PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ALTERAÇÕES CITOGENÉTICAS EM CRIANÇAS RESIDENTES EM REGIÕES ECOLOGICAMENTE ADVERSAS DO CAZAQUISTÃO

CYTOGENETIC CHANGES IN SCHOOLCHILDREN RESIDING IN ECOLOGICALLY ADVERSE REGIONS OF KAZAKHSTAN

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Received 01 June 2020; received in revised form 18 June 2020; accepted 04 July 2020

RESUMO

No Cazaquistão, devido ao fortalecimento do papel geopolítico na arena internacional e ao desenvolvimento industrial ativo, os impactos ambientais negativos se intensificaram nos últimos anos. Muitas regiões são ambientalmente desfavoráveis e correm o risco de um aumento na incidência de vida nessas condições da população. Inúmeros desvios na saúde da população criam condições para o polimorfismo genético e o crescimento de certos grupos de doenças característicos de regiões com poluição ambiental. A poluição ambiental a longo prazo é a razão do aumento da taxa do processo de mutação e do volume da carga genética na população humana. O objetivo deste estudo é mostrar a influência de fatores ambientais dominantes em regiões distantes do Cazaquistão sobre distúrbios citogenéticos no corpo da geração mais jovem. Estudantes de diferentes escolas com idades entre 16 e 18 anos que moram perto do local de teste de Semipalatinsk, na região leste do Cazaquistão e na região de Aral, na região de Kyzylorda, participaram dos estudos. Os resultados do estudo mostram que as células epiteliais orais em dois terços dos adolescentes examinados que vivem em condições ambientalmente desfavoráveis, próximas ao local do teste de Semipalatinsk ou na região do Mar de Aral, no Cazaquistão, revelaram uma ampla gama de distúrbios citogenéticos. A maior porcentagem de violações, associada à formação de micronúcleos, protrusões e apoptose, foi detectada em estudantes urbanos que vivem a 150 km do local do teste de Semipalatinsk. Ao comparar os dados de estudantes urbanos e rurais, foi revelado o desenvolvimento predominante de desordens citológicas, como destruição nuclear (cariorexia, cariólise e apoptose) em adolescentes rurais, o que indica o impacto negativo contínuo do local de teste de Semipalatinsk fechado e a salinização do Mar de Aral sobre a saúde da geração mais jovem de crianças.

Palavras-chave: *Ecologia, Saúde do adolescente, Local de teste de Semipalatinsk, Priaralye, Distúrbios citogenéticos*.

ABSTRACT

In Kazakhstan, due to the strengthening of the geopolitical role in the international arena and active industrial development, negative environmental impacts have intensified in recent years. Many regions are environmentally unfavorable and carry the risk of an increase in the incidence of living in these conditions of the population. Numerous deviations in the health of the community create the conditions for genetic polymorphism and the growth of certain groups of diseases characteristic of regions with environmental pollution. Long-term environmental pollution is the reason for the increase in the rate of the mutation process and the volume of the genetic load in the human population. The purpose of this study is to show the influence of dominant environmental factors in distant regions of Kazakhstan on cytogenetic disturbances in the body of the younger generation. Students from different schools aged 16 to 18 years living near the Semipalatinsk test site of the East Kazakhstan region and in the Aral region of the Kyzylorda region took part in the studies. The results of the study show that the oral epithelial cells in two-thirds of the examined adolescents living in environmentally unfavorable conditions either near the Semipalatinsk Test Site or in the Aral Sea region of Kazakhstan revealed a wide range of cytogenetic disorders. The most significant percentage of violations associated with the formation of micronuclei, protrusions, and apoptosis, was detected in urban students living 150 km from the Semipalatinsk Test Site. When comparing the data of urban and rural students, the predominant development of cytological disorders was revealed, such as nuclear destruction (karyorrhexis, karyolysis, and apoptosis) in rural adolescents, which indicates the ongoing negative impact of the closed Semipalatinsk Test Site and the salinization of the Aral Sea on the health of the younger generation of children.

Keywords: *Ecology, Adolescent health, Semipalatinsk Test Site, Priaralye, Cytogenetic disorders*.

1. INTRODUCTION:

Currently, there is an increase in the incidence of environmental pollution, with industrial waste entering the water, soil, air, and food. Changes in the quality of the living environment lead to the disruption of numerous evolutionarily established mechanisms of interaction between man and nature. Such shifts violate the ecology of the external and internal environments of a person and lead to disease (UNECE Environment Division, 2013).

In Kazakhstan, environmental problems also pose dangerous risk elements for human life and health, including the long-term effect of radioactive contamination in the area near the former Semipalatinsk Nuclear Test Site and the pollution of the environment with toxic compounds as the Aral Sea dries up. These problems, due to their significance, have a global resonance (Nazarbayev *et al*., 2016).

The East Kazakhstan Region in Kazakhstan has existed since 1932 and includes the territories of 15 districts. The Altai and Tarbagatai mountains are located in the region, and it contains more than 885 rivers, carrying over 40% of all water reserves in Kazakhstan. About 1.5 million inhabitants of more than 15 nationalities live here (Nazarbayev *et al*., 2016). Over the past 20 years, the mortality rate from neoplasms among the adult population in the East Kazakhstan Region has been 20-30% higher than the average national level (Yan, 2019). Radioactive pollution of the environment continues to be considered the cause for this high mortality, the sources of which are the Semipalatinsk Test Site and the enterprises of the nuclear-industrial complex. For 40 years, until 1989, about 456 nuclear tests were carried out at the abovementioned test site, including 30 ground and 86 air tests (Nazarbayev *et al*., 2016). The consequences of such tests are a still increased level of radioactive infection and genetic mutations, with a high incidence for the population of several generations living near the landfill (Grosche *et al*., 2017). With industry concentrated in this region, 154 industrial enterprises annually emit 294 thousand tons of toxic chemicals into the environment. For example, in the territory of Semey, the maximum concentration of heavy metals exceeds the permissible standards for copper, lead, and chromium by 100 times, for zinc by 300 times, and for cobalt and nickel by 50 times (Gazaliev, 2016). Currently, at the state level, a number of programs have been adopted to improve the region's ecology and disease prevention measures (Nazarbayev *et al*., 2016). As a result of sanitary and hygienic standards over the past ten years, the incidence rates of many diseases in the population have decreased in the Semipalatinsk region. Still, over the years, the level of endocrine disorders has remained 30-40% higher than both the regional average and the national average (in 2011, 1255.5 in Semey vs 954.3 in the republic; in 2017, 1389.7 in Semey vs 976.4 in the republic) (Ministry of Health of the Republic of Kazakhstan, 2011).

Regarding the Kazakhstani part of the Aral Sea region, the following problems should be highlighted. The intensive development of irrigation since 1960 in both Kazakhstan and Central Asia as a whole has led to a steady decline in the level of the Aral Sea, the deterioration of ecosystems, the development of aridization, and significant desertification in the region. As a consequence of such processes, there have emerged problems, including a high content of mineral salts in the water and soil and the depletion and pollution of water resources of the Aral Sea region (Crighton, Barwin, Small, & Upshur, 2011; Novikov & Kelly, 2017; White, 2013).

The environmental problems of the region have led to wide-scale and regional climate changes in large areas of the country due to an increase in the greenhouse effect, an increase in the concentration of carbon dioxide in the atmosphere, and salt and dust emissions, which have impacted human living conditions in the Aral Sea region. According to a number of authors, in the Kazakhstani part of the Aral Sea region, over the past 20 years, demographic processes have significantly deteriorated due to an increase in total and infant mortality. The leading causes of this mortality increase are diseases of the circulatory system and respiratory and digestive organs (Ni, Tonkobaeva, & Ilyasova, 2013; Toleutay, 2017).

In recent years, according to official statistics, in the Republic of Kazakhstan, there has been a general decrease in the incidence of disease in the population (Ministry of Health of the Republic of Kazakhstan, 2011, 2017, 2018). This has been primarily due to the widespread provision of modern equipment to medical facilities in cities and outpatient networks in districts, which has increased the diagnostic capabilities of local health care. Nevertheless, in many regions of the country, a high incidence of disease in the population remains, which can be explained by the environmental pollution of the living environment. Environmental pollution is one of the main reasons for the increase in the rate of the mutation process and the volume of the genetic load in human and animal populations, as evidenced by the growth in the number of hereditary and multifactorial diseases, congenital pathologies, and malformations, which are especially pronounced in ecologically disadvantaged regions (Landrigan & Fuller, 2015).

One of the indicators of the state of the body is cytogenetic homeostasis, which is determined using a micronucleus test, which, essentially, calculates the frequency of cells with micronuclei in the epithelial cells of the buccal epithelium. The micronuclear test is included in many manuals (Hayashi, 2016) and is actively used in many studies, in particular, to determine the environmental load that can cause damage to the genetic apparatus in humans and animals (Baranov, 2019; Yurchenko, Krivtsova, & Podolnaya, 2008). The popularity of this test is because it is fast, non-invasive, and economically viable, allows an intravital screening of the examined individuals an unlimited number of times, and does not require special equipment for cell culture or enhanced sterility.

An indicator of genetic disorders in interphase nuclei is not only the frequency of the micronuclei but also the sum of cytological disorders, such as binuclear cells, protrusions, and destructive changes in nuclei. Micronuclei are formed mainly from fragments of chromosomes lacking a centromere during the formation of chromosome aberrations or due to lagging at the anaphase stage of the total number of diverging chromosomes. During mitosis, this material enters one of the daughter cells and forms one or more micronuclei. They can also be formed by the whole chromosome as a result of the violation of the spindle of division (Kolmakova, Belik, Morgul, & Sevryukov, 2013).

Protrusions, like micronuclei, can be formed by fragments of chromosomes or due to lagging when the fission spindle is broken by whole chromosomes, the nuclear membrane of which is connected to the membrane of the central nucleus. A broken egg protrusion looks like a micronucleus connected to the nucleus by a nucleoplasmic bridge. It is assumed that nuclear protrusion can also be formed by budding of the interphase nuclei (Sarto *et al*., 1987).

Proliferation indicators are the presence in the sample of binuclear cells and nuclei with a circular notch (a groove that is equal to or partially shifted to one of the poles of the groove). This anomaly is likely to arise in the process of incomplete mitosis as a result of damage to the spindle of division (Holland *et al*., 2008).

Indicators of nuclear destruction are the presence of cells with karyorrhexis, karyolysis, and apoptotic bodies. They are observed in diseases of accumulation, inflammation, as well as after exposure to chemicals and radiation (Holland *et al*., 2008).

Determining the genetic status of the most vulnerable population – adolescents from 16 to 18 years old, living in different regions of the country according to environmental stress – will show the presence of cytological disorders at the cellular level, which can lead to various health problems in the future. Undoubtedly, under the conditions of scientific and technological progress on a global scale, there is a high probability of an increase in ecogenetic pathology, which requires strengthening the measures to prevent diseases caused by an unfavorable living environment due to the growing role of new pollution factors (Landrigan & Fuller, 2015). The research results will bring a new understanding of the problem of the deteriorating health of the younger generation according to the ecology of the living environment.

Hence, the purpose of this study is to show the influence of dominant environmental factors in distant regions of Kazakhstan on cytogenetic disorders in the bodies of the younger generation.

2. MATERIALS AND METHODS:

The study participants were schoolchildren living in cities and villages located 150-200 km from the Semipalatinsk Test Site and the Aral Sea region of Kazakhstan, 150-200 km from the Aral Sea. A total of 182 high school students aged 16 to 18 years were examined, of which 81 were schoolchildren living near the Semipalatinsk Test Site of the East Kazakhstan Region, and 101 were students living in the Aral Sea region of the Kyzylorda Region. At the time of the study, all students were healthy. According to the requirements of the local ethics commission, informed consent was obtained from each participant and their parents. The cytogenetic studies carried out were comparative in nature, allowing a new understanding of the nature of their appearance, regardless of the type of dominant environmental load, and the long-term effects of the radioactive contamination or mineralization of the atmosphere and drinking water.

For an analysis of the cytogenetic smear preparations, samples of buccal epithelium were taken by scraping epithelial cells from the inner surface of the pupils' cheeks with a plastic spatula. Next, the epithelial cells were resuspended in 100 μl of phosphate buffer (AMRESCO) or saline, evenly distributed over the surface of the slide, and dried in air. Two preparations were prepared (right, left cheek) from each student. The preparations were stained with 10% Romanovsky–Giemsa solution for 10 min (Stefanović *et al*., 2017).

The micronuclei frequency was calculated using a Zeiss Axioscop 40 microscope at a magnification of 16x10. Photo documentation of the most specific disorders of the analyzed epithelial cells was carried out. The cytogenetic analysis of the buccal epithelium was conducted for 1000 to 3000 cells in each sample. The frequency of cytogenetic disorders was calculated as a percentage per 100 cells analyzed. When conducting a cytogenetic examination, all violations of the structure of epithelial cells that differed from normal morphology were recorded. The data obtained were processed using Microsoft Office Excel software. To compare the average values, a student's t-test was conducted using the formula (Eq. 1):

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}} \quad \text{(Eq. 1)}$$

where M_1 and M_2 are the arithmetic means of the compared indicators, and m_1 and m_2 are the average errors of these indicators. The number of degrees of freedom was found according to the formula (Eq. 2):

$$F = (n_1+n_2) - 2 \quad \text{(Eq. 2)}$$

after which the student criterion was determined from the Table at a significance level at $p \leq 0.01$. (Chaliev & Ovcharov, 2007).

To examine the schoolchildren, permission was obtained from the Local Ethics Commission (LEC) of the Kazakh National Medical University, S.D. Asfendiyarova, extract from protocol No. 3 (80) of 02/27/2019.

3. RESULTS AND DISCUSSION:

The cytogenetic analysis of buccal epithelial cells of the oral cavities of students living in the Aral Sea region of Kazakhstan and near the Semipalatinsk Test Site of the East Kazakhstan Region showed the presence of epithelial cells containing a few micronuclei (m/i), cells with a protrusion of nuclear material, binuclear cells, and epithelial cells with apoptotic bodies and karyolysis. Figures 1 – 4, respectively, show images of normal epithelial cells and cells with various changes, namely with two nuclei, a protrusion of nuclear material, and epithelial cells with apoptotic bodies and karyolysis, as detected in the students.

When comparing the data of urban students in both regions, it was found that the frequency of cells with cytogenetic disorders in adolescents living in the city of Semey, located near (143 km) the Semipalatinsk Test Site, is 7 times higher than that of schoolchildren in the Aral Sea region (Table 1).

In rural schoolchildren living in settlements located 200 to 300 km from the Semipalatinsk Test Site, the frequency of cells with cytogenetic disorders was 1.6 times higher than in rural teenagers of the Aral Sea region living 200 km from the Aral Sea. In terms of proliferation, no significant differences were observed in both regions (Table 1). Based on the changes in the structure of the nucleus, it was found that urban schoolchildren living in the city of Semey (East Kazakhstan Region, Kazakhstan) had a 20 times higher frequency of nuclear destruction than urban pupils in the Aral Sea region. However, in rural schoolchildren living near the nuclear test site (East Kazakhstan Region, Kazakhstan), the frequency of nuclear destruction was 4 times lower than that of rural students in the Aral Sea region (Table 1).

In general, adolescents in both studied regions showed a high level of cytogenetic changes. The maximum level of micronucleus frequency was found in 90% of the examined adolescents, 2-3 nuclei were found in the genetic material of 54% of the examined schoolchildren, and protrusions were found in 41% of students, regardless of the place of residence and environmental load. Nevertheless, in the students from Semey, located near the Semipalatinsk Test Site, the cytogenetic changes associated with nuclear destruction (karyorrhexis, karyolysis, and apoptosis) were found to be significantly higher than in the adolescents in the Aral Sea region. The presence of a high frequency of such violations in the younger generation indicates that the long-term effect of environmental pollution by radioactive contamination products has been preserved despite the fact that the nuclear test site has been closed since 1991.

It is known that buccal cells have a limited ability to recover DNA compared to peripheral blood lymphocytes and, therefore, can more accurately reflect the age-related event of genomic instability in epithelial tissue (Grewal, Jindal, & Chauhan, 2013; Ladnova, Istomin, Kurochitskaya, & Silyutina, 2016). Being in direct contact with inhaled and absorbed genotoxic agents and metabolites of various chemicals, epithelial tissues are the first to express the genotoxic effects of these agents. The frequency of occurrence of micronuclei reflects the destruction of chromosomes under the action of adverse factors long before the development of clinical symptoms of pathological disorders of the body's functions (Grewal *et al*., 2013; Podrimaj-Bytyqi *et al*., 2018; Thomas *et al*., 2009; Weng & Morimoto, 2009).

As a result of a cytogenetic analysis of oral epithelial cells, various cytogenetic disorders were revealed in two-thirds of the examined adolescents from 16 to 18 years old. The most significant percentage of violations associated with the presence of micronuclei was detected in urban students living 130 km from the former Semipalatinsk Nuclear Test Site. The data obtained are consistent with the high incidence of disease among adolescents in this region, as cited by official sources. The official statistical data for the teenage population of the East Kazakhstan Region of Kazakhstan for the period 2010-2017 showed a high incidence of neoplasms as well as a cardiovascular, respiratory and nervous system, and congenital anomalies at a rate 1.5-2 times higher than the average national level (Ministry of Health of the Republic of Kazakhstan, 2011, 2017, 2018).

When comparing the results of the study of buccal epithelium samples of urban and rural students of the Aral Sea region (Kyzylorda Region, Kazakhstan), the likelihood of developing cytogenetic disorders in rural adolescents, such as micronuclei, proliferation and nuclear destruction, including karyorrhexis, karyolysis, and apoptosis, which also indicates a negative effect, is revealed concerning the impact of the ecology of the region of residence on the health of the younger generation. This is confirmed by the official statistics for the period 2010-2017 and by the total morbidity of adolescents aged 15 to 17 years in the Kyzylorda Region, which shows that the number of sick adolescents is 45% more in rural areas (10509.4 cases) than in the city (7218.0 cases). Moreover, over the past seven years, the incidence of adolescent diseases of the blood and blood-forming organs, as well as immune disorders and iron deficiency anemia, has remained significantly high, exceeding the national average by 2 times (in 2017, 3,792.2 cases in the Republic of Kazakhstan against 7976.8 cases in the Kyzylorda Region per 100 thousand teenagers) (Ministry of Health of the Republic of Kazakhstan, 2017).

The changes in the chromosomal material of the cells of the body of the younger generation shown in this work reflect a whole set of cytogenetic changes up to the destruction of the cell nucleus, regardless of the type of pathological environmental factor. Adolescents living in places located near the former Semipalatinsk Test Site, as well as the adolescent population of the Aral Sea region, suffer from pollution and drought in the region due to the drying out of the Aral Sea, whereby identical changes in cytogenetic indices with slight deviations in one direction or another were revealed. Considering that the main period of active pollution of the ecology of the regions where the adolescents lived was in the 1970-80s (Stepanov, 2016), the revealed changes at the chromosomal level show the long-term negative effect of environmental pollution on the health of the younger generation (UNESCO, 2013).

Currently, the acceleration of technological progress is creating more zones of ecological disadvantage, which is increasing the incidence of disease among the child population. This is not only occurring in the present but will also, as our study shows, cause cytogenetic changes in the cells of the bodies of future generations. In different countries of the world, the genetic vulnerability of populations to various environmental challenges has been shown (Panico *et al*., 2020; Zani *et al*., 2020). Mexican researchers investigated and showed the effect of gasoline vapors contained in exhaust gases on the human genome. They revealed cytogenetic disorders such as micronucleus frequency, karyolysis, karyorrhexis, and binuclear cells in the buccal epithelium, similar to our results. The authors suggest that this can cause carcinogenic diseases (Martinez-Valenzuela *et al*., 2017). Genomic instability in the buccal epithelial cells of children living in one of the contaminated regions of Brazil has also been shown. The authors indicated a high level of cytogenetic disorders, also similar to our data, in children under the influence of the abnormal ecology of the region, namely the pollution of water resources (Alpire, Cardoso, Seabra Pereira, & Ribeiro, 2019). Indian researchers, assessing the health of children, also indicated genetic damage, such as damage to telomere length, micronuclei, and urothelial cells in samples of the buccal epithelium, as a result of drinking water from underground sources with increased content of heavy metals that are especially dangerous for health, such as arsenic (Chatterjee *et al*., 2018). In Russia, in the Altai Territory, which is 500 km or more distant from the Semipalatinsk test site, an increased level of cytogenetic abnormalities and incidence of adolescent endocrine diseases and congenital anomalies has been maintained for 40 years (Kolyado, Plugin, & Konovalov, 2017; Muldagaliev & Konovalov, 2018). Increasingly, they write about the impact of climate change in Central Asia on the health and gene pool of the population (Danielyan, Nazaretyan, Kosyan, & Nersisyan, 2017; Montgomery, 2016; Mullerson, 2014).

As can be seen from the literature, the presented research results are consistent with the data of various authors on a global scale, thereby introducing a new insight into the problems of the increased incidence of disease among the child population in regions where the peak of environmental problems was observed 40-50 years ago. The revealed cytogenetic disorders in the younger generation highlight the need to reduce the environmental burden in all regions to preserve the health of children, for both current and future generations.

4. CONCLUSIONS:

Ecological dysfunction of the environment has a distant negative effect on the health of the younger generation and contributes to the development of cytogenetic disorders at the cellular level:

1. The maximum level of cytogenetic disorders associated with increased micronuclei and proliferation of nuclei in cells was detected in students living 150-200 km from the Semipalatinsk nuclear test site. The data are consistent with official data for 2010-2018 on a high incidence of adolescents in this region with diseases of the cardiovascular, respiratory and nervous systems, neoplasms and congenital anomalies exceeding 1.5-2.0 times the national average.

2. An extensive spectrum of cytogenetic disorders was detected in the overwhelming majority of the Aral Sea teenagers living 150-200 km from the dried Aral Sea, the territory of which is characterized by desertification and significant salinization of the soil and remaining water. The micronuclei, the presence of 2-3 micronuclei, the proliferation of the nucleus and the destruction of the cell nucleus in the form of karyorrhexis, karyolysis and apoptosis were revealed in the buccal epithelium of the adolescent population, which indicates the continuing negative effect of the dried Aral Sea on the genetic material of the young generation of this region.

The presented data on cytogenetic changes in children born much later than peak environmental pollution emphasize the need for constant monitoring of their health and assessment of their genetic status, since environmental and human health issues are interrelated and are one of the urgent problems of our time. Given that the current study is preliminary, further analysis with a wider selection of children from different regions of the country is of scientific interest and opens up prospects for the future.

5. ACKNOWLEDGEMENTS:

The study was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan as part of grant No. AR05132033.

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__Table 1.__ The average frequency of cytogenetic disorders in the buccal epithelium of the oral cavity in students aged 16 to 18 years living in the Aral Sea region (Kyzylorda Region, Kazakhstan) and 150 km from the Semipalatinsk Test Site (East Kazakhstan Region, Kazakhstan)

Indicator / Region	**300 km from Lake Aral Priaralye (Kyzylorda Region, Kazakhstan)**		**130-300 km from the Semipalatinsk Test Site (East Kazakhstan Region, Kazakhstan)**	
	city n[a]=50	**village n[a]=51**	**city n[a]=35**	**village n[a]=46**
Indicators of cytogenetic disorders				
Micronuclei, %	0.85±0.06	0.92±0.06	4.15±0.19*	1.29±0.05*
Protrusions, %	0.02±0.01	0	0.71±0.05*	0.36±0.03*
2-3 m/i[a], %	0.12±0.02	0.18±0.01	2.07±0.08*	0.16±0.02*
Sum	0.99±0.01	1.10±0.01	6.93±0.07*	1.81±0.02*
Proliferation rates				
Binuclear cells, %	0.61±0.04	0.56±0.03	0.66±0.05*	0.45±0.02*
Sum	0.61±0.04	0.56±0.03	0.66±0.05*	0.45±0.02*
Nucleus destruction indicators				
Apoptosis, %	0.05±0.01	0.24±0.01	1.00±0.08*	0.06±0.01*
Integral Evaluation	1.65±0.01	1.90±0.01	8.59±0.14*	2.32±0.03*

Note: [a] accordingly: n - the number of students, m/i – micronuclei;
* p≤0.01, when compared with the data of students in the city and villages of the Aral Region.

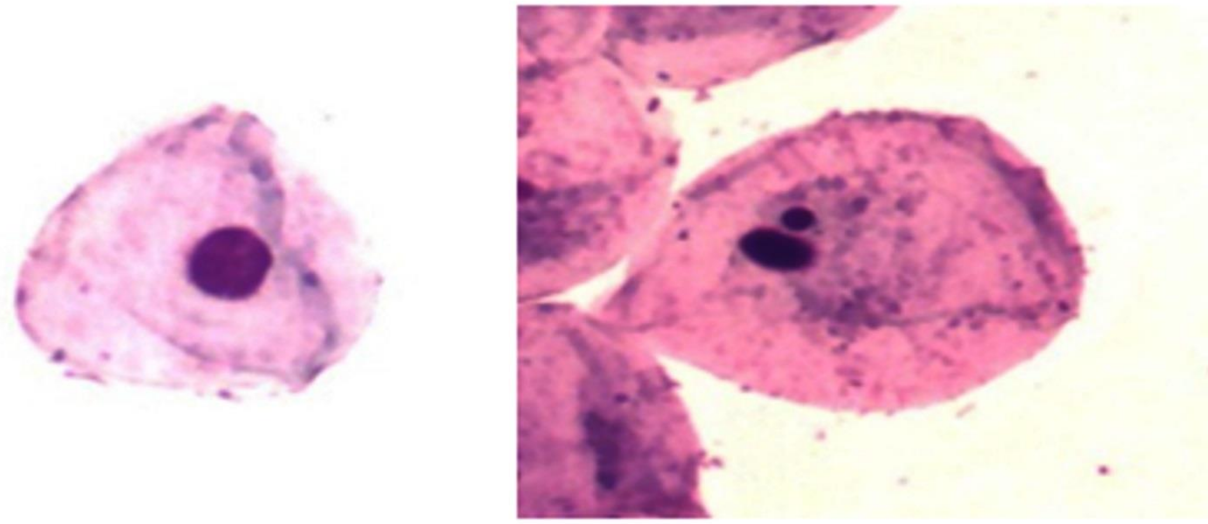

Figure 1. *Normal cell and cell with one micronucleus of the buccal epithelium of the oral cavity (x160)*

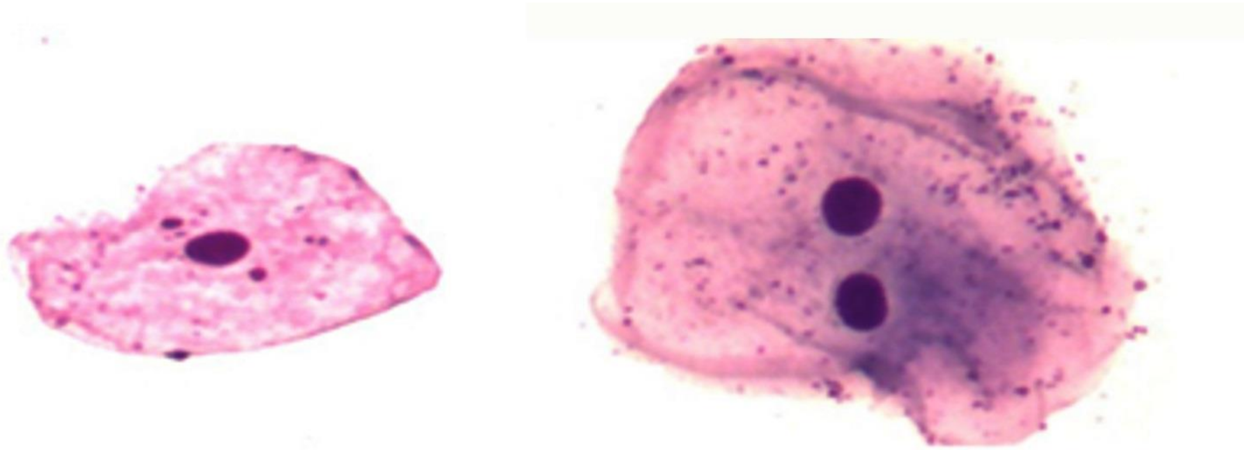

Figure 2. *Cells of the buccal epithelium of the oral cavity with several small and two large micronuclei (x160)*

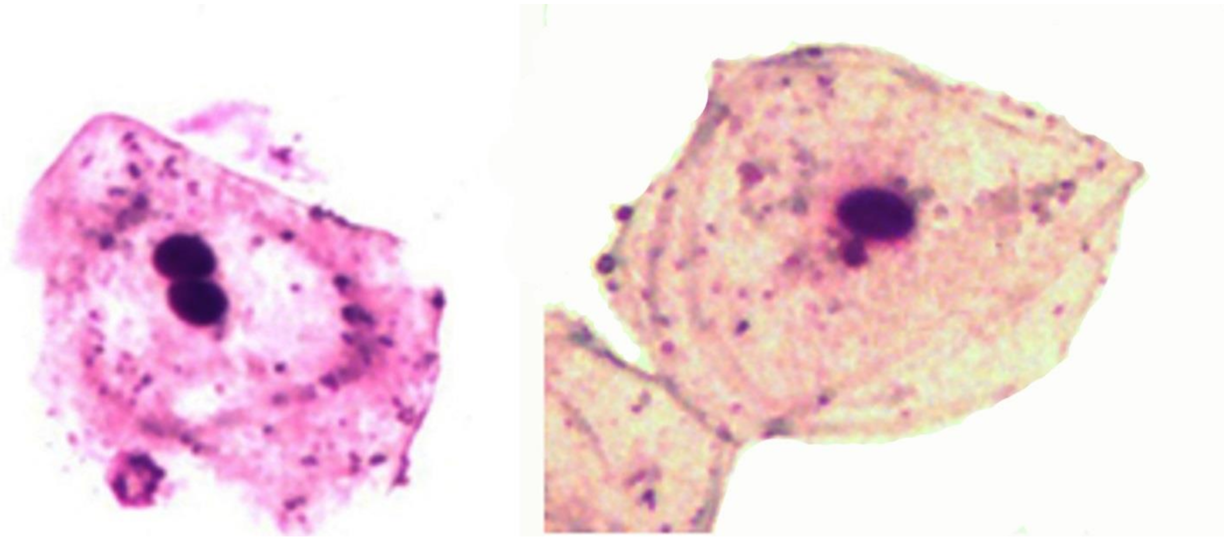

Figure 3. *A core with a circular notch and with a protrusion of the “broken egg” type (x160)*

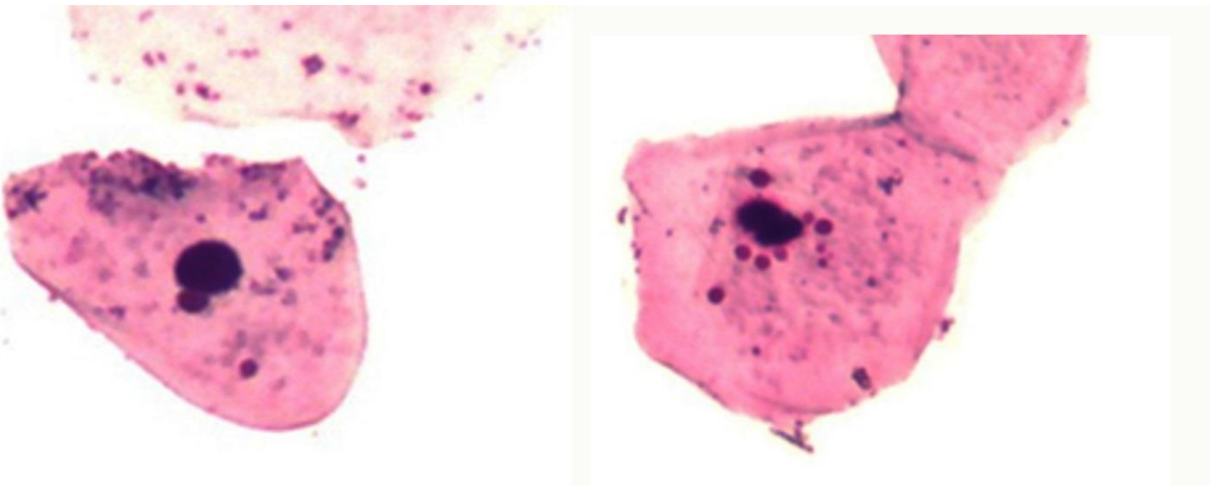

Figure 4. *Cells of the buccal epithelium of the oral cavity with a protrusion of the "bubble" type and with apoptotic bodies (x160)*