ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The value of exfoliative cytology in the diagnostic of oral mucosa changes in diabetes mellitus

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Abstract

In this study, we performed microscopic qualitative analyses of the oral epithelium cytological smears in potential early phase of diabetes and in type 1 and 2 diabetic patients *versus* a healthy control group. The cytological assessment of the oral changes was realized on superficial and profound smears, from jugal and ventral tongue mucosa and it was based on the comparison between three staining methods [Papanicolaou, APT (polychrome tannin blue)-Drăgan and Hematoxylin–Eosin (HE)]. Cytological changes of oral cells population were correlated with the type, duration and complications of diabetes. Oral flora was also evaluated. Irrespective the staining used, we found a clear dividing line between the control group and the real diabetic patients. In all diabetes cases (independently of the type of smear, harvest site, clinical form of disorder and present complications), cells presented alterations both at the level of cytoplasm and nucleus. Dyschromasia, cytolysis, different degrees of fatty degenerescence, binucleated cells, hyperchromasia, nuclear enlargement with modified nuclear/cytoplasmic ratio, were the most frequent findings. There were no discrepancies in the cellular aspects of type 1 or 2 diabetic patients' smears or between the control group and the potential prediabetic status patients. Findings were interpreted as oral epithelium reactive changes induced by the disease. We concluded that exfoliative cytology alone is of low value as a diagnostic and prognostic tool in the diagnosis of diabetes mellitus (DM); it detects the reactive changes induced by the disease, but it makes no differences between DM types or degree of severity and does not allow by qualitative analysis alone to detect abnormalities in early diabetes.

Keywords: diabetes mellitus, oral exfoliative cytology, cytological staining, squamous cell alterations.

Introduction

Diabetes mellitus (DM), one of the oldest diseases known to man (firstly reported in an Egyptian manuscript about 3000 years ago [1]) is a common disorder with great morbidity and mortality [2]; in 2012, it represented the eighth leading cause of death among both sexes and the fifth leading cause of death in women [3]. The global prevalence of diabetes is increasing at an amazing pace (more than 151% until 2030 according to *World Health Organization* [WHO] estimation [2]) due to many factors, mainly aging, obesity and physical inactivity [3]). The human and financial costs (around 4000 $ per person per year) of the disease cannot be underestimated [3] and any precocious detection technique represents therefore an important medical target.

DM represents a combination of heterogeneous disorders with episodes of hyperglycemia and glucose intolerance (result of insulin absence, defective insulin action, or both [4], so the current clinical practice in diagnosing the disease is the use of the derived parameters: fasting plasma glucose level ≥126 mg/dL, or 2-hour plasma glucose level ≥200 mg/dL, during an oral glucose tolerance test [5]. The introduction of urine strips and glucometers made the disease more easy to detect and more manageable for the patient [1].

It is well known that lesions of oral mucosa may be representative clues in different systemic diseases (leukemia, lues) [6]. The clinical manifestations of DM in oral cavity are numerous (xerostomia, burning mouth syndrome, halitosis, dental caries, infection and inflammation: periodontal disease, periapical abscesses, gingivitis and candidiasis [7–10]. However, microscopic researches regarding specific cytological modifications of oral cells in diabetes are not much investigated. Over time, very few assumptions [7, 11, 12] were made about this subject. To emphasize these changes might be a possible alternative method for *early diabetes diagnostic*, providing the possibility of exfoliative cytology implementation in public health programs [7, 13]. The more so, as, according to Drăgan-Lungulescu, diabetes is accompanied by oral changes that can even precede the classic symptomatic triad of polyphagia, polydipsia and polyuria [13]. The application of oral exfoliative cytology as a non-aggressive and quick method of diagnostic is therefore more appropriate in DM than is biopsy (the most frequent technique used for the diagnosis of oral cavity lesions), considering the risk of infection and the reduced healing capacity demonstrated in this disease [7].

Classification of DM is based on etiology and presentation: type 1, insulin-depending diabetes, is secondary

to autoimmune mediated destruction of β cells, resulting in insulin deficiency [7]; type 2, non-insulin dependent, familial type, is a component of metabolic syndrome, resulting from target tissue resistance to insulin [4, 7, 12, 14]. The long-term disease (especially type 1 DM) is associated with different aggravating conditions. The major, microvascular complications of DM (diabetic retinopathy and nephropathy), implying a severe form, arise due to ischemia [15], and derangements in the regulatory systems of carbohydrates, lipids and proteins storage/mobilization [4]; over time, high glucose concentrations promote the formation of non-enzymatic advanced glycosylated end products with cell damage [16]; different levels injured cells suffered morphological changes that, accordingly, should be correlated with the severity of the disease [16].

The composition and role of normal microbial flora of the oral cavity have been attested by many studies [8, 17–20]. The oral cavity forms an indispensable part of the human microbiome, due to its unique and diverse microflora distributed in various niches (periodontal crevice and pockets, tongue dorsum and other mucosal surfaces) [20]. The mouth harbors at least six billion bacteria representing more than 700 species [18, 19]. Diabetes is associated with oral candidiasis [8] and oral inflammations [10], which in all could damage the mucosa, thus promoting cellular alterations.

The aims of the study were complex: to assess morphological alterations of diabetic oral mucosa squamous cells by means of three cytological staining methods: Papanicolaou, APT (polychrome tannin blue)-Drăgan and Hematoxylin–Eosin (HE); to compare the reliability of these methods in emphasizing any possible characteristic features related to the disease and interpreted as a pertinent pathognomonic element; to correlate these possible alterations to DM type, duration (early) and evolution (mild or complicated forms); finally, to speculate the role of DM metabolic imbalance in excessive bacterial colonization of the oral mucosa.

Materials and Methods

We investigated 30 adult patients: 10 apparently healthy (the potential prediabetic status patients; their glycemia values were at the upper limit, ≥120 mg/dL, and they had a family history of diabetes, mainly type 2) and 20 with both DM forms (type 1 and type 2), in different stages of the disease: 10 cases of non-insulin dependent diabetes (NIDD) – five mild forms and five complicated forms, and 10 cases of insulin-dependent diabetes (IDD) – five mild forms and five complicated forms. The diabetic patients were either taking oral hypoglycemic agents or a combined treatment containing also insulin. The duration of the disease ranged from three to 30 years. All patients were selected from the Clinical Center of Diabetes, Nutrition and Metabolic Diseases (Emergency County Hospital, Cluj-Napoca, Romania).

The control group consisted of 30 healthy individuals (with glycemia values <120 mg/dL, and no personal history of diabetes).

Biochemical and hematological measurements were done to exclude other systemic diseases. Smokers, alcoholics, persons with oral lesions, gingivitis and periodontal disease were excluded from all groups, to prevent all side effects that these injuries could determine on oral mucosa.

Each patient consented to a protocol approved by the Medical Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca. Name, age, gender and relevant medical history were completed in a pro forma inventory. All the investigated persons were young people, with an age ranged from 20 to 35 years old.

In order to evaluate the cellular alterations induced by DM, exfoliative cytology was used for the analysis of oral mucosa.

The patients gargled with normal saline and the oral cavity was dried with a gauze swab to remove saliva in excess and surface debris. Cells were collected by superficial and profound brushing of the jugal and lingual (ventral tongue) mucosa, relatively distant sites in oral cavity (in an attempt to prevent the extension of a possible local inflammation). Ten gently (to avoid bleeding) scratching rotations of each patient mucosa with a Cytobrush® (Figure 1a) were realized. This type of brush is frequently used, being an adequate tool for oral cytology; it facilitates the sampling, with good results in terms of obtained cells number, quality and uniform distribution on slides. Four major types of smears (120 slides) were obtained: superficial and profound jugal smears and superficial and profound lingual ones, consequently divided among the type of patients (non-diabetic, possible prediabetic, type 1 and type 2 diabetic) and clinical forms (mild or complicated). Identical cellular fields were harvested together by the brush translation movement between the slides (Figure 1b). The cells were immediately spread on a glass slide and fixed with 96% solution of ethylic alcohol. Due to their great number, the slides were labeled with a code identifying each investigated group. After drying, smears from all cases were colored using three techniques: Papanicolaou, APT-Drăgan and HE (the study was finally done on 360 slides).

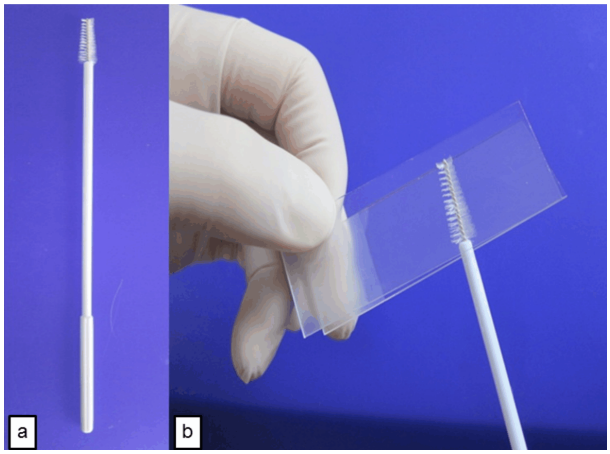

Figure 1 – ***(a) The Cytobrush®; (b) Smearing of the cells onto the glass slides.***

Papanicolaou polychromatic coloration is the most popular staining for cytology, especially for cervical and

vaginal smears [21]. It can be performed automatically or manually, according to the steps summarized in Table 1.

Table 1 – *Papanicolaou staining steps*

Isopropyl alcohol	3 minutes
Rinse	3 minutes
Hematoxylin solution	3 minutes
Rinse	2 minutes
Isopropyl alcohol	40 seconds
Orange G	3 minutes
Isopropyl alcohol	30 seconds
EA 50	3 minutes
Isopropyl alcohol	30 seconds
Absolute alcohol	30 seconds
Drying and mounting of the smear with a low pH Canada balm.	

When performed properly, the Papanicolaou-stained specimen should display hues from the entire spectrum of colors. The cell nuclei are blue to black. Cells with a high content of keratin are yellow. Thus, superficial cells are orange to pink; intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink [21].

APT-Drăgan is an original cytological Romanian staining technique [13], currently performed in our country for over 20 years [22, 23]. It has a good affinity for squamous epithelium and a strong correlation with Papanicolaou method [13].

The main technical steps of APT-Drăgan staining are summarized in Table 2.

Table 2 – *APT-Drăgan staining steps (Drăgan-Lungulescu, 2004 [13])*

Polychrome blue solution	15 seconds
Washing with tap water or distilled water	15 seconds
1% Tannin solution differentiation	15 seconds
Drying and mounting of the smear with a low pH Canada balm.	

APT-Drăgan staining highlights all the components of the oral epithelial cells: the nucleus is stained in blue, the nucleoli are blue-violet or pink and the intranuclear inclusions staining in pale blue. This method divides the cells in four types, depending on the cytoplasmic staining: alpha (pink staining), beta (pale blue to green), gamma (combination of pink and pale blue to green) and delta (clear cytoplasm, no chromatic affinity); the cytoplasm tinctoriality is a mirror of the cell functional state at that specific moment [13].

Hematoxylin–Eosin (HE) is the principal staining in histology, being widely used for medical diagnosis, in tissue specimens. From a technical point of view, is the most easy staining to perform, requiring only the two solutions (working solution of Hematoxylin and Eosin), tap water and a mounting stand [24]. We used it as a gold standard, comparatively to the other two typical cytological stains.

Cytological assessment on the identical cellular fields, either on superficial or profound smears, was similar compared based on the comparison between the elements provided by the three staining methods (overlapping cellular details, identified specific features). Cytological changes of oral cells population were correlated with the type, duration and complications of diabetes. Oral flora was also evaluated.

Results

According to the Bethesda System 2001 for cytology reporting and taking into consideration the smear total cell number, all specimens in our study were satisfactory [21]. The predominant cells in both type of smears and both sites of harvest were of superficial and intermediate type (rare parabasal cells were found in profound smears), with similar morphology. All stainings outlined the epithelial cells and their nuclei (Figure 2).

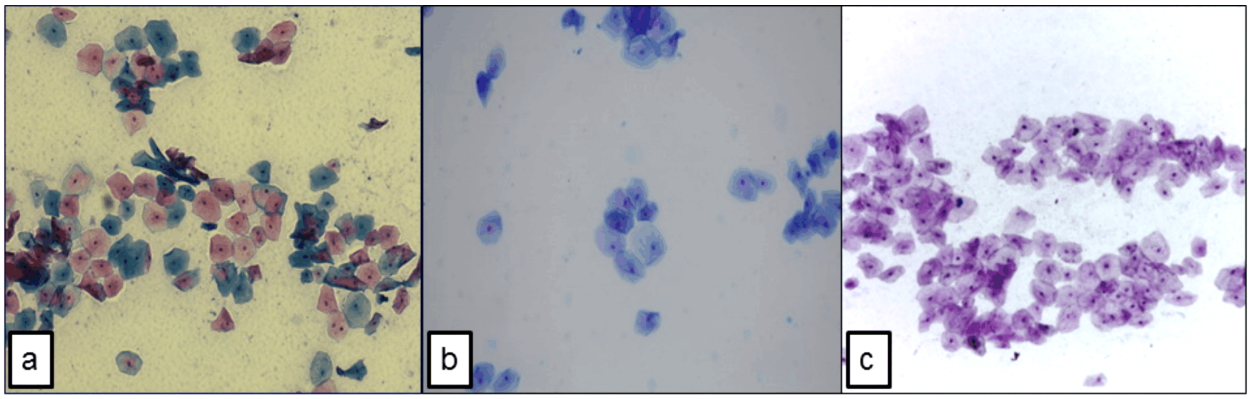

Figure 2 – *Normal oral smear: (a) Papanicolaou staining, ×200; (b) APT-Drăgan staining, ×200; (c) HE staining, ×200.*

In order to be assessed, the alterations of diabetic oral squamous cells were grouped by staining.

Papanicolaou staining

The cells in Papanicolaou staining were highlighted according to the layer they originate in. Most of them were superficial (orange colored, due to their content of keratin), with only a few intermediate elements (stained in green), and rare parabasal cells (stained in green-blue) in profound harvested specimens.

There were no important differences between the control group and potential prediabetic status patients regarding the cells phenotype. The nuclei of epithelial cells were in both cases small and compact with an adequate nuclear/cytoplasm ratio (Figure 3).

In mild forms of diabetic patients, intracytoplasmic

granulations of keratohyalin could be properly highlighted (Figure 4, a and b), but in complicated forms their number decreased.

Superficial binucleated cells were present in clusters (Figure 4c). A higher nuclear/cytoplasmic ratio was found along with nuclear dystrophy changes in almost all cases of DM (Figure 4d). Most of the nuclei were pale and uniformly stained, but focal hyperchromasia could also be encountered.

In all five cases of uncomplicated DM, type 1 and 2, the cells were discolored, with lipid droplets (Figure 4d). In three cases of complicated type 1 DM, superficial cells displayed a clear perinuclear ring, karyorrhexis and karyolysis (Figure 4e).

APT-Drăgan staining

The results obtained with this staining parallel the cellular findings obtained with Papanicolaou method.

In the control group and potential prediabetic status patients, the cytological aspects were normal, in both types of smear, and both sites of harvest. The nuclei were small and compact, with an adequate cytoplasmic/nuclear ratio (Figure 5).

In all diabetic patients, fatty degenerescence of the cytoplasm and nuclear changes were found.

The mild forms were characterized by the presence of peripheral cytoplasmic granulations, fatty degenerescence of the cytoplasm, binucleated cells, and higher nuclear/cytoplasmic ratio in contrast to small, dystrophic nuclei.

In *complicated forms of type 2 DM*, an increased number of α and β cells of patients presented cytolysis and consecutively, free nuclei. Lipid vacuoles were also identified in the cytoplasm of these patients; some cells had only small lipid droplets, why others had large lipid vacuoles, occupying a large amount of the cell volume. These modifications are accompanied by the fading of the nuclear membrane (mostly in β and γ cells) or hyperchromasia.

In *severe type 1 DM*, the cellular changes were more important; both types of smears presented cells with dyschromasia, lysis, vacuolization. The number of keratohyalin granules was decreased.

The lipid droplets seemed to be bigger, irregular; initially situated perinuclear, they extend to the rest of the cytoplasm, or form large vacuoles. The nuclei alterations (fading of the nuclear membrane, chromatic condensation, karyorrhexis, karyolysis) were in great number (Figure 6).

Hematoxylin and Eosin staining

With the cytoplasm and its components colored in pink-red, there were no cellular differences in terms of the originating layer. The nuclear chromatin was highlighted at high resolution.

No differences were found between the control group and the potential prediabetic patients.

In *all diabetic* patients, eosinophilic intracytoplasmic granules along with keratohyalin granules (larger, stronger and pink-red stained) have been observed (Figure 7, a–c).

The cells displayed a variety of chromatin condensation, ranging from mild (pale blue nucleus) to aggregates of chromatin just below the nuclear membrane (in complicated forms of type 1 DM). Intranuclear clear inclusions and binucleated cells were also found.

A number of two jugal superficial smears of *complicated type 1 DM* displayed a strong inflammation, consisting of neutrophils (predominantly) and lymphocytes (Figure 7d).

Microbial flora

The control group and the potential prediabetic status patients did not present a reach microbial flora on the smears.

Directly linked with the severity of DM, excessive microbial flora (consisting of cocci, diplococci, bacilli, diplobacilli) was demonstrated by all three stains. No fungal mycelia were seen.

In *mild forms of DM types*, isolated epithelial cells were completely covered in bacteria (especially cocci), also displaying enlarged nuclei, without suspicious elements of dysplasia.

In *complicated forms of type 1 DM*, three smears revealed clusters of epithelial cells covered in cocci or bacilli, associated with neutrophils (Figure 8).

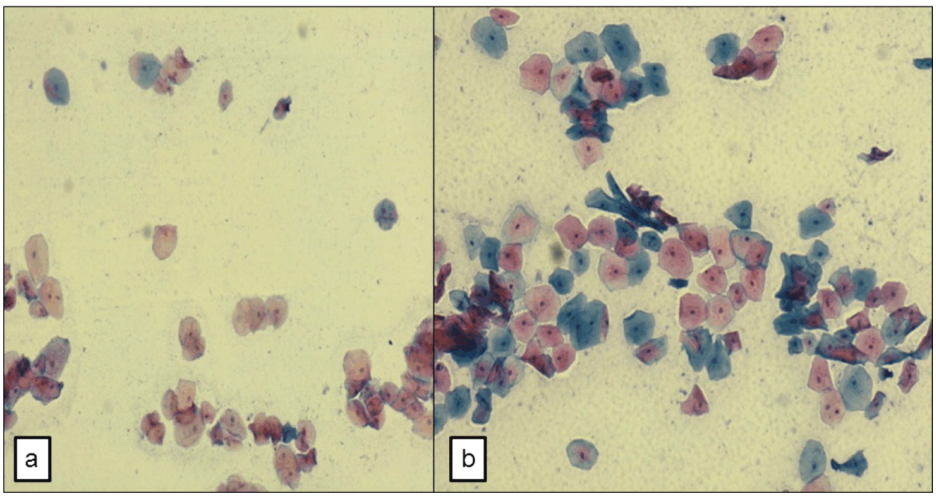

Figure 3 – ***(a) Superficial jugal specimen in control group; (b) Superficial lingual specimen in possible prediabetic status patient. Papanicolaou staining, ×200.***

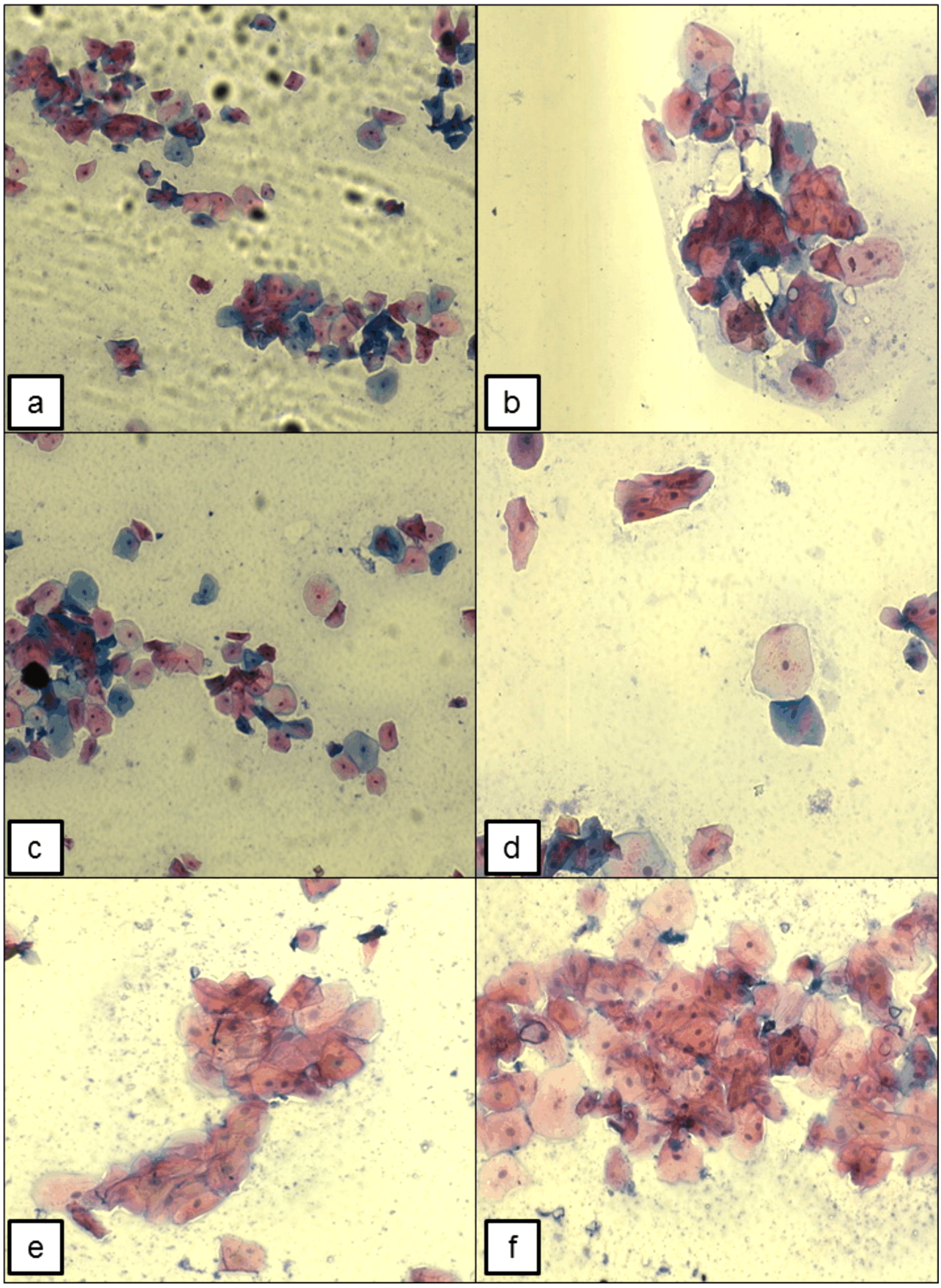

Figure 4 – ***Cell changes in Papanicolaou staining: (a) Intracytoplasmic granulations (type 2 DM, mild form, superficial jugal, ×100); (b) Intracytoplasmic granulations (type 2 DM, mild form, superficial jugal, ×200); (c) Rare binucleated cells (type 1 DM, complicated, superficial jugal, ×200); (d) Pale nucleus staining, with a higher than normal nuclear/ cytoplasmic ratio (type 2 DM, complicated form, superficial jugal, ×400); (e) Nuclear enlargement, along with small, fading nuclei, binucleation, and bacterial flora (type 1 DM, complicated, superficial jugal, ×400); (f) Discolored cells with lipid droplets; the nuclei are modified (binucleation, focal hyperchromasia, dystrophy, higher nuclear/cytoplasmic ratio); excessive bacterial flora (type 1 DM, uncomplicated, profound lingual, ×400).***

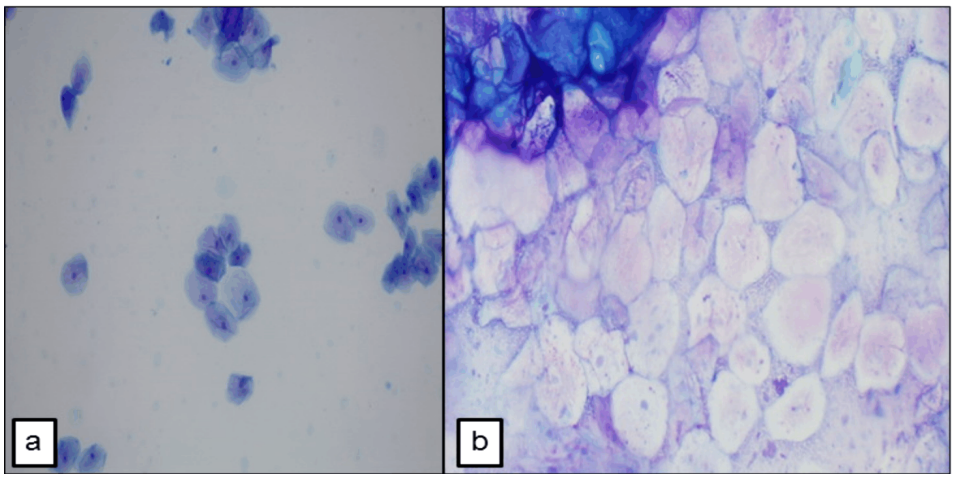

Figure 5 – ***Normal smear in APT-Drăgan staining, superficial jugal: (a) ×400; (b) ×1000.***

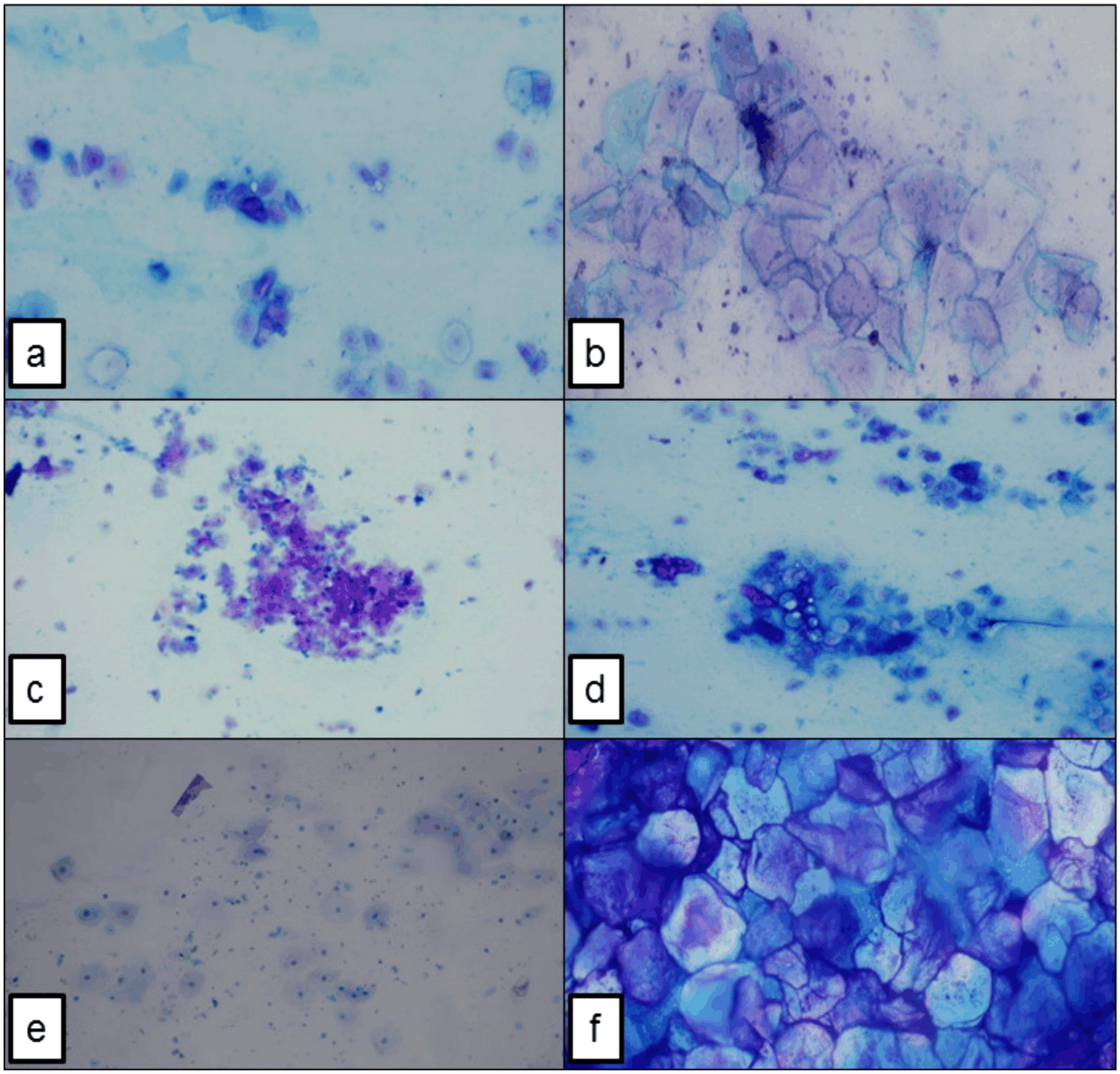

Figure 6 – ***Cell modifications in APT-Drăgan staining: (a) Lipid droplets (type 1 DM complicated form, jugal superficial, ×200); (b) Dystrophic nuclei (type 1 DM, mild form, jugal superficial, ×400) (c) Alpha and beta cells with loss of cellular membrane and free nuclei (type 2 DM, complicated form, jugal superficial, ×100); (d) Large lipid droplets (type 2 DM, complicated form, jugal profound, ×100); (e) Cytolysis and free nuclei (type 1 DM, mild form, lingual superficial, ×200); (f) Beta/gamma intermediary cells, with orthochromatic cytoplasm, type I granulations or agranulated cells and pyknotic or loss of the nuclei (type 1 DM, complicated form, jugal, ×400).***

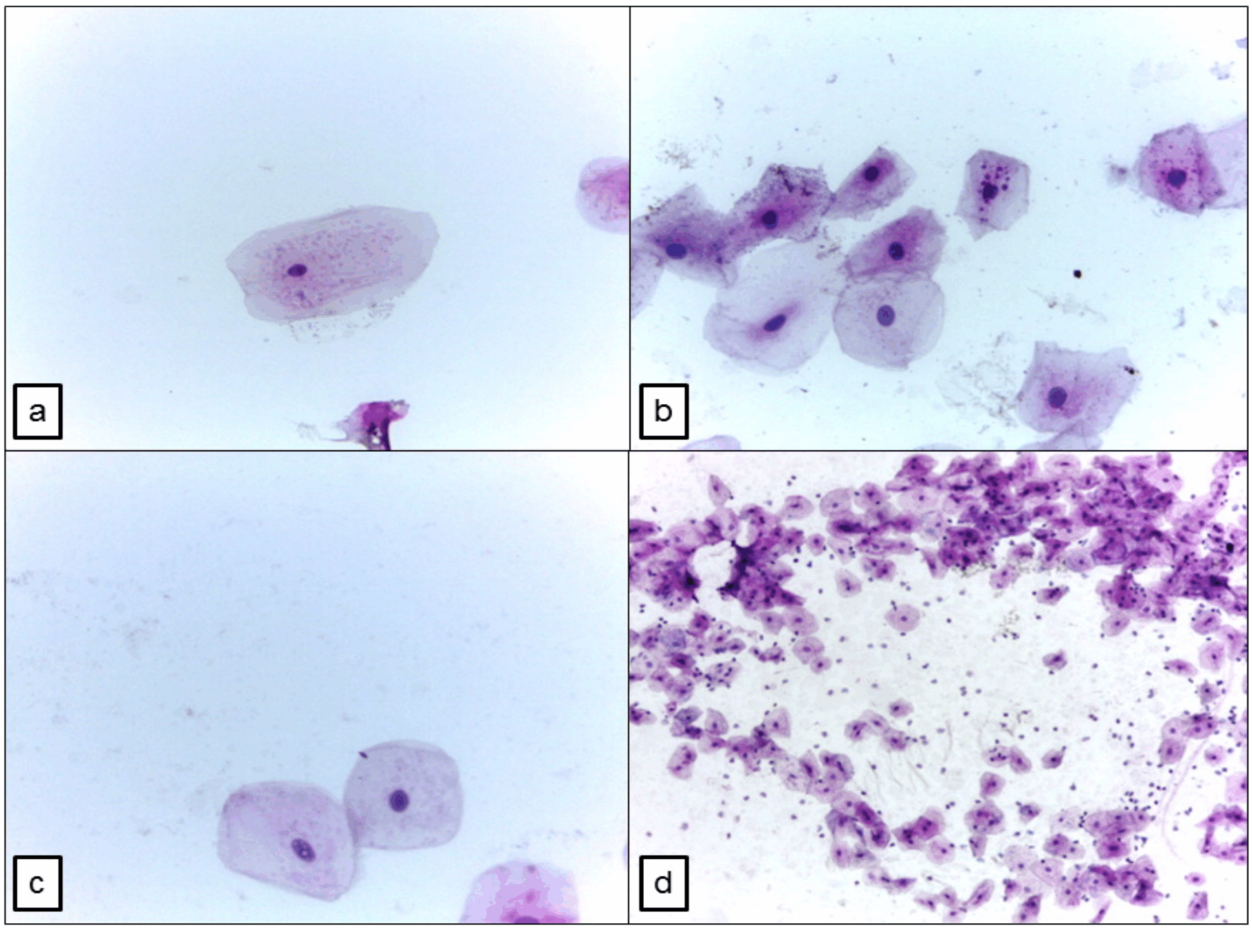

Figure 7 – ***Cells changes in HE staining: (a) Eosinophilic intracytoplasmic granules (jugal superficial, ×400); (b) Intranuclear clear inclusions (jugal superficial, ×400); (c) Keratohyalin granules (jugal superficial, ×400); (d) Inflammatory cells along with epithelial cells (type 1 DM, complicated, jugal superficial, ×100).***

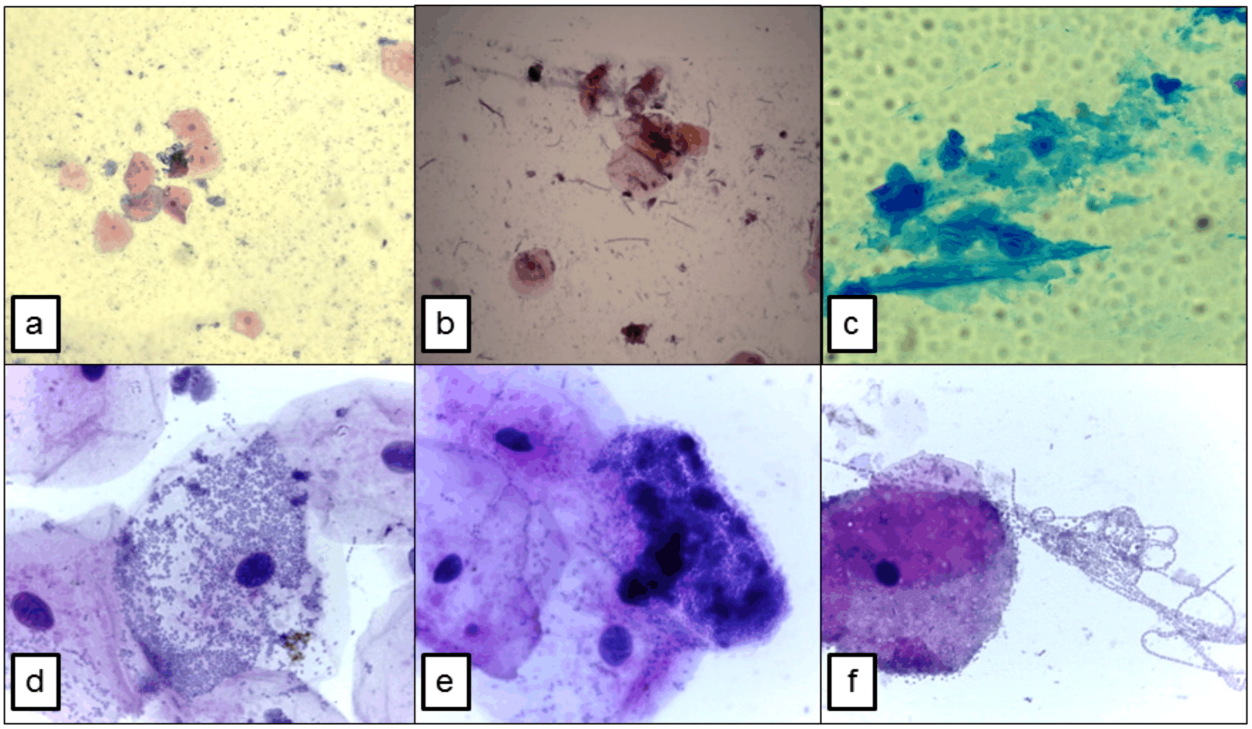

Figure 8 – ***Microbial flora seen on oral smears: (a) Mixed flora composed of cocci and bacilli (Papanicolaou staining, ×100); (b) Lactobacilli (Papanicolaou staining, ×200); (c) Various microbacterial types – cocci, diplococci, diplobacilli (APT-Drăgan staining, ×100); (d) Coccobacilli covering an epithelial cell (HE staining, ×400); (e) Micrococcus sp. colony (HE staining, ×1000); (f) Strands of Streptococcus sp. and Micrococcus sp. covering an epithelial cell (HE staining, ×1000).***

Discussion

The current use of exfoliative cytology in gynecology diagnostic, prevention and control of cervical cancer, have determined increasingly more authors to investigate the role of this method in detecting and monitoring oral mucosa changes driven by cancer [15] or systemic diseases [12, 25].

Exfoliative cytology is a non-invasive clinical technique that has the advantage to be relatively simple, rapid, non-aggressive, painless and anesthesia free [7, 15]. In oral cavity, it allows a fairly reliable assessment of suspicious lesions, it is well tolerated by patients and has no side effects [7, 15]. Any general practitioner, pediatrician or dentist could perform this method during routine oral examination [7, 15], so it has great potential to be developed as a screening procedure for national programs in many pathologies [7, 12, 15].

DM is fastly expanding and nearly one-third of cases are undiagnosed [7, 12]. Additional tools that can improve the rate of its detection can only be welcome. Since literature data do not provide much information about the oral changes in DM [7, 26], the main purpose of this research was to investigate these alterations and to emphasize the relevance of exfoliative cytology in the diagnosis.

In this study, we performed microscopic qualitative analyses of the oral epithelium cytological smears in potential early phase of diabetes and in type 1 and 2 diabetic patients.

Different cellular features were observed, that well correlates with other authors reports [7, 12, 15, 27]. Irrespective the staining used, we found a clear dividing line between the control group and the real diabetic patients. In all diabetes cases (independently of the type of smear, harvest site, clinical form of disorder and present complications), cells presented alterations both at the level of cytoplasm and nucleus. Dyschromasia, cytolysis, different degrees of fatty degenerescence, binucleated cells, hyperchromasia, nuclear enlargement with modified nuclear/cytoplasmic ratio, were the most frequent findings. There were no discrepancies in the cellular aspects of type 1 or 2 diabetic patient's smears, that was also similar with other studies [7, 12, 15]. However, in the severe, complicated forms of type 1 diabetes cases, the frequency of these alterations was higher onto the microscopic fields. Like in other research [7, 12, 15], all these findings were interpreted as oral epithelium reactive changes induced by the disease. The more so, as in order to avoid interferences, all patients included in the study were thoroughly chosen and the cytological smears were prepared from two different regions of oral cavity (like other research protocols demanded [7, 15]).

Literature data mention differences in the number of nuclear changes (karyorrhexis) or cytoplasm vacuolization between the smears harvested from the tongue and other oral cavity regions that were not visible in our study. The cause may be the different impact of hyperglycemia relative to the various locations [15]. With spectral cytopathology it was demonstrated that cells from the tongue and the floor of buccal mucosa possessed different biomolecules and a different biochemistry compared to other oral sites [15].

In order to improve the value of cytology and the quality of diagnostic, in most literature studies [7, 12, 15, 27] researchers used quantitative methods like cytomorphometry; yet, their more objective results were in conformity with our findings; the parameters involved were nuclear area (NA), cytoplasmic area (CA) and nuclear/cytoplasmic ratio (N/C); there were statistical significant differences between healthy subjects and diabetic patients but no statistical significant differences between type 1 and 2 diabetic patients [7, 14, 15]. In most of these papers, cellular polymorphism was reported, the decrease in size of nucleus and cytoplasm or in the number of cytological diagnostic layers being related to the atrophic changes observed in diabetes and clinically emphasized by the possible formation of superficial ulcerations or other lesions [28]. The oral epithelium atrophy in diabetic patients was also revealed in our research by the small, fading nuclei, and the decreased number of keratohyalin granules (connected to a delay in recovery process of keratinizing cells of this area following systemic illnesses [12]. Moreover, in both locations, the parabasal cells on the smears were rare, fact mainly interpreted as an arrest in cells proliferation.

Until now, no other study in literature took into consideration a group of potential prediabetic status patients. Cells alterations observed in this group could have been served as DM early diagnosis and would have represented an important aspect of health care. However, in our study, there were no differences in the aspect of oral smears compared to the control group.

Regarding the three stainings used in our research, even if they stained cells and their components in a suitable manner, they could not distinguish between the types of diabetes or its uncomplicated/complicated forms. However, each of them proved its reliability in highlighting specific components and alterations, which makes their association beneficial for research and a complete investigation of oral mucosa cells. The use of Papanicolaou staining brings the advantage of discriminating cells by the layer they originate in. Most of the cells examined on our smears were of superficial origin, so most alterations were seen in this type of cells. It was the only staining that evidentiated the clear perinuclear rings in the complicated forms of type 1 DM and obviously highlight the parabasal layer cells. APT-Drăgan method proved efficient in emphasizing lipidic components of the cytoplasm, regardless of size, which was relevant in estimating the degree of cell degenerescence. It is also the only method that selectively stains intracytoplasmic granulations, peripheral cytoplasmic granulations being found, in relation with the cell maturation degree and differentiation [13]. Nuclear changes as pyknotic nuclei could also be easier assessed using this staining. HE staining emphasized best the variability of chromatin disposition inside the nucleus. Various arrangement of the chromatin was seen in the examined cells, from uniform stained nuclei to aggregates of chromatin. The small eosinophilic cytoplasmic granulation and the clear intranuclear inclusions could be observed only by this technique and interpreted in the context of reactive changes. Inflammation, present in few cases was strongly evidenced by this staining method.

One of the goals of our research was to outline if

possible, a pertinent, pathognomonic marker for DM that could serve for the screening of this disease. This could facilitate a proper and quick diagnosis, in conditions of speed and numerous and cellular smears, demanded by this type of program. Unfortunately, all the changes obtained were of nonspecific type and could occur in a large number of conditions. There are cytomorphometric studies in the literature that refer to the age and gender of the patients, showing that oral mucosa non-specific changes may be observed in the context of advanced age or related to gender (the NA and CA parameters can be much higher in females than in males groups) [15]. Xerostomia, a typical feature of DM (due to hyperglycemia, dysfunctionalities in the mechanism of saliva secretion and general dehydration), can also generate significant oral mucosa abnormalities (decrease of CA). Inflammation is able to determine non-specific alterations (increase NA and reduce CA in young cells [15] too, and in oral cavity), diabetes is frequently associated with chronic inflammation (gingivitis, stomatitis, periodontal disease, ulcerations, cheilitis, glositis, lichen planus, lichenoid reaction [28]). Likewise, candidiadis, often connected to diabetes, determine important changes of epithelial cells (increase in NA, decrease in CA, cytoplasmic vacuoles and perinuclear rings) [8, 15].

All these conditions and possibly many more (oral cancer, endocrine disorders or chronic respiratory diseases) may overlap and mask the changes induced by diabetes itself (in particular in terms of national programs of screenings, where the patients are not selected).

The lower degree of antibacterial defense driven by type 1 and 2 DM influences the oral flora that become excessive in this disease [28–30]. The spread of bacteria increases with the gravity of disease, associated disorders, immunity of patient and predisposing factors. Literature data mention that in all complicated forms, due to the poor metabolic control of the patients, the most frequently found bacterial infection was given by *Streptococcus* species (*salivarius*, *mutans*, *lactis*, *pneumoniae*, *pyogenes*, *viridans*), *Staphylococcus aureus*, *Branhamella catarrhalis*, *Escherichia coli*, *Actinobacillus actinomycetemcomitans*, *Bacteroides oralis*, *Serratia* [28–31].

In our research all three staining methods highlighted the excessive flora, seen either in the background of slides or covering the cells. Some of the non-specific nuclear and cytoplasmic alterations may have been caused by this microbial flora. Little flora was seen on the slides of the control group or potential diabetic patients.

Fungal infection, in particular candidiasis can be a feature of diabetes, but on the smears from our study, no typical aspects of *Candida* sticks were seen, so the clear perinuclear rings (that can signal its presence on Papanicolaou staining) were interpreted in the context of cells reactive changes.

Although oral exfoliative cytology has disadvantages, the accuracy of the method was recently augmented by additional, different diagnostic techniques as molecular analysis that can increase her popularity [15]. The spectral cytopathology may be of aid in DM diagnosis, the biochemical organization of the cells providing information about a pathological condition, before it can be morphologically revealed [15].

Conclusions

Exfoliative cytology alone is of low value as a diagnostic and prognostic tool in the diagnosis of DM; it detect the reactive changes induced by the disease, but it makes no differences between DM types or degree of severity and do not allow by qualitative analysis alone to detect abnormalities in early diabetes. A number of factors that influence the morphology of oral epithelial cells should be taken into account when assessing diabetic changes, because they can generate identical microscopic picture or they can mask the original alterations caused by the disease.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: October 30, 2015

Accepted: December 2, 2016