Cytological analysis of the periodontal pocket in patients with aggressive periodontitis and chronic periodontitis

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Abstract

Background: Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of this study was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis (AP) and chronic periodontitis (CP). **Materials and Methods:** Patients aged 24–54 years, of whom 41 were diagnosed with AP, 40 with CP, sub-classified as mild, moderate and severe periodontitis, and 40 healthy individuals who were the control group. Samples of the epithelium of the periodontal pocket were taken for the cytological study. **Results:** Superficial and intermediate cell values were significantly greater in patients with AP than in patients with CP or the control group. Histiocyte number was higher in patients with CP than in those with AP, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with CP, and there were differences between patients with periodontitis and the control group. **Conclusions:** The cytological study demonstrated that patients with AP had greater tissue damage, shown by the increase in intermediate and superficial cells of the epithelium of the periodontal pocket compared to the group of healthy subjects and to a lesser extent, to patients with CP. Only superficial cells made it possible to differentiate the sub-stages of the disease.

Keywords: Exfoliative cytology, gingivo-periodontal diseases, histiocytes, intermediate cells, periodontal pocket, surface cell

Introduction

Gingival-periodontal diseases include a set of pathologies that affect the protective and insertion tissues of teeth. Their onset is caused by the presence of specific biofilm and its metabolic products.[1] A wide range of clinical pictures arises from a complex interrelationship among bacterial aggression, intrinsic host response, and risk factors or disease modifiers.[2]

Traditional diagnosis is done by measuring different clinical and radiographic indices: Probing depth (PD), attachment level (AL), and bleeding on probing (BOP) and/or spontaneous bleeding, ulceration, exudate, and suppuration; however, these methods only provide information on past destruction.[3,4] Given the severity of aggressive periodontitis (AP) and its tendency to advance, early detection is a major concern.

Variations in the onset, severity, and clinical characteristics can be used to recognize and describe different forms of periodontitis including early-onset periodontitis now called AP, and adult or chronic periodontitis (CP). Both can be sub-classified as laser periodontitis (LP), moderate periodontitis (MP), and severe periodontitis (SP).[5,6]

Aggressive periodontitis usually appears early in life, which means that the etiological agents were able to produce clinically detectable levels of disease within a fairly short time. This is crucial for understanding these diseases because it implies infection by highly virulent microflora or a high level of susceptibility of the subject to periodontal disease. Nevertheless, AP may appear at any age. Its diagnosis requires the exclusion of systemic diseases that can severely deteriorate host defenses and lead to premature loss of teeth.[7]

Chronic periodontitis is usually more prevalent in adults but may also be found in children and youths. The extent of the destruction is consistent with the presence of local factors. The progression of CP is usually slow or moderate, but there may be short periods of rapid destruction. It may be modified by systemic conditions such as diabetes, smoking, and stress.[1]

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Given the severity of AP and its tendency to advance, early detection is a major concern. During the migration process of epithelial cells of the gum from deep layers toward more superficial layers, there is a series of differentiation and maturation events. The formation of a layer of keratin which protects the gingival from direct mechanical, chemical, and bacteriological stimuli, thus preserving its integrity, is a phenomenon that translates into cytological changes which can reflect the degree of periodontal health.[8]

Oral exfoliative cytology includes the study and interpretation of the features of naturally or artificially exfoliated cells from the oral mucosa. It consists of observing the morphology of superficial epithelial cells under the microscope after sampling, fixing, and staining. It is a simple, nonaggressive, relatively painless technique that patients accept well. Ogden *et al*., 1990, suggest that these quantitative techniques based on evaluating parameters such as variations in the size of the nucleus and the cytoplasm and alterations in the nucleus/cytoplasm ratio can increase the diagnostic sensitivity of exfoliative cytology.[8]

The use of exfoliative cytology could be used as a complementary diagnosis method because it is easy to perform and allows repeated sampling without destroying the integrity of the tissue. Cytological study of healthy gum shows exfoliated surface cells and a smaller proportion of intermediate cells from the stratum spinosum.[9] There are also a few leukocyte-type transitory cells with defense functions, red blood cells, and microbial flora.[10]

The aim of this study was to analyze cytological changes of the periodontal pocket in patients with AP and CP in their clinical sub-stages LP, MP, and SP.

Materials and Methods

Subjects

We worked with patients aged 24–54 years, of whom 41 had AP (ages 24.0 ± 9.0) (21 male and 20 female), and 40 had CP (ages 58.3 ± 7.0) (13 male and 27 female). The control or clinically healthy group was made up of 40 healthy individuals (ages 44.0 ± 10.0) (18 male and 22 female).

Clinical and dental records were prepared for all subjects, and they signed informed consent for cytological sampling. An ethical clearance was obtained before the study by the Ethical Committee of the institution in compliance with the Declaration of Helsinki of 1975 and the WHO standards for observational studies.

Dental clinical parameters

Periodontal diagnosis was performed by a single calibrated examiner. It included: Plaque index (PI),[11] gingival index (GI),[12] PD, AL, and BOP using a Marquis-type periodontal probe (Hu-Friedy, NC, USA). A radiographic study was performed to detect bone loss.

Patients were classified according to clinical and radiographic criteria into four categories, for both AP and CP:

1. Clinically healthy: PD $<$ 3 mm, AL $<$ 2 mm, PI $<$ 20%, GI $<$ 1 mm, BOP (−) and bone loss (−)
2. LP: 3 $\leq$ PD $<$ 5 mm, 2 $\leq$ AL $<$ 4 mm, PI $\geq$ 20%, GI $\geq$ 1 mm, BOP (+) and bone loss (+)
3. MP: 5 $\leq$ PD $<$ 7 mm, 4 $\leq$ AL $<$ 6 mm, PI $\geq$ 20%, GI $\geq$ 1 mm, BOP (+) and bone loss (+)
4. SP: PD $\geq$ 7 mm, AL $\geq$ 6 mm, PI $\geq$ 20%, GI $\geq$ 1 mm, BOP (+) and bone loss (+).

Inclusion criteria for patients were: Individuals of either sex at least 20 natural teeth, two sites with attachment loss in each quadrant >3 mm. For the control group, absence of periodontal disease was added.

Exclusion criteria for both the patients and the control group were: Smoking, systemic diseases, chirurgic and nonchirurgic periodontal therapy, use of antibiotics, steroidal or nonsteroidal anti-inflammatory agents, all in the 6 months prior to the study. Although smoking, medication, and systemic diseases may affect in different ways the development of the different periodontitis and are frequently associated with chronic disease, these factors were excluded. In this way in order to the cytological founds may be exclusively related to the periodontal diagnose.

Cytological analysis

Samples were collected from the soft wall of the periodontal pocket and gingival crevice of patients and control subjects, respectively, using specific curettes (Hu-Friedy, NC, USA), after removing supra-gingival bacterial plaque. Samples were obtained from the places with mostly higher periodontal indices. The samples were fixed in 96° alcohol and stained using the Papanicolau method.[13] The following parameters were observed under a trinocular microscope (Carl Zeiss Axiostar, Göttingen, Germany): Desquamation of surface and intermediate cells, polymorphonuclear neutrophils (PMN), histiocytes, and microbial flora. They were assessed semi-quantitatively as: Absent – 0; Scarce – 1(+); Moderate – 2(++); Abundant – 3(+++).

Statistical analysis

The Kruskal–Wallis test for qualitative variables was used to determine the differences between the AP and CP diagnoses. When there were differences between the groups, the ANOVA one-way test was applied, and when the differences were significant, Tukey's test was used. Data were analyzed using SPSS software (SPSS Inc., v 13.0, Chicago, IL,USA).

Results

Among the patients selected, those with AP were on average much younger than those with CP, confirming the influence of age on the characteristics of the two types of periodontitis.

For the control group, we selected individuals of ages between those of the patients with AP and CP [Table 1]. Of all the patients with AP ($n = 41$), 25% ($n = 10$) were diagnosed with LP, 27% ($n = 11$) with MP and 48% ($n = 20$) with SP. For CP ($n = 40$), 25% ($n = 10$) were diagnosed with LP, 25% ($n = 10$) with MP, and 50% ($n = 20$) with SP.

Regarding the cytological study, Figure 1 shows the surface cells and the exfoliated cells of the intermediate layer of patients with AP [Figure 1a], PC [Figure 1b] and a control individual [Figure 1c]. The first (surface cells) show increased cell volume, imprecise edges and blurry appearance of the nucleus, in a patient with periodontitis compared to the control group. The exfoliated cells are of the same size or slightly smaller than surface cells. They are rounded or polygonal, some with folded edges, slightly thicker cytoplasm than in surface cells; the nucleus is central and round. The cells in the intermediate layer are slightly enlarged in AP [Figure 1a], and CP [Figure 1b] compared to the control group [Figure 1c].

The semi-quantitative difference for surface cells was significant ($P < 0.001$) when AP was compared to CP and the control group [Figure 2]. In turn, within AP, there were significant differences ($P < 0.001$) among LP, MP, and SP. Patients diagnosed with CP showed significant differences ($P > 0.05$) between MP and the control group.

The semi-quantitative study of intermediate cells showed statistically significant differences ($P < 0.001$) between AP and CP, and between AP and the control group [Figure 3]. There was no difference ($P > 0.001$) in the quantity among groups LP, MP, and SP for patients diagnosed with AP and CP.

Figures 4a-c show PMNs and histiocytes of the soft wall of the periodontal pocket in patients with AP, CP, and control individual, respectively. The PMNs have a polymorphic nucleus and numerous granules in their cytoplasm with differential staining. Histiocytes appear as large, multi-nucleate cells. More PMNs and histiocytes are observed in AP [Figure 4a] than in CP [Figure 4b] and control [Figure 4c].

Table 1: Characteristics of patients with periodontitis and control subjects

Groups	*n*	Diagnosis (*n*)	Age (x±SE)	Sex (*n*)	
				Female	Male
Aggressive periodontitis	41	Mild (10)	24.0±9.0	5	5
		Moderate (11)		5	6
		Severe (20)		10	10
Chronic periodontitis	40	Mild (10)	58.3±7.0	5	5
		Moderate (10)		6	4
		Severe (20)		12	8
Control	40		44.0±10.0	22	18

SE: Standard error

The statistical analysis of PMNs showed no significant difference ($P > 0.001$) between AC and CP, although there were differences ($P < 0.001$) between stages LP, MP, and SP compared to the control group in AP [Figure 5a]. In CP patients, MP and SP only differed significantly ($P < 0.001$) from the control group.

Statistical analysis showed much higher values for histiocytes in CP ($P < 0.001$) than in AP [Figure 5b]. There were statistically

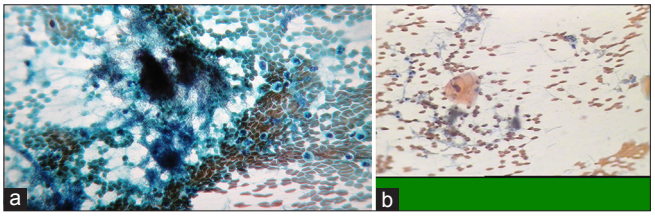

Figure 1: Surface cells and intermediate cells in patients with aggressive periodontitis (a) chronic periodontitis (b) and control group (c) ×400 Pap

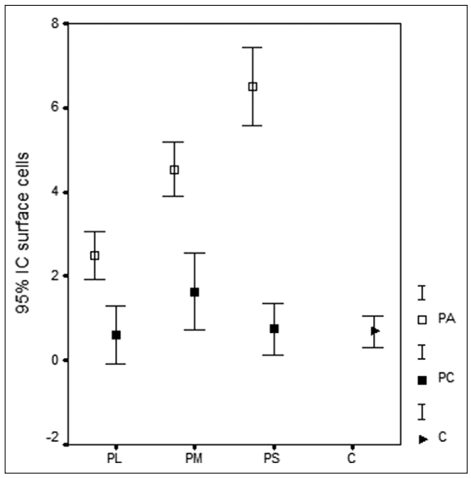

Figure 2: Semi-quantitative analysis of surface cells in patients with aggressive periodontitis, chronic periodontitis, and control group (c)

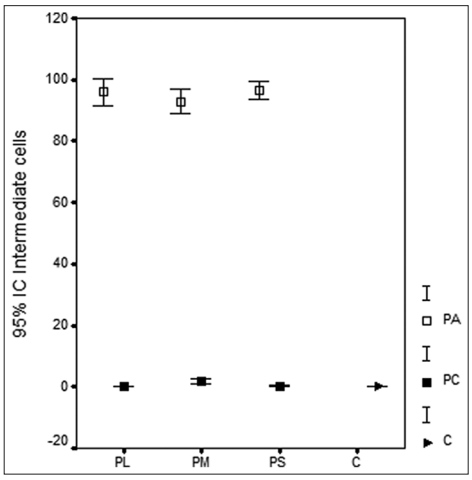

Figure 3: Semi-quantitative analysis of intermediate cells in patients with aggressive periodontitis and chronic periodontitis and control group (c)

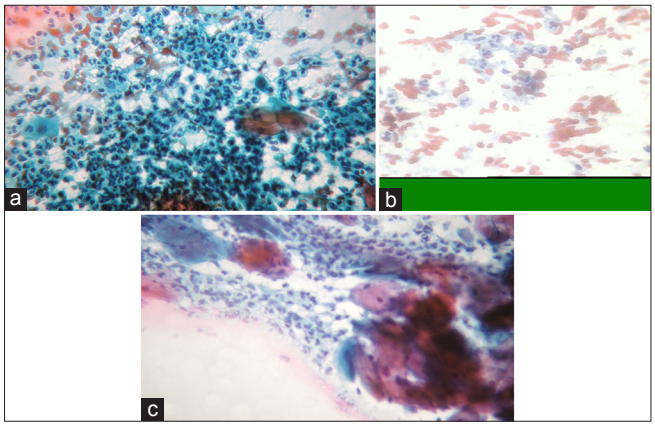

Figure 4: Neutrophil polimorhphonucleate leukocytes and histiocytes in patients with aggressive periodontitis (a) chronic periodontitis (b), and control group (c) ×400 Pap

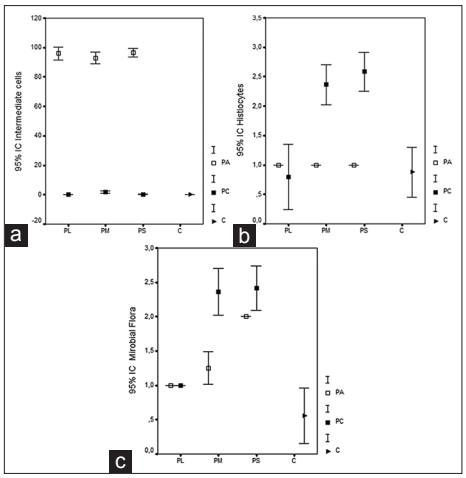

Figure 5: Semi-quantitative analysis of neutrophil polimorhphonucleate leukocytes (a) histiocytes (b) and microbial flora (c) in patients with aggressive periodontitis and chronic periodontitis and control group (c)

significant differences ($P < 0.001$) in histiocytes between patients with AP and the control group. Histiocytes in patients with CP, MP, and SP, differed significantly ($P < 0.001$) from patients with LP, and there were significant differences among all three stages and the control group.

There were statistically significant differences ($P < 0.001$) [Figure 5c] among groups LP, MP, and SP in patients diagnosed with AP, as well as between them and the control group ($P < 0.001$). In patients with CP, MP, and SP differed significantly ($P < 0.001$) from LP and the control group.

Regarding sex and age of patients, no significant difference ($P > 0.001$) was found in the number and type of exfoliated cells or microbes (results not shown).

Discussion

The extent to which the tissues are affected determines the LP, MP, and SP clinical pictures. These pictures, as well as gingivitis, can be studied biochemically, cytologically, and histologically using specific markers. Exfoliative cytology, which is a straightforward noninvasive diagnostic method that can be applied during inflammation, can be considered as a more practical technique to evaluate the oral mucosa. It collects superficial desquamated cells, which can easily be analyzed microscopically. Desquamation of gingival epithelium depends on mitotic activity of basal cells, their enzyme processes, and mechanical irritations of gingival tissue. In recent years, exfoliative biopsy has been introduced with good results in specification and diagnosis of the lesions buccales. A few studies have used exfoliative cytology to evaluate changes in the oral mucosa in gingival inflammation and periodontal disease.[14]

In periodontal inflammatory processes, there is a reduction of keratinization. Lange 1965 suggests an increase in the young cells in the deepest layers of the epithelium such as intermediate and prepyknotic cells, reducing the number of cells containing keratin particles. According to that study, there is a direct relationship between the degree of inflammation and reduction in keratinization.[15] Endo *et al*. 2008, found a reduction of keratinization in superficial inflammations of the gingival. They assume that this enables gingivitis to be diagnosed using cytological methods.[16]

Our study found more superficial and intermediate cells in patients with AP than in patients with PC and the control group. These results differ from those of other authors, who found fewer of these cells in patients with chronic periodontal disease.[17] The presence of intermediate cells in the proportion in which they appear in these studies might indicate the onset of periodontal disease in the preclinical, as yet asymptomatic stage.

Many authors conclude that exfoliative cytology has a certain value in the diagnosis of precancerous lesions, and particularly in determining their prognosis.[18-20] There is currently no cytological study on chronic and aggressive periodontal disease; nevertheless, our research has shown that the cytological study of the soft wall of the pocket shows tissue damage as an increase in inflammatory cells, cells from deep layers of the epithelium, and disease-related microorganisms.

Some studies have shown cell changes in the oral mucosa of patients with systemic diseases such as endocrine disorders and respiratory diseases.[19,20] Other researchers have found cytomorphometric changes in the cells of the oral mucosa of smokers, similar to those found in diabetics, caused by the chronic inflammation of the oral mucosa. Changes in the oral mucosa similar to those appearing in individuals with type 2 diabetes have also been found in patients with Vitamin B12 and folic acid nutritional deficiencies.[21-23]

Histological studies on tissue samples show significant differences in the percentage of collagen fibers and type of cell population such as histiocytes and PMNs, in patients with periodontal disease and gingivitis compared to healthy individuals, and also between both groups of patients.[23] Our study showed a high level of histiocytes in MP and SP for CP compared to the control group, as expected, since they are immune cell types that protect the body against infection.

Our results showed higher values for PMNs and microbial flora in patients with CP and AP than in the group of healthy patients, providing evidence of the cellular immune system defense in these groups of patients. Although the control group was made up of clinically healthy individuals, cytological examination showed the presence of PMNs, histiocytes and microbial flora, due to the fact that under normal conditions, the gingival have an inflammatory infiltrate that makes up about 5% of the volume of the connective tissue.[23]

The analysis considering sex and age showed no significant difference in exfoliated cells, coinciding with the results of Séguier *et al*. 2000.[23] Due to the presence of ulceration and mucosal erosion present in stomatitis and gingivitis, squamous cell layer is partially or completely replaced by cells from deeper layers. These cells may vary in size and shape, being mostly increased in their nuclear size, with multiple ovoid nuclei and poorly conserved cytoplasm.[24]

The use of exfoliative cytology for obtaining samples on which to apply sophisticated diagnosis techniques, cytomorphometry, analysis of DNA content, and molecular analysis seems to be gaining ground as a reliable method for the diagnosis of oral cancer in its earliest stages. The main advantages of exfoliative cytology are that the technique is rapid, relatively painless, easy to perform allows repeated sampling of biological material without destroying tissue integrity, which means it can be performed repeatedly in preventive screening programs and during routine dental examinations.[13]

Cytological studies of the periodontal pocket of patients with clinically diagnosed AP and CP had greater tissue damage as shown by the increase in cells from the deep epithelium layers, inflammatory cells, and microbial flora compared to the group of healthy subjects. Patients with AP had more surface and intermediate epithelium cells from the periodontal pocket than CP patients and the group of healthy subjects. In patients with AP, surface cells increased with the severity of the disease.

Conclusions

The cytological study showed that patients with AP had greater tissue damage, shown by the increase in intermediate and superficial cells of the epithelium of the periodontal pocket compared to the group of healthy subjects and to a lesser extent, to patients with CP. Only superficial cells made it possible to differentiate the sub-stages of the disease.

References

1. Page RC. The pathobiology of periodontal diseases may affect systemic diseases: Inversion of a paradigm. Ann Periodontol 1998;3:108-20.
2. Ebersole JL. Humoral immune responses in gingival crevice fluid: Local and systemic implications. Periodontol 2000 2003;31:135-66.
3. Castro CE, Koss MA, López ME. Biochemical markers of the periodontal ligament. Med Oral 2003;8:322-8.
4. Armitage GC. Analysis of gingival crevice fluid and risk of progression of periodontitis. Periodontol 2000 2004;34:109-19.
5. Lindhe J. Examination of patients with periodontal disease. In: Clinical Periodontology and Implant Dentistry. 3rd ed.. Copenhagen: Munksgaard; 1998, p. 383-95.
6. Tonetti M, Mombelli A. Periodontitis precoz. In: Lindhe J, Karring T, Lang N, editors. Periodontología Clínica e Implantología Odontológica. 3rd ed.. Madrid: Ed Médica Panamericana; 2000. p. 229-60.
7. Tonetti MS, Mombelli A. Early-onset periodontitis. Ann Periodontol 1999;4:39-53.
8. Ogden GR, Cowpe JG, Green MW. Quantitative exfoliative cytology of normal buccal mucosa: Effect of smoking. J Oral Pathol Med 1990;19:53-5.
9. Diniz-Freitas M, García-García A, Crespo-Abelleira A, Martins-Carneiro JL, Gándara-Rey JM. Applications of exfoliative cytology in the diagnosis of oral cancer. Med Oral 2004;9:355-61.
10. Montgomery PW. Study of exfoliative cytology of normal human mucosa. J Dent Res 1951;30:12-6.
11. Silness J, Löe H. Periodontal disease in pregnancy. II Correlation between oral hygiene and periodontal conditions. Acta Odontol Scand 1964;22:121-35.
12. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
13. Belgaumi U, Shetty P. Leishman Giemsa cocktail as a new, potentially useful cytological technique comparable to Papanicolaou staining for oral cancer diagnosis. J Cytol 2013;30:18-22.
14. Obradović R, Kesić L, Mihailović D, Jovanović G, Antić S, Brkić Z. Low-level lasers as an adjunct in periodontal therapy in patients with diabetes mellitus. Diabetes Technol Ther 2012;14:799-803.
15. Lange D. Comparative histologic and cytologic studies on the human gingival marginal tissue. Dtsch Zahnarztl Z 1965;20:1035-47.
16. Endo H, Rees TD, Kuyama K, Matsue M, Yamamoto H. Use of oral exfoliative cytology to diagnose desquamative gingivitis: A pilot study. Quintessence Int 2008;39:e152-61.
17. Sugerman PB, Savage NW. Exfoliative cytology in clinical oral pathology. Aust Dent J 1996;1:71-4.
18. Jones AC, Pink FE, Sandow PL, Stewart CM, Migliorati CA, Baughman RA. The cytobrush plus cell collector in oral cytology. Oral Surg Oral Med Pathol 1994;1:95-9.
19. Alberti S, Spadella C, Francischone T, Assis G, Cestari T, Taveira L. Exfoliative cytology of the oral mucosa in type II diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 2003;32:538-43.
20. Pérez-Sayánsm M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara-Vila P, Gándara Rey JM, *et al*. Exfoliative cytology for diagnosing oral cancer. Biotech Histochem 2010;85:177-87.

21. Zimmermann ER, Zimmermann AL. Effects of race, age, smoking habits, oral and systemic disease on oral exfoliative cytology. J Dent Res 1965;44:627-31.
22. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO. The effect of tobacco smoking and of betel chewing with tobacco on the buccal mucosa: A cytomorphometric analysis. J Oral Pathol Med 1999;28:385-8.
23. Séguier S, Godeau G, Brousse N. Immunohistological and morphometric analysis of intra-epithelial lymphocytes and Langerhans cells in healthy and diseased human gingival tissues. Arch Oral Biol 2000;45:441-52.
24. 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.

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