Cytological features of buccal epithelium in patients of various ages

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Abstract. The article describes the potential of buccal cells investigations. The authors presented buccal epithelium application in non-invasive diagnosis of early human aging; identified common cytological features of buccal epithelium for different ages; revealed the accumulation of cytogenetic abnormalities (epithelial cells with micronuclei, protrusions of the nucleus) and degenerative-dystrophic changes (perinuclear vacuole, condensed chromatin, karyorexis, karyolysis) with age. These findings reflect the predominance of apoptosis over reparation in the process of aging.

Introduction

It is known that the aging rate of an organism can significantly depend on the lifestyle of a particular person, environmental conditions, hereditary factors, etc. Relating to the same age group patients may have a different state of the body, the severity of aging processes, manifestations of various diseases [1, 2, 3]. For example it was found that the difference between the biological and chronological age in patients with diabetes is 4.76 years for one year of diabetes. The largest difference of 8.41 years was found in patients with a combination of arterial hypertension, diabetes mellitus and atherosclerosis [2].

Most age-related changes are associated with the progression of functional instability in organs and tissues. This requires promising definitions of biological age and the pace of the human body development based on laboratory and instrumental assessment of the structure and functions of tissues. In different studies of the biological age and rate of body aging can be found more than 150 biochemical, endocrinological, morphological, immunological, anthropometric and clinical physiological parameters [2, 3, 4]. In this regard the urgent task is searching for biological objects (tissues, cells, biological fluids) for the timely diagnosis of signs of accelerated aging.

The oral cavity is an accessible area for the study of aging markers (predictors). Non-invasive methods can be used to obtain such research objects as oral fluid, gingival fluid,

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saliva, buccal cells, dental plaque, etc.

Buccal cells can be considered as a border zone between the external and internal environment of the body. Changes in the functional activity of buccal epithelial cells largely reflect the state of both local and systemic homeostasis of the body or its impairment during aging. For this reason researchers studied buccal cells to diagnose the state of the body using various methods [2, 5, 6, 7, 8].

In the last decade the methodology of using buccal cells in the determination of biological age has been developed. It was based on electrokinetic characteristics (electro motility, speed, electronegativity) of cells in vitro using microelectrophoresis of nuclei. The possibility of buccal cells application in this case is due to the availability for production, the presence of a large nucleus and high vitality in vitro [2].

In another study buccal cells were used as a material to determine the rate of aging of an organism using a telomere test. By real-time PCR in blood lymphocytes (a traditional object) and buccal cells the absolute length of telomeres was measured in healthy people and patients with Alzheimer's disease. It was found that buccal cells can be an alternative material for telomere testing. Their advantage over lymphocytes is the non-invasiveness of the production [2].

According to some data, the morphological features of buccal cells nuclei can be used to indirectly determine the biological age. An analysis of the literature showed that in elderly healthy donors the number of micronuclei in buccal cytology can increase by 366%, the number of heterochromatin - by 45.8%, the number of cells with karyorexis - by 439%, the number of cells with a displaced nucleus - by 233% compared with similar indicators in young people. According to the authors, the differences between morphological characters of buccal cells nuclei during normal and accelerated aging reflect the systemic processes of DNA damage, proliferation and apoptosis of buccal epithelial cells during aging [2, 5, 6, 7, 8].

At the same time in another study of age-dependent changes in buccal cells an increase in the number of buccal epithelial cells with micronuclei was found from 0.53% in young people to 0.87% in elderly people [9, 10, 11]. In forensic practice a method for determining age by the size of buccal epithelial cells is presented. It is described by the formula: Estimated age = -0.0516 (cell size) + 57.363 [12]. In addition it is known that the number of cells with micronuclei is used to assess the genotoxicity of various factors [13]. Also, the micronuclear test has found application in assessing the activity of inflammatory periodontal diseases, the effectiveness of dental treatment [6, 14, 15].

In addition for the diagnosis of aging rates, buccal epithelial cells were studied using the method of immunohistochemistry to verify signal marker molecules. Previously a correlation was found between the expression of cell cycle markers and age-related pathology. For example in a culture of buccal epithelial carcinoma cells a change in the expression of the following markers of cell aging and apoptosis was found: *p21(CIP1), p27(KIP1), p16(INK4a), Bax, Fas,* and *Bcl-2-like protein.* Among these proteins *p16(INK4a)* is a recognized marker of cell aging and various age-related pathologies [2, 3].

Thus, buccal epithelium is an accessible material that allows to evaluate a person's biological age by various indicators, track the process of accelerated aging with age-related pathology and the effectiveness of the treatment.

The purpose of the study was to compare the cytological characteristics of buccal epithelial cells in patients of various age groups (children, young people, the elderly and senile).

Matherials and Methods

To study the buccal cytology, 4 groups of patients were recruited in accordance with the WHO age classification. Group 1 consisted of children (up to 18 years old, 231 people, male and female), group 2 - patients of young age (18-44 years old, 121 people, men and women), group 3 - elderly patients (60-74 years, 16 people, men and women), group 4 - patients of senile age (75-90 years, 5 people, men and women).

Patients of the study groups underwent a comprehensive dental examination (interrogation, examination, additional research methods). Buccal smears were collected from apparently healthy individuals. Buccal cells were taken using a disposable sterile cytobrush (Rambrush, type D, mod. 2). The material was applied to glass slides. After fixation in 95% alcohol, the smears were stained using Leishman stain. The level of cytoplasmic and karyological abnormalities in cells were evaluated. After that, the following integral indices of the buccal cytogram were calculated:

cytogenetic index (Ic) - the sum of cells with micronuclei, protrusions;

proliferative index (Ip) - the sum of binuclear cells (including those with dual nuclei);

apoptosis index (Iap) - the sum of cells with condensed chromatin, karyorexis, karyopicne, karyolysis, apoptotic bodies;

the index of cytogenetic disorders accumulation (Iac) - (Ic × Ip / Iap) × 100;

reparative index (RI) - the sum of the cells with karyorexis, karyopicne / dual-core cells + cells with micronuclei.

Statistical data processing was carried out in the software product «Gretal». To compare the average values of the indices of the independent groups, the nonparametric Mann-Whitney test was used. Differences in indicators were taken as significant at $p < 0.05$.

Results and Discussion

As a result of the buccal cytology, a number of patterns have been established that reflected the appearance of degenerative-dystrophic changes in the nucleus in patients with increasing age. In particular, an increase in the frequency of formation of perinuclear vacuoles and signs of decay of the nucleus (karyopicnosis) have been established with increasing age of patients.

Table 1. The results of buccal cytology in patients of various ages.

Signs	Age			
	Children	Young	Elderly	Senile
Micronuclei, Me (Q1;Q3)	0 (0; 0)	0 (0; 1,6) p=0,04	0 (0; 0,2) p=0,02	0 (0; 0) p=0,05
Protrusions, Me (Q1;Q3)	0 (0; 0)	0 (0; 1,5) p=0,03	0,2 (0; 0,35) p=0,03	0,5 (0,35; 0,95) p=0,01
Binuclear cells, Me (Q1;Q3)	0,8 (0; 1,0)	1,4 (0,8; 2,0) p=0,01	0,6 (0,25; 1,0) p=0,04	1,0 (0,95; 1,08) p=0,04
Karyolysis, Me (Q1;Q3)	0 (0; 0)	2,4 (1,1; 4,8) p=0,02	1,4 (0,5; 2,1) p=0,01	1,05 (0,58; 1,9) p=0,03
Karyorexis, Me (Q1;Q3)	0 (0; 0,9)	0,6 (0; 2,2) p=0,01	1,2 (0,8; 1,5) p=0,02	1,0 (0,8; 1,05) p=0,04
Karyopicnosis, Me (Q1;Q3)	0 (0; 1,0)	0,8 (0,4; 1,4) p=0,04	1,0 (1,0; 2,0) p=0,05	1,45 (1,23; 1,65) p=0,03
Apoptotic bodies, Me (Q1;Q3)	0 (0; 0)	0,2 (0; 0,6) p=0,07	0 (0; 0) p=0,01	0 (0; 0) p=0,01
Condensed chromatin, Me (Q1;Q3)	3,4 (2,7; 3,8)	1,9 (0,8; 3,6) p=0,03	0 (0; 0,6) p=0,01	0,35 (0; 0,78) p=0,02
Perinuclear vacuole, Me (Q1; Q3)	0 (0;0)	0,6 (0,2; 1,0) p=0,04	1,0 (0,6; 1,55) p=0,02	1,6 (1,25; 1,93) p=0,04

Note: in table 1 p - in comparison with a group of children under 18 years of age (p <0.05).

The values of the integral indices of the buccal cytogram calculated by the medians are presented in table 2.

Table 2. Integral buccal cytogram indices in patients of various age groups.

Indices	Age				p
	Children	Young	Elderly	Senile	
Cytogenetic index (Ic)	0	0	0,20	0,50	p<0,05
Proliferative index (Ip)	0,80	1,40	0,60	1,00	p<0,05
Apoptosis index (Iap)	3,30	6,00	3,60	3,85	p<0,05
The index of cytogenetic disorders accumulation (Iac)	0	0	3,30	12,98	p<0,05
Reparative index (RI)	0	2,14	3,5	2,05	p<0,05

Note: in table 2 p - in comparison with a group of children under 18 years of age (p <0.05).

The analysis of values of the cytogenetic index and the index of cytogenetic disorders accumulation showed their increase with age which seems quite logical. At the same time the total number of cells with abnormalities of the nucleus increases with the patients' age. Our data reflect the occurrence of systemic processes of DNA damage, a general tendency to increase degenerative-dystrophic changes in cells and the predominance of apoptosis over repair processes. Such changes may be due to an increase in the number of concomitant somatic diseases in patients with age and long-term using of various drugs.

Thus, the results of this buccal cytogram revealed regular signs of human aging, determined by studying buccal cells. They are similar to those described in world literature. Along with this the obtained data showed the severity of age-related changes to a lesser extent compared to the data of other authors.

Conclusion

Thus, as a result of this study, it was found that the accumulation of cytogenetic abnormalities of buccal epithelial cells occurs mainly in the elderly and senile. Other changes reflecting proliferative activity and apoptosis may be wave-like. On this basis, it can be assumed that the buccal cytogram reflects age-dependent processes and can serve as an adequate tool for studying the mechanisms of aging.

Aging is associated with changes that lead to progressive, irreversible deterioration of the functional capacities of several tissues and organs. Our study demonstrated the effect of age on the histological and apoptotic behavior of oral mucosa cells.

Age estimation is one of the essential factors in establishing the identity of an individual. Among various methods exfoliative cytology is a unique, noninvasive technique involving simple and pain-free collection of intact cells from the oral cavity for microscopic examination.

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