International Journal of Advanced Science and Technology
Vol. 29, No. 11s, (2020), pp. 3128-3134

Cytogenetic And Cytotoxic Indicators Of Buccal Epithelium In Patients With Chronic Recurrent Aphthous Stomatitis

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ABSTRACT

In the Republic of Uzbekistan, the frequency of chronic recurrent aphthous stomatitis among all diseases of MOP is 6.22% and tends to increase, therefore, the study of the cytogenetic and cytotoxic parameters of buccal epithelium in patients is of undoubted practical interest [5].

The aim of the study was to study the cytogenetic and cytotoxic parameters of buccal epithelium in patients with chronic recurrent aphthous stomatitis.

Recurrent aphthous stomatitis accounts for up to 90% of all violations of the integrity of the oral mucosa encountered in dental practice. Thus, a significant excess of cytogenetic and karyological disorders was established in patients with a scarring form of the disease in all periods of chronic recurrent aphthous stomatitis.

KEY WORDS: *chronic recurrent aphthous stomatitis, stomatitis, recurrent aphthous stomatitis, dentistry*

INTRODUCTION

Current epidemiological data on recurrent aphthous stomatitis (ASD) indicate a high prevalence of the disease, and its frequency according to different authors is from 35.0 to 60.0% [1,12,15]. According to WHO, it affects up to 20% of the population.

Recurrent aphthous stomatitis accounts for up to 90% of all violations of the integrity of the oral mucosa encountered in dental practice [10].

Chronic recurrent aphthous stomatitis (CPAS) is a chronic inflammation of the oral mucosa characterized by recurrent ulcerations that affect predominantly non-keratinized mucous membranes. Chronic recurrent aphthous stomatitis (CPAS) is one of the most common painful conditions of the oral mucosa observed among patients. They are recurrent ulcers with multiple, different size, round shape with protruding edges, with yellow or gray floors and surrounded by erythematous halos [5,10,12].

The etiology of recurrent aphthous stomatitis has not been fully established, which leads to significant difficulties in treatment and its low effectiveness.

Relapsing aphthous stomatitis (ASD) due to a significant incidence of a marked decrease in incidence, a pronounced tendency to the development of frequent exacerbations and persistent relapses, and the absence of anti-relapse treatment methods is a serious problem in therapeutic dentistry [5,10,15].

In recent years, the attention of researchers as a material for non-invasive express diagnostics has attracted buccal epithelium. Buccal epithelial cells, like all epithelial cells of the mucous membranes, take an active part in the system of humoral cell homeostasis. Participation in the immune response and intercellular interactions is ensured by the secretion of a number of signaling molecules that ensure the maintenance of humoral homeostasis [2,3,6,13,14]. Deviations of morphological, physical, chemical and biochemical parameters that occur under the influence of various exogenous and endogenous factors lead to changes in epithelial differentiation recorded morphologically (size, nature of nuclei and granules, signs of cytolysis), as well as a change in charge on the surface of the nucleus of a living cell, which in turn, violates the electrokinetic properties of the nuclei. It is proposed to take these features into account when screening for the state of health, stressful effects, harmful environmental factors, somatic pathology, and the biological age of a person [4,7,8,9,11,16].

Despite the emergence of new molecular genetic methods in recent decades, the micronuclear test not only does not yield its position, but also continues to be actively used. Registration of cells with micronuclei in their composition is a practically significant and highly informative diagnostic indicator of many diseases, which makes it possible to predict their course with sufficient probability and makes it possible to monitor their correction.

In the Republic of Uzbekistan, the frequency of chronic recurrent aphthous stomatitis among all diseases of MOP is 6.22% and tends to increase, therefore, the study of the cytogenetic and cytotoxic parameters of buccal epithelium in patients is of undoubted practical interest [5].

The aim of the study was to study the cytogenetic and cytotoxic parameters of buccal epithelium in patients with chronic recurrent aphthous stomatitis.

MATERIALS AND METHODS

A total of 143 patients with a clinically verified diagnosis of chronic recurrent aphthous stomatitis were examined. Fibrinous - 100 patients and scarring - 43 patients of the disease form were isolated depending on the clinical form of the disease. The control group consisted of 40 patients of average age 38.01 ± 1.59 years, of comparable gender and age without pathology of SOP.

The selection of materials for the study, its staining and analysis were performed in accordance with the recommendations set forth in the article by Kalayev et al. [4]. A spatula, pre-treated with alcohol, made scraping from the mucous membrane of both cheeks above the line of closure of the teeth. Smears were dried in air and then stained with azure-eosin according to Romanowsky – Giemsa. Separate intact cells were selected for analysis.

Statistical processing of the results of the study was carried out using Student's criterion. The data are presented as M ± m, where M is the arithmetic mean value, and m is the error of the mean. The difference was considered statistically significant at $p < 0.05$.

RESULTS

The results of cytogenetic karyological studies of buccal epithelial cells in patients with various clinical forms of chronic recurrent aphthous stomatitis are presented in the table. It has been established that in patients with chronic recurrent aphthous stomatitis, cells with micronuclei and various forms of nuclear protrusion are more common.

In this case, the frequency of occurrence of cytogenetic disorders is determined by the stage of the disease and the clinical form. The maximum frequency of cytogenetic disorders was recorded during the period of exacerbation of the disease.

So, in patients with a fibrous form of the disease, the frequency of detection of micronuclei exceeded the control value by 288.3% ($P < 0.01$); with scarring - by 486.67% ($P < 0.001$); the corresponding frequency of broken egg type protrusion cells was 260.06% ($P < 0.01$) and 410.0% ($P < 0.01$); with protrusions of the "bubble" type - 256.6% ($P < 0.001$) and 480.0% ($P < 0.01$); and protrusions of the tongue type — 332.0% ($P < 0.01$) and 488.0 ($P < 0.001$) (Fig. 1).

Prolonged monitoring of the cytogenetic effects in buccal epithelium in the dynamics of the epithelization of aphthous elements on the CRM showed a high level of cell chromosomal apparatus disturbances during the period of convalescence. So, in patients with the fibrinous form of the disease, the frequency of micronuclei exceeded the parameters of the control group by 195.00% ($P < 0.001$); with protrusions of the “broken egg” type - by 145.0% ($P < 0.01$); with protrusions of the "tongue" type - by 128.0% ($P < 0.01$); the corresponding excesses in the scarring form were 273.30% ($P < 0.001$); 185.0% ($P < 0.001$); 200.0% ($P < 0.01$) (table, Fig. 1).

Analysis of the total frequencies of cytogenetic disturbances during the convalescence period showed that the total values exceed the level of the control group with the fibrous form by 152.89% ($P < 0.001$); with scarring - by 240.79% ($P < 0.001$) (table).

During the period of clinical remission, with the complete cessation of aphthous elements and visible changes in SOP in the buccal epithelium, cytogenetic disorders persisted, more pronounced in patients with a scarring form. So, in the fibrinous form, the number of cells with micronuclei exceeds the control value by 23.33% ($P < 0.05$); with protrusions of the “broken egg” type - by 45.0% ($P < 0.05$); and protrusions of the "bubble" type - by 30.0% ($P < 0.05$) and with protrusions of the "tongue" type - by 44.0% ($P < 0.05$); the total frequency of nuclei with cytogenetic disorders exceeded the control value by 34.07% ($P < 0.05$); the levels of cytogenetic disorders in the scarring form were significantly higher ($P < 0.01$) and exceeded the values of the control group by 41.67%, respectively ($P < 0.01$); 200.0% ($P < 0.01$); 106.67% ($P < 0.01$); 92.0% ($P < 0.01$) and 77.78% ($P < 0.01$) (table, Fig. 1).

Currently, the opinion has been firmly established that cells with micronuclei and micronuclear anomalies, with protrusions of the nucleus, with a circular notch are indicators of cytogenetic disturbances arising from the influence of endo- and endogenous factors on the nuclear apparatus of chromosomes, leading to fragmentation of chromosomes, budding of interphase nuclei, or removal from DNA nuclei, a shift in the location of chromatin in the nucleus, a shift in the position of interphase chromosomes, a violation of chromosome aberrations during meytosis, a violation of the spindle division, etc. [8].

The study of proliferation indicators of the nucleus showed a significant increase in cells with two nuclei and a circular notch in both studied clinical forms, more pronounced during an exacerbation of the disease in patients with a scarring form. So, during the period of exacerbation, the total frequency of cells with proliferation of the nucleus exceeded that of the control group with fibrinous form by 106.0% (P <0.01), with a scarring form - by

260.0% (P <0.001); the level of proliferation of buccal epithelial cells decreased slightly during the convalescence period and exceeded the control values by 720% (P <0.01) and 168% (P <0.01), respectively; and was minimal during the period of remission: the corresponding excesses were 34.0% (P <0.01) and 56.0% (P <0.01) (table; Fig. 2).

When studying the signs of early nuclear destruction, it was found that the average values of indicators of early nuclear destruction in all periods of the disease with a high degree of statistical significance (P <0.05) exceed the values of the control group.

The maximum values of destructive changes in the nucleus were found during the period of exacerbation, the average values of signs of early nuclear destruction in patients with a fibrous form exceeded the control values by 168.40% (P <0.01) and with a scarring form by 291.67% (P <0, 01); during the convalescence period, the levels of early nuclear destruction somewhat decreased and exceeded the control values by 97.40% (P <0.01), respectively, and 171.88% decreased and exceeded the control values

Table 1. Cytogenetic and karyological indicators of buccal epithelial cells in patients with various clinical forms of chronic recurrent aphthous stomatitis depending on the period of the disease

Fig. 1. Cytogenetic indicators of buccal epithelium in patients with chronic recurrent aphthous

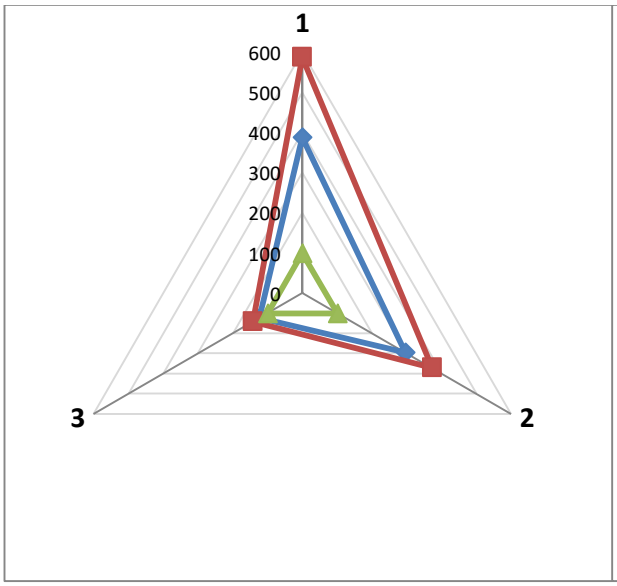
Index,%	Midst		Reconvalescence		Remission		Control
	Fibrinous	Scarring	Fibrinous	Scarring	Fibrinous	Scarring	
Cytogenetic indicators, cell frequency with…							
Microkernels	2,33±0,11•	3,52±0,14•∧	1,77±0,06•x	2,24±0,10•∧x	0,74±0,03•⊙	0,85±0,03•⊙∧	0,6±0,02
broken egg protrusions	0,72±0,02•	1,02±0,05•∧	0,49±0,02•x	0,77±0,03•∧x	0,29±0,01•⊙	0,60±0,03•⊙∧	0,2±0,01
bubble protrusions	1,07±0,03•	1,44±0,06•∧	0,65±0,03•x	0,84±0,04•∧x	0,42±0,02•⊙	0,62±0,02•⊙∧	0,30±0,008
tongue protrusions	1,08±0,04•	1,47±0,07•	0,57±0,02•x	0,75±0,03•∧x	0,36±0,01•⊙	0,48±0,02•x∧	0,25±0,011
The total frequency of cells with cytogenetic lesions	4,90±0,21•	7,30±0,31•∧	3,41±0,16•x	4,60±0,21•∧x	1,81±0,07•⊙	2,55±0,12•⊙∧	1,25±0,05
Proliferation indicators, cell frequency with…							

stomatitis (in% relative to the control).

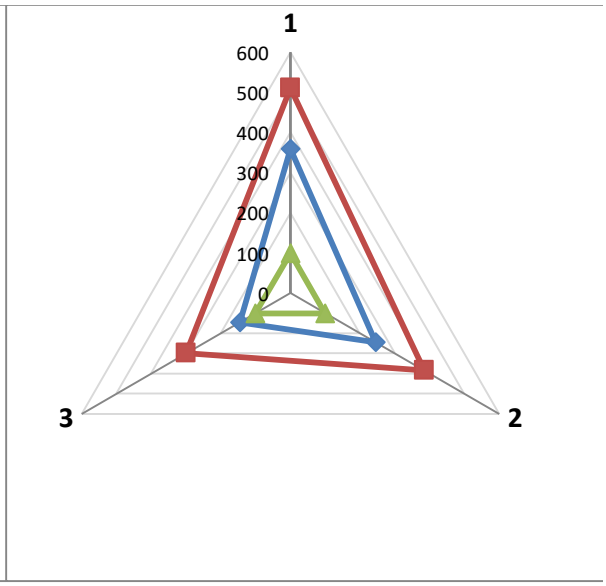
Two cores	0,52±0,02●	0,91±0,04●^	0,34±0,01●X	0,72±0,03●^X	0,27±0,04●⊙	0,38±0,01●⊙^	0,20±0,01
Circular notch	0,61±0,03●	0,88±0,03●^	0,52±0,02●X	0,61±0,02●^X	0,46±0,02●⊙	0,42±0,02●⊙^	0,30±0,01
The total number of cells with proliferation of nuclei	1,13±0,04●	1,80±0,07●^	0,86±0,03●X	1,34±0,05●^X	0,67±0,03●⊙	0,78±0,03●⊙^	0,50±0,02
Early stage of cell destruction, cell frequency with…							
Perinuclear vacuole	3,16±0,14●	4,17±0,20●^	2,46±0,11●X	2,97±0,13●^X	2,00±0,05●⊙	2,11±0,04●⊙^	1,45±0,06
Chromatin condensation	1,10±0,05●	1,84±0,08●^	0,68±0,03●X	1,11±0,05●^X	0,39±0,02●⊙	0,59±0,02●⊙^	0,26±0,01
Vacuumization of the nucleus	0,92±0,04●	1,51±0,07●^	2,65±0,02●X	1,13±0,04●^X	0,37±0,02●⊙	0,58±0,02●⊙^	0,21±0,01
The total frequency of cells with nuclear destruction	5,18±0,24●	7,52±0,37●^	3,80±0,17●X	5,22±0,25●^X	2,77±0,12●⊙	3,28±0,15●⊙^	1,92±0,08
Completion of nuclear destruction, cell frequency with…							
Karyorexis	0,29±0,01●	0,46±0,01●^	0,23±0,01●X	0,34±0,01●^X	0,16±0,008●⊙	0,21±0,01●⊙^	0,12±0,005
Karyopiches	0,59±0,02●	0,84±0,03●^	0,40±0,02●X	0,51±0,02●^X	0,33±0,014●⊙	0,32±0,01●⊙^	0,25±0,01
Karyolysis	0,37±0,01●	0,73±0,03●^	0,26±0,01●X	0,36±0,01●^X	0,16±0,007●⊙	0,25±0,01●⊙^	0,10±0,04
Apoptotic cells	4,54±0,22●	5,44±0,22●^	2,82±0,12●X	3,40±0,16●^X	1,97±0,08●⊙	2,46±0,10●⊙^	1,25±0,05
The total frequency of cells with complete destruction of the nucleus	5,80±0,27●	7,47±0,31●^	3,71±0,16●X	4,61±0,21●^X	2,63±0,12●⊙	3,24±0,16●⊙^	1,72±0,06
The total number of aberrant cells	17,03±0,81●	24,09±1,02●^	11,52±0,51●X	14,96±0,71●^X	7,45±●⊙	9,76●⊙^	5,49±0,22

Note: ● - p <0.05 with respect to the control group; ^ - p <0.05 with respect to the fibrous form; X - p <0.05 with respect to the peak period; ⊙ - p <0.05 with respect to convalescence.

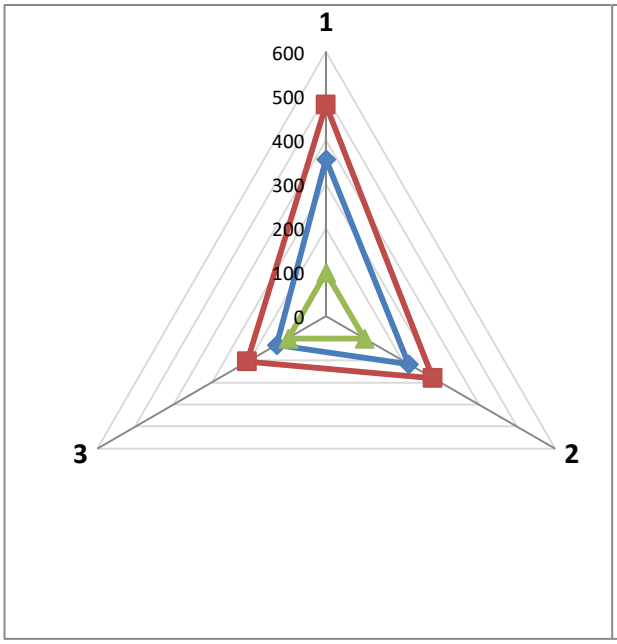
Micronucleated cells



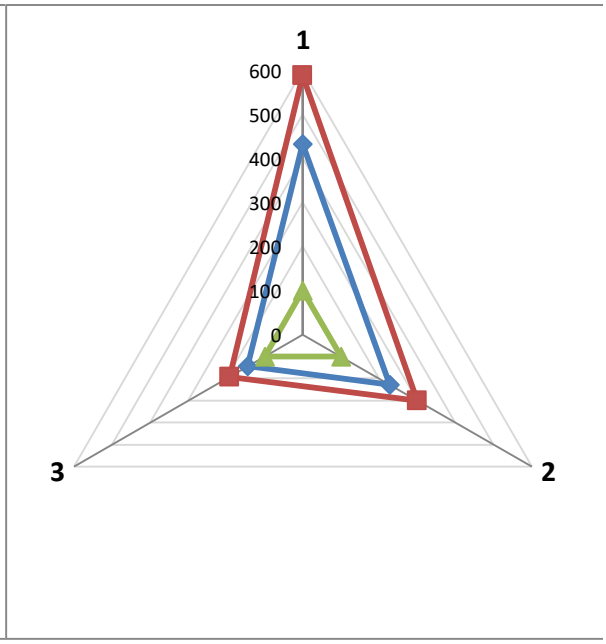
Broken Egg Protrusion



Bubble protrusion



Tongue type protrusion



Note: 1 - exacerbation; 2 - convalescence; 3 - remission; fibrinous form; scarring form; control - 100%

Respectively, 97.40% (P <0.01); during the period of clinical recovery, the levels of early nuclear destruction were minimal; excesses by 44.27% decreased and exceeded control values by 97.40% (P <0.05), respectively, and 70.83% decreased and exceeded control values by 97.40%, respectively (P <0.01).

It should be noted that in patients with a scarring form of chronic recurrent aphthous stomatitis, all forms of early nuclear destruction were significantly more often relatively fibrotic: nuclei with perinuclear vacuole, chromatin condensation, and nucleus vacuolization.

At the same time, with the scarring form of chronic recurrent aphthous stomatitis, nuclei with complete nuclear destruction (karyopicnosis, karyorexis, karyolysis, and aktosis cells) were more often reduced and exceeded the control values, respectively, by 97.40% (P <0.01).

Moreover, the total frequency of cells with complete destruction of the nucleus exceeded the control value in all periods of the disease.

Involution of aphthous elements was accompanied by a significant decrease in cells with nuclear destruction. So, during the period of exacerbation, the total frequency of cells with complete destruction of the nucleus exceeded the indices of the control group with the fibrous form - by 237.21% (P <0.01); with scarring - by 334.30% (P <0.01). The corresponding excesses in the convalescence stage were 115.70% (P <0.01) and 168.02 (P <0.01); in remission, respectively, 52.91% (P <0.01) and 88.37 (P <0.01).

An increase in nuclei with initial and complete destruction of the nucleus indicates activation of necrotic processes in tissues, destruction of cell membranes, violation of their barrier function and cell necrosis [4].

A comparative analysis of the total number of aberrant cells corresponded to the general tendency of individual cytogenetic and karyological changes, with a maximum of registration during the period of exacerbation and a decrease during the involution of the disease.

So, in the acute phase of chronic recurrent aphthous stomatitis, the total number of aberrant cells in the fibrous form exceeded the control level by 210.20% (P <0.01); with a scarring form, it exceeded the control level by 338.80% (P <0.01). The corresponding excesses during the convalescence period were 110.99% (P <0.01) and 170.50% (P <0.01); during remission - 35.70% (P <0.01) and 77.78% (P <0.01) (table 1).

CONCLUSION

Thus, a significant excess of cytogenetic and karyological disorders was established in patients with a scarring form of the disease in all periods of chronic recurrent aphthous stomatitis.

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