GEORGIAN MEDICAL NEWS

ISSN 1512-0112

No 1 (262) Январь 2017

ТБИЛИСИ - NEW YORK



ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

No 1 (262) 2017

Published in cooperation with and under the patronage of the Tbilisi State Medical University

Издается в сотрудничестве и под патронажем Тбилисского государственного медицинского университета

გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან თანამშრომლობითა და მისი პატრონაჟით

> ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ ТБИЛИСИ - НЬЮ-ЙОРК

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Версия: печатная. Цена: свободная. Условия подписки: подписка принимается на 6 и 12 месяцев. По вопросам подписки обращаться по тел.: 293 66 78. Контактный адрес: Грузия, 0177, Тбилиси, ул. Асатиани 7, III этаж, комната 313 тел.: 995(32) 254 24 91, 995(32) 222 54 18, 995(32) 253 70 58 Fax: +995(32) 253 70 58, e-mail: ninomikaber@hotmail.com; nikopir@dgmholding.com

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IMPACT OF SMOKING HABITS ON THE STATE OF CHROMATIN AND MORPHOLOGY OF BUCCAL EPITHELIAL CELLS AMONG MEDICAL STUDENTS

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Smoking is a destructive habit, but is not perceived in society as a very harmful habit. People mostly are poorly aware of the hazards associated with smoking; in particular, among the students of medical higher educational institutions the percentage of smokers is high [1,16].

Professional position of physician provides an excellent opportunity to conduct propaganda about the dangers of smoking, because doctors are in contact with a large part of the population and, therefore, have the opportunity to help people change their behavior, give advice and recommendation to stop smoking, to answer questions related to the consequences of tobacco consumption. Even brief advice of health care professional about the harmful effects of smoking and importance of quitting is one of the effective methods of reducing smoking. However, the health worker himself should be an example for patients. If the recommendation is given by the doctor who smokes the patient's motivation to stop smoking is reduced [15]. Unfortunately, the smoking habit is not rare among medical students. The data of experimental observations on cytological changes, induced by smoking, presented in anonymous form, may be an additional argument among medical students against smoking habit.

In the number of works the changes in buccal cells of smokers were shown. For instance, oral keratinocytes were evaluated using flow cytometry. Among smokers compared to non-smokers, the DNA damages were registered. Namely, the DNA adducts (8-hydroxy-2'-deoxyguanine (8-OHdG) and benzo[a]pyrene (B[a]P)) were indicated. Other lesions, as increased DNA content, aneuploidy, percentage of cells in (S) and G(2)+(M) phases, and also apoptosis were registered[9].

The mean AgNOR numbers per nucleus in the group of smokers was higher than among non-smokers (p<0.001). The mean nuclear area (NA) of cells of smokers group @ *GMN*

was significantly higher, than among non-smokers group (p<0.05). Authors hypothesize that results of both AgNOR counts and nuclear area show that cellular proliferation issignificantly higher in the group of smokers and this can indicate shift towards dysplasia[14].

Experimental results revealed that NA, nuclear perimeter (NP), minimal nuclear diameter (ND-min), and maximal nuclear diameter (ND-max) values of the buccal mucosa cell nuclei of smokers were higher than those of non-smokers. The difference was statistically significant in the case of NA, ND-min, and ND-max values [6].

Mean NA for smokers was elevated compared with nonsmokers. Mean cell area(CA) in smokers was decreased as compared to non-smokers but the difference was not statistically significant. Also, NA/CA ratio was elevated in smokers group. Authors suggest that the increase in NA and NA/CA ratio, and also decrease in CA are due to smoking [3].

An increase in NA, the NA/CAratio, and ratio of maximum NDto minimum ND were observed, while a decrease in CA were observed in lateral surface of the tongue, buccal mucosa and floor of the mouth of smokers and also waterpipe users (p≤0.001). No statistically significant differences were observed in percent of karryorhexis, vacuolization of cytoplasm, and two- or multilobed nuclei in oral mucosa of smokers, waterpipe users (p=0.8), and normal individuals (p=0.9) in buccal mucosa, tongue, and mouth floor areas. The parallel analysis revealed that the percentage of inflammation and Candidacontamination among smokers (p<0.001) and waterpipe users (p=0.002) were higher than among non-smokers[10]. The statistically significant decrease in CA and an increase in NA and NA/CA ratio were observed in tobacco users[7]. Changes in buccal cell nucleus morphology among smoking students also were shown in [2]. The quantities of nuclei with tongue- and broken egg-type protrusions, micronuclei, binuclear cells, as well as a significant decrease in CA and increase in NA and NA/CA ratio were detected in the group of smokers as compared to the control group. In authors' opinion, these changes indicate the presence of local inflammation resulting from the toxic effects of smoking products on the oral mucosa [2].

At the same tine no differences between groups of 9 smoking and non-smoking women in micronuclei test in buccal cells weredetected [4]. At the same time significantly high frequency of micronuclei (p<0.01) and binucleate cells (p<0.05) were observed in the group of smokers as compared to non-smokers. A dose-response relationship was observed between smoking and DNA damage [17]. In the study of 90 individuals of which 46 were smokers and 44 were non-smokers was found a statistically significant increase in the micronuclei quantity among smokers (p<0.05) [5]. The mean percentage of aneuploid nuclus wasstatistically higher in the group of smokers (39.3%) [13].

The purpose of the present work was to investigate the morphology of buccal cells in relation to smoking habits among students. In this investigation the special attention is paid to changes in the state of chromatin condensation, previously non-studied comparatively among smokers and non-smokers. Also we investigated in comparative aspect the group of hookah smokes, this group of smokers previously not attracted the attention of many researchers.

Material and methods. The students of the Faculty of Dentistry and of the Medical Faculty of the Kharkiv National Medical University were used as goodwill donors of buccal epithelium cells. The study was performed in accordance with the European Convention on Human Rights and Biomedicine (1997) and also of Declarations and Recommendations of the First, the Second and the Third National (Ukrainian) Congresses of Bioethics (Kiev, Ukraine, 2001, 2004, 2007) and Ukrainian legislation. All participants gave written informed consent prior to participation in the research. The research was approved by the Board of Bioethics of the V.N.Karazin Kharkiv National University.

The 81students with different smoking habits were examined. Cells of buccal epithelium were obtained from donor's cheek were obtained by scraping with sterile blunt spatula. Cells were stained by 2% orcein solution in 45% acetic acid [11]. Cells were photographed at a magnification x 400 by microscope MICMED-7 (Russia). Morphometric measurements were performed on cell photographs. Insample of cells of each donor were assessed 100cells and the mean values of all parameters were calculated. The heterochromatin granules quantity (HGQ) cell nucleus area (NA), cell area (CA), nucleus perimeter (NP), and cell perimeter (CP) were assessed. All results were processed statistically by Student method. In Fig. 1-5 are presented mean values and standard errors of mean. The variants which significantly differ from control (p>0.05) are marked by asterisk (*).

Results and their discussion. It was conducted a survey among 115 volunteer students of 2nd, 3^d year of study of the Dental Faculty and of 5th year of study of the Medical Faculty of Kharkov National Medical University, among which 66 (57.3%) girls and 49 (42.6%) of young men aged 17-25 years old.

According to the survey among smokers: 18% smokers start smoking up to 14 years old, 32% of students - from 14-16 years old, 40% at the age of 17-19 years old, and 10% started smoking after 19 years old.

Distribution of smokers by the number of cigarettes consumed daily shows that 78% of medical students smoked less than 10 cigarettes per day, 18% – about 20 cigarettes, more than one pack of up to 4% of all students participating in the survey.

All students taking part in experiment (n=103) were divided in connection to their smoking habits into 6 groups: consuming light cigarettes (L) n=8; consuming heavy cigarettes (H) n=15; united group of smokers of cigarettes n=23; consuming hookah (water-pipe) n=13; consuming both hookah and cigarettesn=6; and non-smoking students n=38.

In Fig. 1 are presented the data on the HGQ in buccal epithelium cells of students of groups with different smoking habits.



Fig. 1. HGQ (heterochromatin granules quantity) in buccal epithelium cells of non-smoking and smoking donors

The data of Fig. 1 demonstrate increase of HGQ, and therefore, chromatin condensation, among smokers of hookah (water pipe), in both variants; hookah only and hookah and also cigarettes. We observed only a tendency of increase of HGQ in cells of cigarette smokers.



Fig. 2. Cell nucleus area (NA) in buccal epithelium cells of non-smoking and smoking donors

In the Fig. 2 are demonstrated data on nuclear area (NA) in cells. In our experiment only among hookah smoker vas observed significant changes in NA. Decrease of NA was detected among smokers of hookah and cigarettes hookah.



Fig. 3. Cell nucleus perimeter (NP) in buccal epithelium cells of non-smoking and smoking donors

In the Fig. 3 are presented data on nuclear perimeter (NP) in different groups of donors. As one can see, the PN proved to be most sensitive to smoking habits and decreased in all groups of smokers. The most decrease is shown for the group of hookah smokers. The NA in our experiment not changed significantly in heavy cigarettes smokers; only tendency of NA decrease was registered. In all other groups of smokers NA decrease was significant. Hookah smokers had the most effect on NA.

In the Fig. 4 are presented data on cell area (CA) among non-smokers and smokers. From the data of Fig. 4 it is seen that the CA decreased in all groups of smokers. Among smokers of heavy cigarettes it was observed only tendency of CA decrease, and in other groups it was significant. The cell perimeter (CP), like the NP, proved to be sensitive to smoking habits, demonstrating decrease in all groups of smokers (Fig. 5).



Fig. 4. Cell area (CA) in buccal epithelium cells of nonsmoking and smoking donors



Fig. 5. Cell perimeter (CP) in buccal epithelium cells of non-smoking and smoking donors

It was previously demonstrated that different stress factors (inhibitors of metabolism, elevated sub lethal temperature, ultraviolet and microwave irradiation) induce the process of heterochromatinization in buccal cell nuclei [12]. Significant increase in the HGQ or heterochromatization in nuclei of buccal cell epithelium in group of hookah smokers (Fig. 1) indicate the fact that cells are in the state of stress connected with factors of hookah (chemical factors of smoke and elevated temperature). As t is known the process of heterochromatinization is connected with decrease of transcriptional activity [8]. The fact that among other groups of smokers only in groups of hookah smokers the increase of heterochromatization was observed, and in these groups NAdecrease was the most indicates the more unfavorable effect of hookah smoking as compared to cigarettes smoking.

The data obtained in this work indicate changes innuclear and cell dimensions of buccal epithelium cells among smokers. As a rule, among smokers are observed decrease of NA and CA, decrease of NP and CP. We connect these processes with elevated rate of apoptosis in buccal epithelium cells connected with smoking. As to CA decrease (Fig. 4), our experimental data are in a good agreement with results of works [2, 3, 7, 10]. Therefore, the observed in this work decrease of NA in buccal cells of smokers (Fig. 2) is in contradiction with results of works [2, 3, 6, 7, 10, 14] demonstrating increase of NA in cells of smokers. We also demonstrated decrease in NP (Fig. 3) that contradicts to results of NP increase in buccal epithelium cells of smokers (Fig. 14]. In our opinion, the only possible explanation of differences between our results and previously reported, may be connected with more intensive smoking in groups of smokers in Kharkiv.

Conclusions. Summing up, the smoking of cigarettes and hookah induces significant decrease in nuclear and cell perimeter and cell area in cells of buccal epithelium. Smoking of hookah induces, besides, the hererochromatization in cell nucleus and the decrease of nuclear area. These data show unfavorable effects of smoking cigarettes and even more harmful effect of hookah smoking.

Conflict of interest. Authors declare no conflict of interest.

REFERENCES

1. Almas K., Maroof F., Mcallister C., Freeman R. Smoking behaviour and knowledge in high school students in Riyadh and Belfast. Odonto-Stomatologie Tropicale. 2002; 25(98): 40-44.

2. Asadov R.I., Morozova E.N., Zabolotnaya S.V., Mikhailik T.A., Morozov V.N. Microscopic features of buccal epithelium in smokers students indo-dravidian race (By E. Hooton). Research Result. Medicine and Pharmacy Series. 2015; 1(6): 50-53.

3. Babuta S., Garg R., Mogra K., Dagal N. Cytomorphometrical analysis of exfoliated buccal mucosal cells: effect of smoking. Acta Medica International. 2014; 1(1): 26-31. 4. Blaszczyk E., Mielzynska-Svach D. Micronucleus assay in epithelial cells from the oral cavity and urinary tract in female smokers and non-smokers. Environmental Biotechnology 10 (2) 2014; 60-65.

5. Farha A.A.S. The effects of smoking on micronucleus frequencies in buccal cells of healthy Iraqi individuals. World Journal of Pharmaceutical Research 2015;4(9): 406-415.

6. Göregen M., Akgül H.M., Gündoğdu C. The cytomorphological analysis of buccal mucosa cells in smokers. Turk J Med Sci. 2011; 41(2): 205-210.

7. Khot K., Deshmane S., Bagri-Manjarekar K., Warke D., Kotak K. A cytomorphometric analysis of oral mucosal changes in tobacco users. Journal of Natural Science, Biology, and Medicine 2015;6 (Suppl 1):S22-S24.

8. Krebs J.E., Goldstein E.S., Kilpatrick S.T. Lewin's Genes XI. Jones and Bartlett Learning, Burlington, MA: 2014; 940.

9. Schwartz J.L., Muscat J.E., Baker V., Larios E., Stephenson G.D., Guo W., Xie T., Gu X., Chung F.L. Oral cytology assessment by flow cytometry of DNA adducts,

aneuploidy, proliferation and apoptosis shows differences between smokers and non-smokers. Oral Oncol. 2003; 39(8):842-854.

Seifi S., Feizi F., Mehdizadeh M., Khafri S., Ahmadi B. Evaluation of cytological alterations of oral mucosa in smokers and waterpipe users. Cell J. 2014; 15(4): 302–309.
Shckorbatov Y.G. He-Ne laser light induced changes in the state of chromatin in human cells. Naturwissenschaften.
1999; 86(9):452-453.

12. Shckorbatov Y. The state of chromatin as an integrative indicator of cell stress. In: New Developments in Chromatin Research, Editors: Neil M. Simpson and Valerie J. Stewart. Chapter 6. Nova Science Publishers, Inc. New York: 2012; 123-144.

13. Souto G.R., Caliari M.V., Lins C.E., de Aguiar M.C., de Abreu M.H., Mesquita R.A. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med. 2010; 39(8):605-610.

14. Usta U., Berberoğlu U., Helvaci E., Altaner Ş., et al. Evaluation of cytological alterations in normal-appearing oral mucosal epithelia of smokers and non-smokers via AgNOR counts and nuclear morphometry. Trakya Univ Tip Fak Derg. 2008; 25(2):110-116.

15. Vinogradov P.B., Medyankin A.V., Solovyov L.N., Dmitruk I.I., Romashevskaya N.Y. The spread of harmful habits among medical students and their relation to it. Problems of social hygiene, and medical history. 1996; 1: 49-51 (in Russian).

16. Vokhmintseva L.V., Yuzenas T.P., Vanyunina V.V., Tereshchenkov A.O. The prevalence of tobacco smoking among students from 1th to 3th years of the Novosibirsk state medical university. Journal of New Medical Technologies 2010; 17(4): 205-208 (in Russian). http://www.medtsu. tula.ru/VNMT/Bulletin/2010/10B4.pdf

17. Yadav A.S., Saini M. Increased frequency of nuclear anomalies in exfoliated buccal mucosa of cigarette smokers. Journal of Entomology and Zoology Studies 2015; 3(2): 7-10.

SUMMARY

IMPACT OF SMOKING HABITS ON THE STATE OF CHROMATIN AND MORPHOLOGY OF BUC-CAL EPITHELIAL CELLS AMONG MEDICAL STUDENTS

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The cells of buccal epithelium were investigated in groups of smoking and non-smoking students. Cell samples were collected by scraping with blunt sterile spatula, stained with orcein and photographed. The GEORGIAN MEDICAL NEWS No 1 (262) 2017

smoking of cigarettes and hookah induces significant decrease in nuclear and cell perimeter and cell area in cells of buccal epithelium. Smoking of hookah induces, besides, the heterochromatization in cell nuclei and the decrease of nuclear area. The data obtained indicate stress reaction in cells (heterochromatinization) and apoptosis-related changes in cells (decrease of nuclear and cell perimeter and cell area). These data show unfavorable effects of smoking cigarettes and even more harmful effect of hookah smoking.

Keywords: cell nucleus; heterochromatin; cell area; nuclear area; cell perimeter, nuclear perimeter; toxic effects of smoking.

РЕЗЮМЕ

ВЛИЯНИЕ КУРЕНИЯ НА СОСТОЯНИЕ ХРОМАТИНА И МОРФОЛОГИЮ КЛЕТОК БУККАЛЬНОГО ЭПИТЕЛИЯ У СТУДЕНТОВ-МЕДИКОВ

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Клетки буккального эпителия исследовали в группах курящих и некурящих студентов. Пробы клеток отбирали, соскабливая посредством тупого стерильного шпателя, окрашивали орсеином и фотографировали. Морфометрические измерения проводились на микрофотографиях клеток. Курение сигарет и кальяна вызывает значительное снижение ядерного и клеточного периметров и площади клеток буккального эпителия. Курение кальяна индуцирует гетерохроматинизацию в ядрах клеток и уменьшение площади ядер. Полученные данные указывают на стрессовую реакцию клеток (гетерохроматинизация) и связанные с апоптозом изменения в них (уменьшение площади ядер и периметра и площади клеток). Эти данные указывают на неблагоприятные последствия курения сигарет и еще более вредное воздействие курения кальяна.

რეზიუმე

მწეველობის გავლენა ლოყის ლორწოვანი გარსის ეპითელიუმის ქრომატინსა და მორფოლოგიაზე სტუდენტ-მედიკოსებში

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მწეველ და არამწეველ სტუდენტებში გამოკვლეულია ლორწოვანი გარსის ეპითელიუმის უჯრედები. უჯრედების ნიმუშები ამოკვეთილი იყო ბლაგვი სტერილური შპატელის მეშვეობით, რის შემდეგაც შეიღება ორსეინით და ფოტოგრაფირებული იქნა. მორფოლოგიური გაზომვები ჩატარდა უჯრედების მიკროფოტოგრაფიებზე. გამოვლინდა, რომ სიგარეტის და ჩილიმის მოწევა იწვევს ბირთვული და უჯრედული პერიმეტრის, უჯრედის ბუკალური ეპითელიუმის ფართობის მნიშვნელოვან შემცირებას. კვლევის შედეგები მიუთითებენ უჯრედების სტრესულ რეაქციაზე და აპოპტოზურ ცვლილებებზე. სტატიის ავტორებს გამოტანილი აქვთ დასკვნა ადამიანის ორგანიზმზე სიგარეტის და ჩილიმის მოწევის საზიანო ზემოქმედების შესახებ.