The aim of the present study was to evaluate gingival epithelial cells by exfoliative cytology in patients with type 2 DM and periodontal disease before and after conservative periodontal therapy.

SUBJECTS AND METHODS

This clinical study was carried out at the Department of Endocrinology and Department of Periodontology and Oral medicine, Nis University, Faculty of Medicine. The study procedures were approved by the Nis University Faculty of Medicine Institutional Ethical Committee (01-2800-7). Patients who had antibiotic, corticosteroid or periodontal therapy in the last 3 months, and smokers were not included in the study in order to eliminate the influence of these conditions on cellular shape and morphology. All of the patients signed an informed consent form.

The group I consisted of 100 patients with periodontal disease and type 2 DM who had a 5 to 10 year history of type 2 DM. The following information was collected from medical records: DM duration (years since diagnosis), patient age and sex. The group II consisted of 100 patients with periodontal disease with no risk factors for DM. The group I consisted of 48 (48%) women and 52 (52%) men; the mean age 62.57±8.57 years and the group II of 50 (50%) women and 50 (50%) men; the mean age 45.68±8.91 years.

After anamnesis and medical records analysis oral examination was done. Patients washed mouths with normal saline for about 5 minutes and then the surface smear was taken with

tip of a lancet from gingival tissue surrounding fully erupted permanent premolar teeth. The smears were transformed to a clean dry glass slide and fixed with 95% ethyl alcohol and prepared with Papanicolaou staining for cytomorphometric analysis. Afterwards, dental plaque removal and full-mouth scaling and root planning was performed in all of the patients. Seven days afterwards, smears were taken again. The morphometric analysis was done using Image program, NU2 microscope (Carl Zeiss, Germany) objective x 63 (NA 0.8).

SPSS software program was used for the statistical analysis. Parameters were shown as mean values (X) and standard deviations (SD). Student t-test, Leven method and Tukey HSD test were used for analysis of difference between groups.

RESULTS

Before therapy ANOVA and Post Hoc analysis revealed higher nuclear area values (NA), optical density (OPTDENS), the standard deviation of the optical density (SDOPTDEN), the volume of the nucleus (PERIMITE), Feret's diameter (FERET), and integrated optical density (IOD) in the group I. The values of maximum optical density (MAXOD) and nucleus circularity (CIRCULAR) were lower in the group I than in the group II, with the level of significance presented in table 1.

Comparing the mean values of the investigated parameters in the group I before and after therapy, t-test showed a statistically significant reduction in mean values of nuclear area, nucleus volume, Feret's diameter, and integrated optical density. Also, a statistically significant increase in all other investigated parameters (optical density, the standard deviation of the optical density and maximum optical density) was noticed with a maximum level of statistical significance ($p < 0.001$). The coefficient of variation of all parameters, except for the standard deviation of the optical density is less than 30, which indicate the homogeneity of the investigated parameters before and after treatment. (Table 2).

After therapy in the group II, t-test for independent samples showed a statistically significant reduction in mean values nuclear area, nucleus volume, Feret's diameter, the integrated optical density with a maximum level of statistical significance ($p<0.001$), and the circularity of nucleus ($p<0.01$). The mean optical density value, standard deviation and maximum optical density showed statistically significant increase ($p<0.001$). Except for the standard deviation of the optical density before and after treatment, all other parameters have the coefficients of variation smaller than 30 – their values are homogeneous before and after treatment. (Table 3).

Comparing groups after therapy ANOVA and Post Hoc analysis revealed higher nuclear area values, optical density, the standard deviation of the optical density, nucleus volume, Feret diameter and integrated optical density in group I. The maximum optical density had higher values in group II. There were no differences in the nucleus circularity between two groups with different levels of significance presented in table 4.

DISCUSSION

In recent years, there has been increasing interest in the role of exfoliative cytology as a standard technique in screening of oral pathologies. Cytomorphometric analysis of exfoliated cells has been suggested as a key approach to identify the cellular changes and many investigators have evaluated different sites in oral cavity of diabetic patients (11, 12). However, data on gingival tissue is scarce. Therefore in our study, cytomorphometric analysis was performed on smears obtained from gingiva of healthy individuals and patients with type 2 DM before and after periodontal therapy in order to illuminate these changes and open the door to future investigations (13-17).

Many investigations suggest that higher NA values and parameters of optical density in cytomorphometric analysis of oral mucosa could be related to increased cellular age in patients with DM. This is thought to be a secondary reaction to ischemia caused by diabetic atherosclerosis (15-18). Hyperglycemia causes agglomeration of advanced glycation end products in the basement membrane of the small blood vessels. These narrowing of the vessel lumen leads to decreased perfusion and decreased sequent cell turnover. This can explain slow keratinization epithelial differentiation processes which lead to an increase in the number of mature cells. Mature cells have large nucleus as a primary characteristic and can be related to higher NA values in our study (16).

Decreased salivation in diabetic patients may lead to dehydration which causes mucosal atrophy (13). Smears from atrophic mucosa usually have cells that are smaller in cell size, but have larger nuclei and give an impression of higher NA values as noticed in our study.

Also, it is thought that acute oxidative stress may be the cause for the development of nuclear cell swelling in patients with DM. It is indicated that altered properties of K+ channels and arachidonic acid metabolites cause ischemic and inflammatory conditions in diabetic patients which are the reason for nuclear swelling and higher NA values (19). A similar pathogenesis could be projected for increase in NA observed in our study.

In our study an increase in NA and parameters of optical density were evident in patients with type 2 DM before and after applied periodontal therapy, a finding similar to other authors (14). Comparing values before and after therapy, higher values were observed before periodontal therapy. There were smaller differences in investigated parameters between groups after therapy suggesting an importance of timely periodontal treatment for the improvement and preservation of periodontal health in diabetic patients. Such observations suggested that periodontal therapy can achieve periodontal health similar to one in the systemically healthy patients.

Patel et al. (11) attempted to define a baseline for pathological smears obtained from gingival tissue and suggested that inflammation is one of the factors that can increase NA and lead to a poorly preserved cytoplasm. They performed conservative periodontal therapy in order to decrease inflammation and detected a range of cellular age in the smears. The investigators suggested that the observed changes are found during periodontal inflammation in young cells. Similar was noticed in our study where higher values were observed before periodontal therapy when inflammation was more pronounced in both investigated groups.

In spite that our study showed that DM type 2 produces cellular changes in the gingival epithelium, this changes are not specific to DM. Cellular alterations are also present in patients with endocrine and respiratory diseases, associated with a decreased rate of keratinization which is the reason for the increase of the nuclear size. Investigators noticed similar changes during keratinization alteration and epithelial dysplasia in smoking subjects (9,20). However, each study differs in the number of cells being counted per slide and type of fixatives. This fact showes a call to establish a guideline for cytological procedure and image analysis standardization in order to obtain more precise results which could be valid to a wider range of clinical situations.

CONCLUSION

Noteworthy cytomorphological differences of the gingiva found in patients with DM type 2 comparing to systemically healthy patients decreased after periodontal therapy. This could suggest the importance of timely therapy for the improvement of general and periodontal health in diabetic patients.

Cytomorphometric analysis of gingiva illuminates what really happens in periodontium in patients with DM. Changes are not specific for DM and further studies with greater sample size

and comparison to other conditions are necessary to define the predictive value of cytomorphometric analysis.

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AUTHORS' NOTE

R Obradovic conceived paper, oversaw data collection, conducted data analysis, wrote manuscript and approved final version. Lj Kesic, A Pejcic and M Igic critically revised manuscript and approved final version. A Petrovic participated in study design, data analysis, interpretation of histological data and approved final version. RA

REFERENCES

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol 2012**; 8:** 228-36.
2. Nathan DM. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: Overview. Diabetes Care 2014; **37:** 9-16.
3. Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. Diab Res Clin Pract 2010; **87:** 15–9.
4. Mealey BL, Rethman MP. Periodontal disease and diabetes mellitus. Bidirectional relationship. Dent Today 2003; **22:** 107-13.
5. Bascones-Martinez A, Matesanz-Perez P, Escribano-Bermejo M, Gonzales-Moles MA, Bascones-Ilundian J, Meurman JH. Periodontal disease and diabetes - Review of the literature. Med Oral Patol Oral Cir Bucal 2011; **16:** 722-9.
6. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. Periodontology 2000 2013; **62:** 59–94.
7. Pérez-Sayánsm M, Somoza-Martín JM, Barros-Angueira F, Reboiras-Lopes MD, Gandara-Vila P, Gandara-Rey JM, et al. Exfoliative cytology for diagnosing oral cancer. J Biotech Histochem 2010; **85:** 177-87.
8. 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:** 335-40.
9. Rivera C, Núñez-de-Mendoza C. Exfoliative cytology of oral epithelial cells from patients with type 2 diabetes: cytomorphometric analysis. Int J Clin Exp Med 2013; **6:** 667-76.
10. Gowdar IM, Almuhaiza M. Diabetes and oral health – A review. Ann Int Med Den Res 2016; **2:** 2-8.

11. Patel PV, Kumar S, Kumar V, Vidya GD. Quantitative cytomorphometric analysis of exfoliated normal gingival cells. J Cytol 2011; **28:** 66-72.

12. Igic M, Mihailovic D, Kesic Lj, Milasin J, Apostolovic M, Kostadinovic LJ, et al. Cytomorphometric and clinical investigation of the gingiva before and after low-level laser therapy of gingivitis in children. Las Med Sci 2012; **27:** 843-8.

13. Babu NA, Masthan KMK, Bhattacharjee T, Elumalai M. Diabetes Mellitus Affect the oral cavity - A review. Global J Pharm 2014; **8:** 166-9.

14. Seifi S, Feizi F, Moazzezi Z, Mehdizadeh M, Zamani B. Evaluation of oral mucosal epithelium in diabetic male patients by exfoliative cytology method. J Diab Metab Dis 2014; **13:** 77-84.

15. Tozoglu U, Bilge OM. Exfoliative cytology of type i diabetic patients. Eur J Gen Med 2010; **7:** 264-8.

16. Prasad H, Ramesh V, Balamurali P. Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol 2010; **27:** 113-7.

17. Nandita KP, Boaz K, Srikant N, Lewis AJ, Manaktala N. Oral epithelium in diabetics: A cytomorphometric correlation. Dent Hypotheses 2014; **5:** 59-65.

18. Parmar D, Sawke GK, Sawke N. A cytomorphometric evaluation of oral mucosal cells in type II diabetics. People J Sci Res 2014; **7:** 22-7.

19. Pannicke T, Iandiev I, Wurm A, Uckermann O, vom Hagen F, Reichenbach A, et al. Diabetes alters osmotic swelling characteristics and membrane conductance of glial cells in rat retina. Diabetes 2006; **55**: 633-9.

20. Göregen M, Akgül HM, Gündoğdu C. The cytomorphological analysis of buccal mucosa cells in smokers. Turk J Med Sci 2011; **41:** 205-10.

Table 1: Mean values (X±SD) of the investigated parameters before therapy

Investigated parameter	**Group I X±SD**	**Group II X±SD**	**ANOVA F**	**p**
NA	99.48±11.69***	76.81±8.50	41.02	0.0000
OPTDENS	0.27±0.05	0.26±0.05	0.38	0.0000
SDOPTDENS	0.02±0.00	0.01±0.00*	4.93	0.0085
MAXOD	0.34±0.07	0.42±0.11**	13.08	0.0000
PERIMITE	38.09±2.66***	33.26±1.92	45.31	0.0000
CIRCULAR	0.83±0.06	0.87±0.03	6.06	0.0030
FERET	13.29±1.70***	11.91±1.10	2.36	0.0981
IOD	26.88±5.77***	20.17±3.96	25.25	0.0000

Group I vs group II; * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

Table 2: Investigated parameters in group I before and after therapy

Investigated parameter	**Before therapy**		**After therapy**		**t**	**p**
Group II	**X±SD**	**CV**	**X±SD**	**CV**		
NA	99.48±11.69	11.76	54.30±5.07	9.34	23.53	0.0000
OPTDENS	0.27±0.05	20.72	0.33±0.08	24.96	4.89	0.0000
SDOPTDENS	0.02±0.00	38.13	0.03±0.01	38.60	6.89	0.0000
MAXOD	0.34±0.07	21.00	0.40±0.10	25.68	3.98	0.0002
PERIMITE	38.09±2.66	6.99	28.03±1.43	5.11	22.14	0.0000
CIRCULAR	0.83±0.06	6.83	0.86±0.03	3.93	0.58	0.5656
FERET	13.29±1.70	12.79	10.09±0.82	8.82	12.29	0.0000
IOD	26.88±5.77	21.48	17.52±4.93	28.16	13.09	0.0000

Table 3: Investigated parameters in group II before and after therapy

Investigated parameter	Before therapy		After therapy		t	p
Group II	**X±SD**	**CV**	**X±SD**	**CV**		
AREA	76.81±8.50	11.07	51.09±5.99	11.74	18.49	0.0000
OPTDENS	0.26±0.05	21.93	0.32±0.08	18.36	12.11	0.0000
SDOPTDENS	0.01±0.00	34.76	0.02±0.01	36.24	7.97	0.0000
MAXOD	0.42±0.11	28.22	0.52±0.12	23.91	7.82	0.0000
PERIMITE	33.26±1.92	5.79	27.64±1.70	6.16	14.63	0.0000
CIRCULAR	0.87±0.03	4.11	0.83±0.06	7.21	2.96	0.0048
FERET	11.91±1.10	9.24	9.9±0.87	8.18	8.75	0.0000
IOD	20.17±3.96	19.66	16.74±2.84	16.96	6.15	0.0000

Table 4: Mean values (X±SD) of the investigated parameters after therapy

Investigated parameter	Group I X±SD	Group II X±SD	ANOVA F	p
NA	54.30±5.07**	512.09±5.99	4.73	0.0102
OPTDENS	0.33±0.06	0.32±0.08	0.33	0.7214
SDOPTDENS	0.03±0.01	0.02±0.01*	4.20	0.0168
MAXOD	0.40±0.10	0.52±0.12***	23.97	0.0000
PERIMITE	28.03±1.43	27.64±1.70	0.97	0.3824
CIRCULAR	0.86±0.05	0.86±0.03	5.43	0.0053
FERET	10.09±0.82	9.9±0.87	19.38	0.0000
IOD	17.52±4.93	16.74±2.84	1.16	0.3176

Group I vs group II: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$