PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ANOMALIAS CARIOLÓGICAS NO EPITÉLIO BUCAL DA CAVIDADE ORAL DOS SERES HUMANOS APÓS A COVID-19

KARYOLOGICAL ABNORMALITIES IN THE BUCCAL EPITHELIUM OF THE ORAL CAVITY OF HUMANS AFTER COVID-19

КАРИОЛОГИЧЕСКИЕ АНОМАЛИИ В БУККАЛЬНОМ ЭПИТЕЛИИ РОТОВОЙ ПОЛОСТИ ЧЕЛОВЕКА ПОСЛЕ КОВИД-19

CHEREDNICHENKO, Oksana[1]*; BAKHTIYAROVA, Sholpan[2]; ZHAKSYMOV, Bolatbeck[2]; KAPYSHEVA, Unzira[2]; PILYUGINA, Anastassiya[1]

[1] Institute of Genetics and Physiology, Laboratory of Genetic Monitoring. Kazakhstan.

[2] Institute of Genetics and Physiology, Laboratory of ecological physiology of human and animals. Kazakhstan.

** Corresponding author*
e-mail: cherogen70@mail.ru

Received 03 January 2022; received in revised form 20 February 2022; accepted 23 February 2022

RESUMO

Introdução: As infecções podem ser/são um factor global de danos mutacionais, causando alterações indesejáveis na hereditariedade, tanto nas gerações presentes como nas futuras. Tudo isto torna o problema da mutagénese infecciosa extremamente urgente e requer uma avaliação atempada do efeito mutagénico e assistência terapêutica e preventiva à população em infecções virais altamente patogénicas. **Objectivo:** o estudo foi para avaliar o efeito da COVID-19 sobre o aparelho genético das células epiteliais bucais em pessoas que tinham sido infectadas pelo vírus corona de gravidade variável. **Métodos:** O estado genético das pessoas 2-3 meses após a infecção com COVID-19 foi estudado por análise cariológica e micronúcleo em células epiteliais vestibulares da cavidade bucal. **Resultados e Discussão:** Foi detectado um aumento fiável dos índices cariológicos (perturbações citogenéticas (micronúcleos), índices de proliferação, índices de destruição do núcleo) no epitélio bucal da cavidade oral. As anomalias estudadas foram detectadas em todos os doentes que foram submetidos à doença, independentemente da gravidade da doença e dos contactos. Em doentes com a forma grave da COVID-19, notou-se um aumento significativo das anomalias citogenéticas não só em comparação com os dados do grupo de controlo, mas também com os índices de doentes com formas moderadas e suaves da doença. **Conclusões:** o contacto com o vírus SRA-CoV-2, independentemente da presença de manifestações clínicas, causa anomalias cariológicas no epitélio vestibular da cavidade oral. Isto indica o seu potencial mutagénico significativo.

Palavras-chave: *epitélio bucal; mutagénese induzida por vírus; micronúcleos; COVID-19; anomalias citogenéticas; infecções virais*.

ABSTRACT

Background: Infections can be/are a global factor of mutational damage, causing undesirable changes in heredity both in the present and in future generations. All this makes the problem of infectious mutagenesis extremely urgent and requires timely assessment of the mutagenic effect and therapeutic and preventive assistance to the population in highly pathogenic viral infections. **Aim:** the study evaluated the effect of COVID-19 on the genetic apparatus of buccal epithelial cells in people who had undergone coronavirus infection of varying severity. **Methods:** The genetic status of people 2-3 months after infection with COVID-19 was studied by karyological and micronucleus analysis in buccal epithelial cells of the oral cavity. **Results and Discussion:** Reliable increase of karyological indices (cytogenetic disorders (micronuclei), proliferation indices, nucleus destruction indices) in the buccal epithelium of the oral cavity was detected. The studied abnormalities were detected in all patients who underwent the disease regardless of disease severity and contacts. In patients with the severe form of COVID-19, a significant increase in cytogenetic abnormalities was noted in comparison with the data of the control group and the indices of patients with moderate and mild forms of the disease. **Conclusions:** contact with the SARS-CoV-2 virus, regardless of the presence of clinical manifestations, causes karyological abnormalities in the buccal epithelium of the oral cavity. This indicates its significant mutagenic potential.

DOI: 10.52571/PTQ.v19.n40.2022.05_CHEREDNICHENKO_pgs_43_53.pdf

Keywords: *buccal epithelium; virus-induced mutagenesis; micronuclei; COVID-19; cytogenetic abnormalities; viral infections*

АННОТАЦИЯ

Введение: Инфекции могут быть/являются глобальным фактором мутационного повреждения, вызывая нежелательные изменения наследственности как в настоящем, так и в будущих поколениях. Все это делает проблему инфекционного мутагенеза чрезвычайно актуальной и требует своевременной оценки мутагенного эффекта и терапевтической и профилактической помощи населению при высокопатогенных вирусных инфекциях. **Цель:** оценить влияние COVID-19 на генетический аппарат букальных эпителиоцитов у людей перенесших короновирусную инфекцию различной степени тяжести. **Методы:** Генетический статус людей через 2-3 месяца после заражения COVID-19 был изучен с помощью кариологического и микроядерного анализа в клетках буккального эпителия полости рта. **Результаты и обсуждение:** Выявлено достоверное повышение кариологических показателей (цитогенетические нарушения (микроядра), индексы пролиферации, индексы деструкции ядра) в буккальном эпителии полости рта. Изученные нарушения были выявлены у всех пациентов, перенесших заболевание, независимо от тяжести заболевания и контактов. У пациентов с тяжелой формой COVID-19 отмечалось значительное увеличение цитогенетических аномалий не только по сравнению с данными контрольной группы, но и с показателями пациентов с умеренной и легкой формами заболевания. **Выводы:** контакт с вирусом SARS-CoV-2, независимо от наличия клинических проявлений, вызывает кариологические аномалии в буккальном эпителии ротовой полости. Это указывает на его значительный мутагенный потенциал.

Ключевые слова: *буккальный эпителий; вирусиндуцированный мутагенез; микроядра; COVID-19; цитогенетические аномалии; вирусные инфекции*.

1. INTRODUCTION:

In recent years, geneticists have been very actively involved in studying the contribution of increasing levels of various environmental genotoxicants to the formation and development of hereditary lesions in the human body. In addition to the action of radiation and chemical factors, the effects of so-called biogenic mutagens, which cause damage to the genetic apparatus of cells, particularly chromosomes, are of great relevance. Various DNA- and RNA-containing viruses are firmly among them. When a virus enters the body, a number ofseveral rearrangements develop in various directions that ultimately induce a genotoxic effect in infected cells in a direct and indirect way. The direct pathway of mutagenic potential of viral infection is associated with the ability of some viruses to integrate into the host genome, which can lead to disruption of gene expression at the insertion sites (Saigo *et al.,* 2008) and, consequently, genome instability in these cells (Sung *et al.*, 2012; Gatza *et al.,* 2005; Kadaja *et al.,* 2009). In addition, the frequency of mutations and hence structural abnormalities (chromosomal aberrations, MN) is also affected by the efficiency of DNA repair mechanisms (Chen *et al.,* 2008) and the immune control system (Abe *et al.,* 2019) due to its blocking by viruses (Jasinski-Bergner *et al.,* 2020) or virus-specific proteins (Chang, 2004; Gooding, 1992; Hamid *et al.,* 2009), impaired elimination system, etc. (Machida *et al.,* 2010). Disruption of these processes, in turn, can lead to higher sensitivity to other mutagens. Of course, all these interactions depend on many factors. The virus species, the type of the course of the infectious process, the nature of DNA damage are important.

The secondary nature of the genotoxic effect of infectious viruses is associated with pathological processes leading to partial or complete disruption of cell metabolism (Torre and Oldstone, 1992), including the presence of reactive oxygen species (ROS) resulting from cytokine activity during chronic inflammation (Pal *et al.,* 2010) and the generation of various endomutagen metabolites that lead to mutagenic effects (Yan *et al.,* 2006; Ilyinskikh *et al*., 2012). The consequences of virus-induced mutagenesis depend on many exo- and endogenous factors.

Under conditions of environmental, epidemiological, and social complications, this can lead to all sorts of effects on the human hereditary apparatus and unpredictable combinations of mutagenic factors of biological, physical and chemical nature. Also, virus-induced mutagenesis is associated with the analysis of the contribution of viruses to the

natural mutation process and the evolution of organisms. From the point of view of assessing possible genetic consequences, the effects of widespread viral infections on humans are of particular interest. Under conditions of acute infection, rapid and massive damage to the genetic apparatus of cells occurs. Even in non-permissive cell systems, viruses and viral nucleic acids induce mutations at the chromosomal and gene level (Fortunato *et al.,* 2000). These findings have been confirmed regarding various viruses - hepatitis (Leite *et al*., 2014; Jiang *et al*., 2016; Ozkal *et al.,* 2005), measles (Nicholos *et al.,* 1965), influenza (Tenshow *et al.* 1989), rubella (George *et al.,* 2019), herpes (Arando-Anzaldo, 1992), retroviruses (Rae and Trobridge, 2013), tick-borne encephalitis (Ilyinskikh NN and Ilyinskikh EN, 2017), AIDS (Shah, 2019). Vaccine preparations (Genghini *et al.,* 2006; Kucerova *et al*., 1980) and viral vectors used for gene therapy (adenovirus, adenoassociated virus, retrovirus, lentivirus) (Rhiannon and Ann 2017).

The last two years have been greatly complicated for mankind by the pandemic coronavirus infection, which has posed many challenges to the prevention and treatment of this infectious disease and all sorts of consequences, including those at the genetic level. One of the indicators of the state of the organism is cytogenetic homeostasis, which manifests itself in the maintenance of the karyotype. The micronucleus test can characterize it, the essence of which consists in counting the frequency of cells with micronuclei (Luzhna *et al.,* 2013). Recently, a non-invasive micronucleus test on buccal epitheliocytes of the oral cavity has been used to study the effects of various genotoxicants on humans (Torres-Bugarın *et al.,* 2014). Being in direct contact with metabolites of food and inhaled substances and pathogens of respiratory infections, oral epithelial tissues are the first to experience their genotoxic effects (Bakhtiyarova *et al.,* 2020). The presence of micronuclei and other nuclear abnormalities in epithelial cells is associated with exposure to genotoxic agents, accelerated aging, risk of oral cancer and neurodegenerative disorders. (Leonardi *et al.,* 2020; Ramírez *et al.,* 2019; Hopf *et al.,* 2019). It appeared relatively recently and quickly became one of the most widely used methods in its field. This is because it is fast enough, non-invasive, cost-effective, allows unlimited lifetime screening of subjects, and does not require special equipment for cell culturing However, the study of the consequences of virus-induced mutagenesis of coronavirus infection, including by this method, has not been described in the literature. In this connection, the aim of our study was: to evaluate the frequency of cytogenetic and karyological abnormalities in buccal oral epitheliocytes of people who had undergone COVID-19 infection of different severity, 2-3 months after recovery.

2. MATERIALS AND METHODS:

2.1. Subjects

The study of patients who underwent COVID-19 was conducted in agreement with the Department of Public Health of Almaty, Kazakhstan. All subjects provided written informed consent. In addition, all subjects filled out a questionnaire concerning age, nationality, work activity, type of diet, diseases, alcohol, and smoking. The study group included 53 patients of the rehabilitation center of phthisiopulmonology "*Kamenskoe Plateau*" (Almaty, Kazakh-stan), 2-3 months after COVID-19 infection. According to WHO criteria and national recommendations, the classification of patients according to the severity of the disease was performed in the medical institution at the place of treatment (WHO, 2020; CPDT, 2021; Terwangne *et al*., 2020). All subjects were not vaccinated. Coronavirus infection was treated according to the severity of the disease and the national protocol. According to medical records, among the 53 patients examined, 15 patients were found to have had a severe form of coronavirus infection (34-74 years old, mean age 57 years), 23 were moderately severe (43-73 years old, mean age 61 years), and 15 were mild (28-64 years old, mean age 52 years). In addition, ten contacts (mean age 50 years) who were PCR-negative for coronavirus infection and had no clinical manifestations of respiratory infection were examined. Samples were collected May-June 2021, and karyological analysis was performed July-August 2021. As a control group, data from buccal epithelium samples from 16 healthy individuals before the coronavirus pandemic (mean age 45 years) were used (2018 study results).

2.2. Kariological analysis

Sampling and preparation of buccal epithelial cell preparations are based on the recommendations (Kalaev *et al.,* 2014; Fenech *et al.,* 2011). Buccal epithelium samples were taken by scraping epitheliocytes from the inner surface of the cheek with a plastic spatula for laboratory studies. Immediately epitheliocytes

were resuspended in 100 µl of physiological solution, evenly distributed on the surface of a slide, and air-dried. The preparations were stained with 10% Giemsa's solution (Merck, Germany) for 10 min. The slides were analyzed on a Zeiss AksioLab A.1, Aksioskop 40 microscopes (Germany) at 16x10, 16x40 magnification. Two preparations were prepared from each subject, and 1000 buccal epithelial cells were examined.

Micronuclei and all abnormalities of the epitheliocyte structure differing from the normal morphology were.

2.3. Statistical analysis

Statistical analysis of the data was performed using Excel. The frequency of detected abnormalities was expressed as a percentage, and the arithmetic mean error and its de-viation were calculated. The data were verified using Student's t-test.

3. RESULTS AND DISCUSSION:

3.1. Results

Table 1 presents the results of the karyological analysis of oral buccal epitheliocytes of the examined groups of people. Three types of karyological disorders were identified - cytogenetic, including cells with micronuclei and protrusions (such as "bubble", "broken egg", "tongue"); proliferation indicators (cells with two nuclei, twin nuclei; nuclei with circular incision) and indicators of core destruction (apoptosis, karyolisis, karyopicnosis, karyorexis) (Jindal, 2017; Fenech *et al,* 2011; Nersesyan and Chobanyan, 2010). The most significant changes resulting from COVID-19 infection on human oral buccal epithelial cells concerned cytogenetic abnormalities and indices of nucleus destruction. The frequency of cytogenetic abnormalities was significantly higher than the control level ($p \leq 0.001$) in all patients infected with COVID-19 and contact subjects. The frequency of cytogenetic abnormalities was 1.6 times higher in pa-tients who had a severe form of the disease than in those who had a moderate or mild form of the infection ($p \leq 0.001$). There was no significant difference in the level of cyto-genetic abnormalities between the contact subjects and the patients who had had the disease in the moderate and mild forms. In all groups of examinees, the ratio of the frequency of cells with one micronucleus and 2-3 micronuclei was within (2-3):1. The most characteristic types of cytogenetic abnormalities detected in the examined people are shown in Figure 1A.

The indices of nucleus destruction in all three groups of patients and contacts significantly exceeded the control level ($p \leq 0.001$). Among the indices of nucleus destruction, cells with apoptotic bodies and karyolysis predominated. Moreover, in the greater part of the over-infected patients, either cells with apoptotic bodies or karyolysis predomi-nated (Figure 1B).

The proliferation indices in all groups of subjects were also statistically significantly ($p \leq 0.001$) higher than the control data, although not as significantly as the cytogenetic disorders and indices of nucleus destruction. Cells with nuclei with circular incisions were the most common (Figure 1C). For the nucleus destruction and proliferation indices, no reliable differences were found in the groups with different severity of the disease and contact subjects.

The integral assessment indicates a 4-5-fold increase in karyological disorders in all COVID-19 survivors. Still, changes were more pronounced in patients who had suffered the disease in a severe form. No statistically significant differences were found in the frequency of detected abnormalities concerning age and sex.

3.2. Discussion

There are three main types of virus-induced damage: cytogenetic damage (chromosomal aberrations, chromosome number changes, micronuclei, chromosome fragmentation, or pulverization); degenerative nuclear disorders; disruption of mitotic cell activity. In the early stages of infection, single-cell damage predominates, and in the later stages, degenerative cell damage predominates.

The results of the studies indicate a significant mutagenic effect of coronavirus infection on human somatic cells and the lack of specificity of the spectrum of virus-induced damage at different degrees of severity of the infection. There is evidence of a relatively rapid cytogenetic response of cells in vivo and in vitro when infected with pathogenic viruses (Gershenson, 1986). Active elimination of the resulting disorders usually occurs within 2.5-3 months (Schramayr *et al.,* 1990; Hamada and Mizuno, 1992). However, there is a prolonged persistence of increased levels of genetic abnormalities in some cases. For example, after

smallpox vaccination, normalization of cytogenetic parameters is observed only after 6 months (Gershenson, 1986). Reconvalescents (hepatitis B) have chromosomal aberrations within a year after recovery. At the same time, they had residual clinical phenomena in the recovery period, which showed that chromosomal aberrations indicate a severe form of viral hepatitis (Leite *et al.,* 2014; Jiang *et al.,* 2016).

The persistence of reliably high levels of cytogenetic abnormalities in patients with Covid-19 infection 2-3 months after the disease, the correspondence of the frequency of abnormalities to the severity of the disease draws attention to the following aspects: after 3 months, there is still no elimination of the resulting lesions. According to the literature (Torres-Bugarın, 2014), micronuclei in epitheliocytes reflect genotoxic events that formed in the dividing basal layer 1-3 weeks ago, i.e., exposure to genotoxicants still occurs. This indicates significant disturbances in the genetic apparatus of sensitive cells, functional disorders of the whole organism, and the prolonged nature of the recovery. This is also evidenced by a significant increase in the frequency of destructive nucleus disorders, especially cells with apoptotic bodies and karyolysis, which are indicators of toxicity/genotoxicity. In people with a predominance of apoptotic cells, there is a significant level of cells with 2-3 micronuclei. This may be since, on the one hand, apoptosis is the main mechanism of the elimination of cells with genetic damage. On the other hand, the presence of 2-3 micronuclei in a cell may be an early stage of apoptosis. (Chen *et al.,* 2008).

The high incidence of karyological abnormalities in contact individuals is noteworthy. Probably, the SARS-CoV-2 virus, regardless of clinical manifestations, has mutagenic potential with respect to buccal epitheliocytes of the oral cavity. This is consistent with the literature that the SARS-CoV-2 virus causes a wide range of non-specific oral mucosal lesions (Sewvandini *et al.,* 2021; Erbaş *et al.,* 2021; Iranmanesh *et al.,* 2021).

Since viruses affect cellular metabolism, one should expect that infectious viruses should disrupt the natural course of the cell cycle (Chen *et al.,* 2008; Isaguliants, 2021). Indeed, another group of abnormalities arising from a viral infection changes in the mitotic mode of cells (proliferation rates), which manifests as stimulation or, more often, suppression of mitotic activity, as well as the appearance of abnormal mitoses. These abnormalities are not related to the direct interaction of the virus with DNA; most likely, it is the effect on final stages of cell division (polyploidization with cytokinetic block disturbance) or damage of division spindle and kinetochore region (Gearhart and Bouchard, 2010). Probably, coronavirus infection does not affect the cell cycle so significantly because this type of disorder exceeds the control level only two times, while indicators of genotoxicity (cytogenetic disorders and nucleus destruction indices) are increased more than five times.

Preventing the appearance of virus-induced genomic instability in infections is currently almost impossible. However, the physiological and genetic characteristics of a given organism play an important role. On this basis, the tactics for correcting genomic instability in viral infections are determined. On the one hand, the application of pharmacological principles of genome protection against virus-induced mutagenesis is required. On the other hand, it is necessary to use the entire arsenal of therapeutic influences to inhibit the processes and increase the efficiency of antimutagenic genome protection systems (Zhanataev *et al.,* 2000).

These studies allow us to consider viruses, and Covid-19 in particular, not only as agents of infectious diseases but also as strong genotoxicants with a wide population prevalence, playing a significant role in hereditary variation. Hence, it becomes evident how infections can become a global factor of mutational lesions, causing undesirable changes in heredity in both current and future generations. All this makes the problem of infectious mutagenesis extremely urgent.

4. CONCLUSIONS:

All patients with COVID-19 disease, regardless of the severity of the disease and contact persons, revealed a significant increase in karyological parameters (cytogenetic disorders, proliferation indices, nucleus destruction indices) in the buccal epithelium of the oral cavity;

A significant increase in cytogenetic abnormalities was observed in patients with severe COVID-19 disease compared to the data of the control group and the indices of patients with moderate and mild disease.

Contact with the SARS-CoV-2 virus, regardless of the presence of clinical manifestations, causeed karyological disorders in the buccal

epithelium of the oral cavity of the examined patientes.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample analyzed in the research period.

5.2. Funding source

This work was supported by the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan under research program OR11465435, “Development and application of new genomic technologies to protect organisms from mutagenic influence, increase the productivity of natural resources and improve the quality of life of the population” and grant no. AR9561591 "Assessment of the potential risk of cognitive and cytogenetic disorders in patients after COVID-19 infection".

5.3. Competing Interests

The authors declare no conflict of interest.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and in agreement with the Department of Health of Almaty, Kazakhstan. The Local Ethical Commission also approved it at the "Institute of Human and Animal Physiology" of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan (Minutes № 3(3) of September 15, 2020).

6.2. Informed Consent

All subjects gave written informed consent for the study.

All authors gave informed consent to the publication of this research work.

7. REFERENCES:

1. Abe, T.; Marutani, Y.; Shoji, I. (2019). Cytosolic DNA-sensing immune response and viral infection. *Microbiol Immunol.,* 63, 51–64. https://doi.org/10.1111/1348-0421.12669
2. Arando-Anzaldo, A. (1992). Evidence for an altered kinetics of DNA excision-repair in cells infected by herpes simplex virus type. *Acta virol.,* 36, 417-427.
3. Bakhtiyarova, S.K.; Zhaksymov, B.; Kapysheva, U.N.; Cherednichenko, O.G. Cytogenetic changes in schoolchildren residing in ecologically adverse regions of Kazakhstan (2020). *Periodico Tche Quimica*, 17(35), 1137-1147.
4. Chang, F.; Re F.; Sebastian, S.; Sazer, S.; Luban, J. (2004). HIV-1 vpr induces defects in mitosis, cytokinesis, nuclear structure, and centrosomes. *MolBiol.,*15, 1793–801.
5. Chen, H.Y.; Tang, N.H.; Lin, N.; Chen, Z.X.; Wang, X.Z. (2008). Hepatitis B virus X protein induces apoptosis and cell cycle deregulation through interfering with DNA repair and checkpoint responses. *Hepatol Res*., 38, 174–182.
6. CPDT (Clinical Protocol for diagnosis and treatment) of "Coronavirus infection COVID-19 in adults" (approved by the Joint Commission on the quality of medical services of the Ministry of Health of the Republic of Kazakhstan on July 2021; Volume 15, protocol № 143).
7. Erbaş, G.S.; Botsali, A.; Erden, N.; Arı,

C.; Taşkın, B.; Alper, S.; Vural, S. (2021). COVID-19-related oral mucosa lesions among confirmed SARS-CoV-2 patients: a systematic review. *International Journal of Dermatology*, https://doi.org/10.1111/ijd.15889.
8. Fenech, M.; Holland, N.; Zeiger, E.; Chang, W.P.; Burgaz, S, Thomas, P.; Bolognesi, C.; Knasmueller, S.; Kirsch-Volders, M.; Bonassi S. (2011). The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells--past, present and future. *Mutagenesis,* 26, 239-245.
9. Fortunato, E. A.; Dell'Aquila, Marie L.; Deborah, H. (2000). Specific chromosome 1 breaks induced by human cytomegalovirus. *Spector PNAS*., 97(2), 853–858.
10. Gatza, M. L.; Chandhasin, Ch.; Ducu, R. I.; Marriott, S. J. (2005). Impact of Transforming Viruses on Cellular Mutagenesis, Genome Stability, and Cellular Transformation. *Environmental and Molecular Mutagenesis*, 45, 304-325.
11. Gearhart, T.L.; Bouchard, M.J. (2010). The Hepatitis B Virus X Protein Modulates Hepatocyte Proliferation Pathways To Stimulate Viral Replication. *J Virol.*, 84, 2675–2686. doi: 10.1128/JVI.02196-09
12. Genghini, R.; Tiranti, I.; Bressa´n, E.; Zamorano Ponce; Ferna´ndez, E.J.; Dulout, F. (2006). Determination of genotoxicity of classical swine fever vaccine in vitro by cytogenetic and comet tests. *Mutagenesis*. 21, 213–217.
13. George, S.; Viswanathan, R.; Sapkal, G.N. (2019). Molecular aspects of the teratogenesis of rubella virus. *Biol. Res.,* 52, 47. https://doi.org/10.1186/s40659-019-0254-3.
14. Gershenson, S.M. (1986). Viruses as environmental mutagenic factors. *Mutation Research/Reviews in Genetic Toxicology*., 167(3), 203-213. https://doi.org/10.1016/0165-1110(86)90030-8.
15. Gooding, L.R. (1992).Virus proteins that counteract host immunex defenses. *Cell.,* 71, 5-7.
16. Hamada, K.; Mizuno, K. (1992). Analysis of chromosome aberrations induced by U-5-RNA. Mutat. Res Fund and Mol. Mech. Mutagen., 267(1), 97-104.
17. Hamid, N. A.; Brown, C.; Gaston, K.; (2009). The regulation of cell proliferation by the papillomavirus early proteins. *Cellular and Molecular Life Sciences*, 66(10), 1700–1717.
18. Hopf, N.B.; Bolognesi, C.; Danuser, B.; Wild, P. (2019). Biological monitoring of workers exposed to carcinogens using the buccal micronucleus approach: A systematic review and meta-analysis. *Mutation Research/Reviews in Mutation Research,* 781, 11-29. https://doi.org/10.1016/j.mrrev.2019.02.006.
19. Ilyinskikh, N.N.; Ilyinskikh, E.N. (2017). Age-Related Peculiarities of Cytogenetic Consequences of Spring-Summer Tick-Borne Encephalitis among Residents of Northwestern Siberia due to Polymorphism of Glutathione S-Transferase Genes. *Advances in Gerontology,* 7, 155-157.
20. Ilyinskikh, N.; Ilyinskikh, I.; Ilyinskikh, E. (2012). Infectious mutagenesis: Cytogenetic effects in human and animal cells as well as immunoreactivity induced by viruses, bacteria, and helminthes. Paperback, Lap lambert Academic Publishing.
21. Iranmanesh, B.; Khalili, M.; Amiri, R.; Zartab, H.; Aflatoonian, M. (2021). Oral manifestations of COVID-19 disease: A review article. *Dermatologic Therapy.* 34, 1, e14578. https://doi.org/10.1111/dth.14578.
22. Isaguliants, M.; Krasnyak, S.; Smirnova, O. Colonna, V.; Apolikhin, O.; Buonaguro, F.M. (2021). Genetic instability and anti-HPV immune response as drivers of infertility associated with HPV infection. *InfectAgentsCancer.* 16, 29. https://doi.org/10.1186/s13027-021-00368-1.
23. Jasinski-Bergner, S.; Mandelboim, O.; Seliger, B. (2020). Molecular mechanisms of human herpes viruses inferring with host immune surveillance. *Journal for ImmunoTherapy of Cancer*, 8. DOI:10.1136/jitc-2020-000841..
24. Jiang, L.; Wu, X.; He, F.; Liu, Y.; Hu, X.; Takeda, S.; Qing, Y. (2016). Genetic Evidence for Genotoxic Effect of Entecavir, an Anti-Hepatitis B Virus Nucleotide Analog. *PLoS ONE*, 11(1): e0147440. https://doi.org/10.1371/journal.pone.014

7440
25. Jindal, S.; Chauhan, I.; Grewal, H.K.; (2013). Alteration in buccal mucosal cells due to the effect of tobacco and alcohol by assessing the silver-stained nucleolar organiser regions and micronuclei. *Journal of Cytology*, 30(3), 174–178.
26. Kadaja, M.; Isok-Paas, H.; Laos, T.; Ustav, E.; Ustav, M. (2009). Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. *PLoS Pathogen,.* 5(4). e1000397. DOI: 10.1371/journal.ppat.1000397.
27. Kalaev, V. N.; Artyukhov, V.G.; Nechaeva, M.S. (2014). Micronucleus test of human oral buccal epithelium: problems, achievements, prospects. Cytology and Genetics, 48(6), 62–80.
28. Kucerova, M.; Polivkova, Z.; Matousek, U.; (1980). Chromosomal aberrations and SCE in lymphocytes of children revaccinated against smallpox. *Mutat. Res.,* 71, 263-267.
29. Leite, S.T.; Silva, M.B.; Pepato, M.A.; Souto, F.J.; Santos, R.A.; Bassi-Branco, C.L. (2014). Increased frequency of micronuclei in the lymphocytes of patients chronically infected with hepatitis B or hepatitis C virus*. Mem Inst Oswaldo Cruz.,* 109, 15-20. DOI: 10.1590/0074-0276140183.
30. Leonardi, S.; Poma, A.MG.; Colafarina S.; D'Aloisio, F.; Scatigna, M.; Zarivi, O.; Mastrantonio, R.; Tobia, L.; Fabiani, L. (2020). Early genotoxic damage through micronucleus test in exfoliated buccal cells and occupational dust exposure inconstruction workers: a cross-sectional study in L'Aquila. *Italy Ecotoxicology and Environmental Safety.,* 203. https://doi.org/10.1016/j.ecoenv.2020.110989.
31. Luzhna, L.; Palak, K.; Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Frontiers in Genetics*, 4, 131. https:// doi=10.3389/fgene.2013.00131.
32. Machida, K.; McNamara, G.; Cheng, K.T.; Huang, J.; Wang, C.H.; Comai, L.; Ou, J.H.; Lai, M.M. (2010). Hepatitis C virus inhibits DNA damage repair through reactive oxygen and nitrogen species and by interfering with the ATM-NBS1/Mre11/Rad50 DNA repair pathway in monocytes and hepatocytes. *J Immunol*.185, 6985–6998.
33. Nersesyan, A.; Chobanyan, N. (2010). Micronuclei and other nuclear anomalies levels in exfoliated buccal cells and DNA damage in leukocytes of patients with polycystic ovary syndrome. *J BUON*., 15(2), 337-339.
34. Nicholos, W.W.; Levan, A.; Aula, P.; Norrby, E. (1965). Chromosome damage associated with the measles virus in vitro. *Hereditas,* 54, 101-118.
35. Ozkal, P.; Ilgin-Ruhi, H.; Akdogan, M.; Elhan, A.H.; Kaçar, S.; Sasmaz, N. (2005). The genotoxic effects of hepatitis B virus to host DNA. Mutagenesis. 20, 147-150.
36. Pal, S.; Polyak, S.J.; Bano, N.; Qiu, W.C.; Carithers, R.L.; Shuhart, M.; Gretch, DR; Das, A. (2010). Hepatitis C virus induces oxidative stress, DNA damage and modulates the DNA repair enzyme NEIL1. J Gastroenterol Hepatol,. 25, 627-634.
37. Rae, D.T.; Trobridge, G.D.; (2013). Retroviral Genotoxicity, Gene Therapy - Tools and Potential Applications, Francisco Martin Molina, IntechOpen, DOI: 10.5772/52530. Available from: https://www.intechopen.com/chapters/43278.
38. Ramírez, M.J.; Minguillón, J.; Loveless, S.; Lake, K.; Carrasco, E.; Stjepanovic, N.; Balmaña, J.; Català, A.; Mehta, P.A.; Surrallés, J. (2019). Chromosome fragility in the buccal epithelium in patients with Fanconi anemia. Cancer Letters., 472, 1-7. https://doi.org/10.1016/j.canlet.2019.12.008.
39. Rhiannon, M.D.; Ann, T.D.; (2017). Viral Vectors: The Road to Reducing. *Genotoxicity, Toxicological Sciences,* 155(2), 315–325, https://doi.org/10.1093/toxsci/kfw220.
40. Saigo, K.; Yoshida, K.; Ikeda, R.; Sakamoto, Y.; Murakami, Y.; Urashima, T.; Asano, T.; Kenmochi, T.; Inoue, I. (2008). Integration of hepatitis B virus DNA into the myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of MLL4 in human hepatocellular carcinoma. *Hum Mutat.,* 29, 703-708.
41. Schramayr, S.; Caporossi, D.; Mak, I.; Jelinek, T.; Bacchetti, S. (1990). Chromosomal damage induced by human adenovirus type 12 requires expression of the E1B 55-kilodalton viral

protein. *J. Virol.*, 64(5), 2090-2095.
42. Sewvandini, A. D.; Ravindra, K. R. (2021). Mucosa, Oral. Saliva and COVID-19 Infection in Oral Health Care. *Frontiers in Medicine*, 8, 340. doi:10.3389/fmed.2021.656926.
43. Shah, S.; Singaraju, S.; Bertin, E.T.; Singaraju, M.; Sharma. A. (2019). Quantification of micronuclei in exfoliated cells of human immunodeficiency virus AIDS-infected female patients. *J OralMaxillofacPathol*., 23(2), 301. https:// doi:10.4103/jomfp.JOMFP_251_17.
44. Sung, W.K.; Zheng, H.; Li, S.; Chen, R.; Liu, X.; Li, Y.; Lee, N.P.; Lee, W.H.; Ariyaratne, P.N.; Tennakoon, C.; Mulawadi, F.H.; Wong, K.F.; Liu, A.M.; Poon, R.T.; Fan, ST; Chan, K.L.; Gong, Z.; Hu, Y.; Lin, Z.; Wang, G.; Zhang, Q.; Barber, TD; Chou, W.C.; Aggarwal, A.; Hao, K.; Zhou, W.; Zhang, C.; Hardwick, J.; Buser, C.; Xu, J.; Kan, Z.; Dai, H.; Mao, M.; Reinhard, C.; Wang, J.; Luk, J.M. (2012). Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet., 44, 765-769.
45. Tentsov, Yu.Yu.; Zuev, V.A.; Rzhaninova, A.A.; Schevchenko, A.M.; Bukrinskaya, A.G. (1989). Influenza virus genetic sequences in the blood of children with congenital pathology of the CNS. Arch Virol., 108, 301-306. DOI: 10.1007/BF01310943.
46. Terwangne, C.; Laouni, J.; Jouffe, L.; Lechien, J.; Vincentouillon, R.; Place, S.; Capulzini, L.; Machayekhi, S.; Ceccarelli, A.; Saussez, S.; Sorgente, A. (2020) Predictive Accuracy of COVID-19 World Health Organization (WHO) Severity Classification and Comparison with a Bayesian-Method-Based Severity Score (EPI-SCORE). Pathogens, 9, 880. doi:10.3390/pathogens9110880. www.mdpi.com/journal/pathogens.
47. Torre, I.-C.; de La Oldstone, MBA (1992). Selective disruption of growth hormone transcription machinery by viral infection. Proc. Nat. Acad. Sci. USA, 89(20), 9939-9943.
48. Torres-Bugarın, O.; Zavala-Cerna, M.; Nava, A.; Flores-Garcıa, A.; Ramos-Ibarra, M. (2014). Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells. Dis. Mar., 210, 302– 315. DOI: 10.1155/2014/956835.
49. WHO (World Health Organization). Clinical Management of COVID-19: Interim Guidance. World Health Organization, 2020. https://apps.who.int/iris/handle/10665/332196.
50. Yan, B.; Wang, H.; Rabbani, Z.N.; Zhao, Y.; Li, W.; Yuan, Y.; Li, F.; Dewhirst, M.W.; Li, CY (2006). Tumor necrosis factor-alpha is a potent endogenous mutagen that promotes cellular transformation. *Cancer Res,.* 66, 11565-11570.
51. Zhanataev, A.K.; Durnev, A.D.; Seredin, S.B. (2000). Antimutagenic activity of afobazole in various regimens of treatment. *Bull ExpBiol Med.,* 130, 1077–1079. https://doi.org/10.1007/BF02688183.

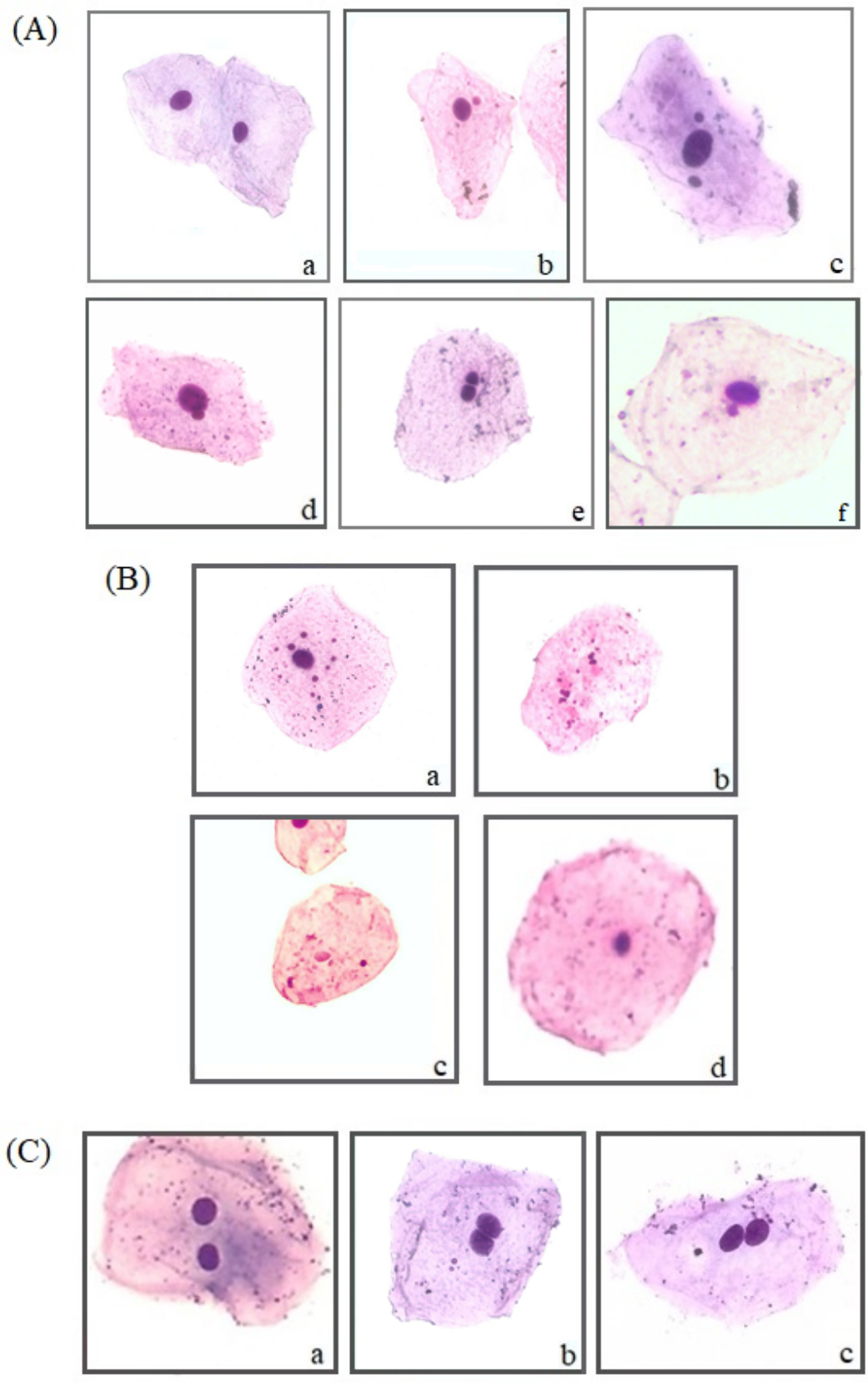

Figure 2. *Types of disorders in buccal epithelium cells of the oral cavity of the examined patients, magnification x 400. (A) Cytogenetic abnormalities (a - normal cell; b, c - cells with one and two micronuclei; d - "vesicle" type protrusion; e - "tongue" type protrusion; f - "broken egg" type protrusion). (B) Indices of nuclei destruction (a - cell with apoptotic bodies; b - karyorexis; c - karyolysis; d – karyopyknosis). (C) Indices of proliferation disorders (a - binuclear cell; b - nucleus with circular notch; c - twin nuclei (amitosis)).*

Table 2. *Frequency of caryological indicators in the buccal epithelium of the oral cavity in people after different degrees of COVID-19 infection.*

Group	Cytogenetic disorders, %	Nucleus destruction indices, %	Proliferation indices, %	Integral score,%
Patients with severe disease	7.79±0.22* **	12.03±0.26*	1.99±0.11*	21.81±0.34*
Patients with moderate disease	4.81±0.13*	13.79±0.22*	1.65±0.08*	20.25±0.25*
Patients with mild disease	4.75±0.18*	12.46±0.29*	1.86±0.12*	19.07±0.34*
Contact	4.06±0.23*	10.33±0.36*	1.49±0.14*	15.88±0.44*
Control	1.4±0.14	2.14±0.17	0.89±0.11	4.43±0.23
* p≤0.001 – reliability of the difference compared to the control indicators				
** p≤0.001 - reliability of the difference compared to other groups of subjects				