8. Lindford A, Valtonen J, Hult M, Kavola H, Lappalainen K, Lassila R, et al. The evolution of the Helsinki frostbite management protocol. Burns. 2017 Nov;43(7):1455-1463. doi: 10.1016/j.burns.2017.04.016.
9. McIntosh SE, Freer L, Grissom CK, Rodway GW, Giesbrecht GG, McDevitt M, et al. Wilderness Medical Society Clinical Practice Guidelines for the Prevention and Treatment of Frostbite: 2024 Update. Wilderness Environ Med. 2024 Jun;35(2):183-197. doi: 10.1177/10806032231222359.
10. Schellenberg M, Cheng V, Inaba K, Foran C, Warriner Z, Trust MD, et al. Frostbite injuries: independent predictors of outcomes. Turk J Surg. 2020 Jun 8;36(2):218-223. doi: 10.5578/turkjsurg.4632.
11. Shoib S, Arif N, Nahidi M, Rumiyya K, Swed S, Yusha'u Armiya'u A. Nagorno-Karabakh conflict: Mental health repercussions and challenges in Azerbaijan. Asian J Psychiatr. 2022 Jul;73:103095. doi: 10.1016/j.ajp.2022.103095.
12. Turner BL, van Dongen TTCF, Berendsen RR, de Jong FJM, Endert EL, van Hulst RA, et al. Frostbite: a treatment guideline for prehospital treatment in a military environment. BMJ Mil Health. 2025 Mar 21;171(2):152-154. doi: 10.1136/military-2023-002380.
13. van Dongen TTCF, Berendsen RR, de Jong FJM, Endert EL, van Hulst RA, Hoencamp R. Frostbite: a systematic review on freezing cold injuries in a military environment. BMJ Mil Health. 2025 Jan 28;171(1):81-85. doi: 10.1136/military-2022-002171.
14. Zaramo TZ, Green JK, Janis JE. Practical Review of the Current Management of Frostbite Injuries. Plast Reconstr Surg Glob Open. 2022 Oct 24;10(10):e4618. doi: 10.1097/GOX.0000000000004618.

Стаття надійшла 11.05.2024 р.

DOI 10.26724/2079-8334-2025-2-92-20-24
UDC 616.31–008.8–022+616.12/.13–002

H.O. Babenia, O.V. Dienha, S.A. Shnaider, G.V. Nikolayeva, V.M. Pochtar, O.E. Korniichuk[1], I.M. Lukovych[2]
State Establishment "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine", Odesa, [1]Dnipro State Medical University, Dnipro, [2]Danylo Halytsky Lviv National Medical University, Lviv

QUANTITATIVE DETERMINATION OF PERIODONTOPATHOGENS IN ELDERLY PATIENTS WITH ATHEROSCLEROSIS

e-mail: annababenya@gmail.com

Dental-plaque pathogens can translocate via the bloodstream, accumulate in atheromatous plaques of various arteries, or directly infect vascular endothelial cells, thereby taking an active part in the progression of atherosclerotic lesions. We performed a quantitative analysis of the "red-complex" periodontopathogens Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia, as well as Aggregatibacter actinomycetemcomitans, Porphyromonas endodontalis, Prevotella intermedia and Fusobacterium nucleatum in gingival crevicular fluid. From every patient three to six of the targeted periodontopathogens were detected when a high-sensitivity threshold was applied. T. forsythia and F. nucleatum were identified in 100 % of samples, P. endodontalis in 77.8 %, P. gingivalis in 44.4 %, and T. denticola, A. actinomycetemcomitans and P. intermedia in 33.3 %. In all study participants, onset and/or progression of atherosclerosis may, to varying degrees, be attributable to the presence of large numbers of periodontopathogens in the oral cavity.

Key words: atherosclerosis, generalised periodontitis, older age, periodontopathogens, polymerase chain reaction.

Г.О. Бабеня, О.В. Дєньга, С.А. Шнайдер, Г.В. Ніколаєва, В.М. Почтар, О.Є. Корнійчук, І.М. Лукович

КІЛЬКІСНЕ ВИЗНАЧЕННЯ ПАРОДОНТОПАТОГЕНІВ У ПАЦІЄНТІВ ПОХИЛОГО ВІКУ З АТЕРОСКЛЕРОЗОМ

Патогени зубного нальоту можуть транслокуватися кровотоком і накопичуватися в атероматозних бляшках різних артерій або безпосередньо інфікувати ендотеліальні клітини судин, беручи, таким чином, безпосередню участь у прогресуванні атеросклеротичних уражень. Нами проведено кількісний аналіз пародонтопатогенів «червоного комплексу» Porphyromonas gingivalis, Treponema denticola та Tannerella forsythia, а також Aggregatibacter actinomycetemcomitans, Porphyromonas endodontalis, Prevotella intermedia та Fusobacterium nucleatum у рідині ясенної часу. У зразках усіх пацієнтів виявлено від 3 до 6 аналізованих пародонтопатогенів під час використання порога високої чутливості. T. forsythia та F. nucleatum виявлені у 100 % зразків, P. endodontalis – у 77,8 %, P. gingivalis – у 44,4 %, T. denticola, A. actinomycetemcomitans та P. intermedia – у 33,3 %. У всіх пацієнтів даного дослідження виникнення та/або прогресування атеросклерозу можуть у тій чи іншій мірі бути обумовлені присутністю великих кількостей пародонтопатогенів у ротовій порожнині.

Ключові слова: атеросклероз, генералізований пародонтит, похилий вік, пародонтопатогени, полімеразно-ланцюгова реакція.

The work is a fragment of the research project "Improving the provision of dental care to the elderly under martial law", state registration No. 0123U103245.

Cardiovascular diseases (CVD) are among the leading causes of mortality in developed countries, accounting for 17.3 million deaths worldwide each year, with an expected rise to 23.6 million by 2030 owing to lifestyle changes and population ageing [15].

Development of atherosclerosis – the principal aetiological factor of CVD – is linked to enhanced penetration of low-density and very-low-density lipoproteins into the arterial subendothelial space, provoking an inflammatory response in the arterial intima [1].

Key risk factors for atherosclerosis include hyperlipidaemia and hypercholesterolaemia, several chronic systemic diseases (e.g., hypertension and diabetes), unhealthy diet and obesity, sedentary lifestyle, smoking, excessive stress, environmental factors and genetic predisposition [7]. More recent studies have demonstrated associations between atherosclerosis and dysbiosis of both gut and oral microbiota. In particular, subgingival periodontal pathogens can translocate haematogenously into atherosclerotic plaques located in different arteries and directly contribute to atherogenesis [12]. Periodontal diseases caused by oral pathogens increase CVD mortality risk by 47 % whereas periodontal therapy and improved oral hygiene lower the risk of atherosclerotic cardiovascular complications [4]. Therefore, dental-plaque pathogens may constitute one of the risk factors for development and/or progression of atherosclerosis and, consequently, CVD.

The purpose of the study was to detect and quantify A. actinomycetemcomitans, P. gingivalis, P. endodontalis, T. denticola, T. forsythia, P. intermedia and F. nucleatum in gingival-crevicular-fluid samples obtained from individuals with atherosclerosis.

Materials and methods. Nine participants (5 women, 55.6 %; 4 men, 44.4 %) aged 61–69 years (mean 66.0±1.03, σ 2.92) with generalised periodontitis (GP) in the context of atherosclerosis were examined. GP stages I–II predominated (66.7 %); stages II and II–III were diagnosed in 33.3 %.

Microbiological samples were collected after an overnight fast and before morning oral hygiene. Sterile endodontic paper points (size 25) were inserted into the gingival sulcus or periodontal pocket for 15 s, transferred immediately into sterile, hermetically sealed 1.5 mL Eppendorf tubes containing 1 mL physiological saline, and transported to the laboratory in a cold-chain container.

Bacterial DNA was extracted with the "DNA-EXPRESS" kit (NPF "Litekh") according to the manufacturer's protocol. Presence and quantity of the seven periodontopathogens were determined by real-time PCR using the "Dentoscreen for Quantitative Analysis, Fluoropol-RV format (OneStep Strip)" kit (NPF "Litekh") on a CFX96 Touch Real-Time PCR system (Bio-Rad, USA) with a high-sensitivity cutoff (> 1 000 bacterial copies per sample). The research was conducted in the molecular genetic laboratory of the State Establishment "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (headed by T.H. Verbytska, PhD). Logarithmic ($\log_{10}$) transformation of bacterial counts was performed in Microsoft Excel 2010; statistical analyses were carried out in Statistica 6.1 (StatSoft Inc., Serial No. AGAR909E415822FA) under Windows XP. Student's *t*-test was used to assess significant differences [2].

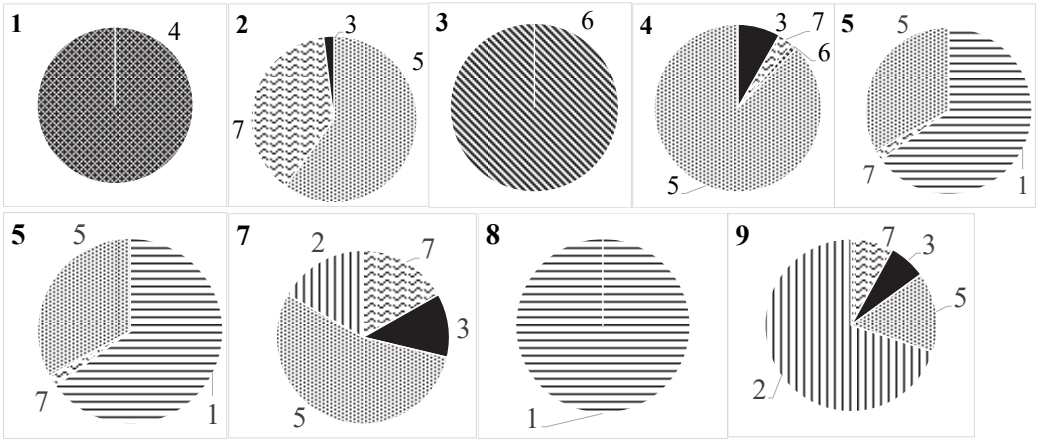
Results of the study and their discussion. A quantitative assessment of seven periodontopathogens was performed in samples from nine patients with atherosclerosis by real-time PCR, applying a high-sensitivity threshold whereby the pathogen was recorded as present if >1000 copies of bacterial DNA were detected in the sample.

The results obtained for the periodontopathogens in individuals with generalised periodontitis on the background of atherosclerosis, before (A) and after (B) logarithmic ($\log_{10}$) transformation of the raw data, are shown in Fig. 1.

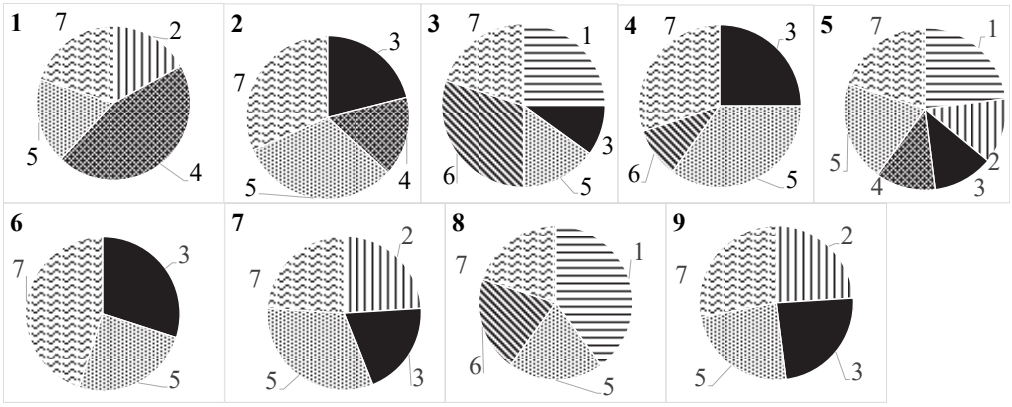
In four patients one of the following pathogens was present in dominant quantities (>95–99 % of all bacteria in the sample): Aggregatibacter actinomycetemcomitans (patient 8), Prevotella intermedia (patient 3), Fusobacterium nucleatum (patient 6) and the red-complex member Treponema denticola (patient 1; Fig. 1A). In three additional patients (2, 4 and 7) the red-complex bacterium Tannerella forsythia predominated (> 50 % of bacterial DNA in the sample; Fig. 1A). This species, together with F. nucleatum (Fig. 1B), was detected in some quantity in every patient; in five patients it accounted for >10 % of the total number of targeted pathogens identified (Fig. 1A). The next most frequent pathogen was Porphyromonas endodontalis, found in 78 % of patients (Fig. 1B), including three in whom it comprised >10 % of the load (Fig. 1A).

The third red-complex member, Porphyromonas gingivalis, was present in samples from four patients (Fig. 1B), but exceeded 10 % only in two of them (patients 7 and 9; Fig. 1A). These two samples also contained appreciable amounts of T. forsythia. The least frequent pathogens – each observed in three of nine patients – were A. actinomycetemcomitans, P. intermedia and the red-complex bacterium T. denticola.

None of the samples contained all seven investigated periodontopathogens. With respect to microbial associations, patients were distributed as follows: a three-species association in one patient; four species in five patients; five species in two patients; and six species in one patient (Fig. 1B).



A



B

Fig. 1. Quantitative distribution of seven periodontopathogens in samples from individuals with generalised periodontitis and concomitant atherosclerosis before (A) and after (B) logarithmic (log_{10}) transformation of the primary data. 1 – Aggregatibacter actinomycetemcomitans; 2 – Porphyromonas gingivalis; 3 – Porphyromonas endodontalis; 4 – Treponema denticola; 5 – Tannerella forsythia; 6 – Prevotella intermedia; 7 – Fusobacterium nucleatum.

Considering the red-complex pathogens separately, the presence of at least one pathogen in a predominant proportion (>50 % of bacterial DNA in the sample) or a combination of two to three red-complex species was observed in 66.7 % of individuals (Fig. 1A, B).

Over recent decades, the cumulative literature has confirmed periodontal disease – particularly periodontitis – as a risk factor for atherosclerotic pathology [9]. Periodontitis is a chronic multifactorial inflammatory disease caused by dysbiosis of the oral microbiota and leads to progressive destruction of the bone and periodontal tissues supporting the teeth [6]. P. gingivalis, T. denticola and T. forsythia constitute the so-called red complex, which is associated with severe clinical manifestations or chronic forms of periodontitis and is thought to play a pivotal role in its pathogenesis [8]. In one study, red-complex species T. denticola and P. gingivalis were detected in 51 % and 45 %, respectively, of coronary-plaque samples from 51 patients with chronic periodontitis [4].

In a comparative analysis of the bacterial composition of atherosclerotic plaques and dental plaque in 81 patients scheduled for coronary-artery bypass grafting or angioplasty, a statistically significant concordance was demonstrated for both P. gingivalis ($p=0.007$) and T. forsythia ($p=0.001$). Moreover, every patient with one or more periodontopathogens in their atherosclerotic plaques had chronic periodontitis [10].

In our study, one red-complex bacterium predominated (>50 % of the total pathogens detected) in 44.4 % of samples – namely T. denticola or *T.* forsythia. We assume that these pathogens may likewise contribute to atherosclerosis progression in those patients. T. forsythia and F. nucleatum were present to some extent in every sample examined.

Previous work has shown that periodontal pathogens can directly infect vascular endothelial cells [4], and that T. forsythia and F. nucleatum enhance the ability of P. gingivalis to invade gingival-epithelial and endothelial cells [14], thereby facilitating direct cellular damage. In vitro, infection of endothelial cells with P. gingivalis induces mitochondrial fragmentation, elevates mitochondrial reactive oxygen species, lowers mitochondrial membrane potential and depletes intracellular adenosine triphosphate [13]. Mitochondrial dysfunction is therefore considered a mechanism by which P. gingivalis accelerates atherosclerosis; endothelial invasion correlates positively with the bacterial load of this pathogen [3].

In the present study, the detection of P. gingivalis at appreciable levels (>10 %) in samples from patients 7 and 9 may be linked to atherosclerosis progression – particularly as both patients also carried high loads of T. forsythia and F. nucleatum, which potentiate P. gingivalis invasion of endothelial cells.

P. endodontalis can also penetrate vascular cells, as shown in vitro [4]. Endodontic lesions involving this species are associated with an increased risk of ischemic heart disease, especially acute coronary syndrome [11]. Significant quantities of P. endodontalis were detected in only 33.3 % of participants (patients 3, 7 and 9), all of whom also harbored red-complex pathogens. Consequently, P. endodontalis did not dominate any sample, precluding conclusions about its direct link with atherosclerosis, although a synergistic effect with other pathogens cannot be excluded.

A meta-analysis of 14 studies on myocardial-infarction patients showed that A. actinomycetemcomitans is the second most frequently detected pathogen in coronary atheromatous plaques after P. gingivalis. In carotid-plaque samples, detection frequencies were 18 % for A. actinomycetemcomitans, 26 % for P. gingivalis and 14 % for P. intermedia [5].

In our cohort, A. actinomycetemcomitans was found in three patients (3, 5 and 8), comprising >50 % and >99 % of the total pathogen load in patients 5 and 8, respectively. P. intermedia likewise appeared in three patients (3, 4 and 8), accounting for >99 % of the load in patient 3. Another study demonstrated a significant correlation between the abundance of P. intermedia in subgingival plaque and in atherosclerotic plaques from patients with ischaemic heart disease [4]. Accordingly, we anticipate the presence of P. intermedia in the atherosclerotic plaques of patient 3 and possibly A. actinomycetemcomitans, F. nucleatum and T. denticola in the plaques of patients 8, 6 and 1, respectively.

Conclusion

All the periodontopathogens investigated are, in one way or another, associated with the risk of developing atherosclerosis or cardiovascular disease. Biological samples from every patient with generalised periodontitis and atherosclerosis contained three to six of the seven targeted pathogens; collectively, all seven were present in the study population. Specifically, T. forsythia and F. nucleatum were detected in every sample, P. endodontalis in 77.8 %, P. gingivalis in 44.4 %, and T. denticola, A. actinomycetemcomitans and P. intermedia in 33.3 %. In 44.4 % of cases one pathogen (F. nucleatum, T. denticola, A. actinomycetemcomitans or P. intermedia) dominated (>95–99 %). Such patients warrant particular attention when exploring the association between specific oral superinfections and the risk of atherosclerosis onset and progression. In at least 66.7 % of subjects, atherosclerosis development may be linked to a substantial presence (>50 %) of a single red-complex pathogen, or to a consortium of two to three red-complex species; this hypothesis, however, requires further confirmation. In all participants, the onset and/or progression of atherosclerosis may be associated with a considerable burden of periodontopathogens in the oral cavity.

References

1. Kazymyrko VK, Silantyeva TS, Dubkova OH, Krylova OS, Kutovyy VV, Ivanitska LM. Ateroskleroz: klitynno-molekulyarni mekhanizmy rozvytku zapalennya v arteriyakh (uzahal′nennya problemy). Likarska sprava. 2022; 1–2: 3. DOI: https://doi.org/10.31640/2706-8803-2022-(1-2)-03 [in Ukrainian].

2. Rohach IM, Keretsman AO, Sitkar AD. Pravylno vybranyy metod statystychnoho analizu – shlyakh do yakisnoyi interpretatsiyi danykh medychnykh doslidzhen. Naukovyy visnyk Uzhhorodskoho universytetu, seriya “Medytsyna”. 2017;2(56):124–128. [in Ukrainian].

3. Aleksijević LH, Aleksijević M, Škrlec I, Šram M, Šram M, Talapko J. Porphyromonas gingivalis Virulence Factors and Clinical Significance in Periodontal Disease and Coronary Artery Diseases. Pathogens. 2022; 11(10): 1173. DOI: 10.3390/pathogens11101173.

4. Czerniuk MR, Surma S, Romańczyk M, Nowak JM, Wojtowicz A, Filipiak KJ. Unexpected Relationships: Periodontal Diseases: Atherosclerosis-Plaque Destabilization? From the Teeth to a Coronary Event. Biology (Basel). 2022; 11(2): 272. DOI: 10.3390/biology11020272.

5. Joshi C, Bapat R, Anderson W, Dawson D, Hijazi K, Cherukara G. Detection of periodontal microorganisms in coronary atheromatous plaque specimens of myocardial infarction patients: A systematic review and meta-analysis. Trends Cardiovasc Med. 2021; 31(1): 69–82. DOI: 10.1016/j.tcm.2019.12.005.

6. Łasica A, Golec P, Laskus A, Zalewska M, Gędaj M, Popowska M. Periodontitis: etiology, conventional treatments, and emerging bacteriophage and predatory bacteria therapies. Front. Microbiol. 2024; 15: 1469414. DOI: 10.3389/fmicb.2024.1469414.

7. Libby P. The changing landscape of atherosclerosis. Nature. 2021; 592(7855): 524–533. DOI: 10.1038/s41586-021-03392-8.

8. Mahdi KA, Abdulridha WM, Mohi A, Al-fahham AA. Pathogenic Bacteria Associated with Periodontitis. International Journal Of Health & Medical Research. 2024; 3(6): 287–290. DOI: 10.58806/ijhmr.2024.v3i06n05.

9. Priyamvara A, Dey AK, Bandyopadhyay D, Katikineni V, Zaghlol R, Basyal B, et al. Periodontal inflammation and the risk of cardiovascular disease. Curr. Atheroscler. Rep. 2020; 22(7): 28. DOI: 10.1007/s11883-020-00848-6.

10. Rao A, D'Souza C, Subramanyam K, Rai P, Thomas B, Gopalakrishnan M, et al. Molecular analysis shows the presence of periodontal bacterial DNA in atherosclerotic plaques from patients with coronary artery disease. Indian Heart J. 2021; 73: 218–220. DOI: 10.1016/j.ihj.2021.01.011.
11. Rus M, Negruțiu BM, Sava CN, Pasca G, Andronie-Cioara FL, Crisan S, et al. The Association Between Periodontal Disease and Acute Coronary Syndrome – A Clinical Analysis. Journal of Clinical Medicine. 2025; 14(7): 2447. DOI: https://doi.org/10.3390/jcm14072447.
12. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: Consensus report. J Clin Periodontol. 2020; 47(3): 268–288. DOI: 10.1111/jcpe.13189.
13. Xu T, Dong Q, Luo Y, Liu Y, Gao L, Pan Y, Zhang D. Porphyromonas gingivalis infection promotes mitochondrial dysfunction through Drp1-dependent mitochondrial fission in endothelial cells. Int. J. Oral Sci. 2021; 13: 28. DOI: 10.1038/s41368-021-00134-4.
14. Zhang Z, Liu S, Zhang S, Li Y, Shi X, Liu D, et al. Porphyromonas gingivalis outer membrane vesicles inhibit the invasion of Fusobacterium nucleatum into oral epithelial cells by downregulating FadA and FomA. J Periodontol. 2022; 93(4): 515–525. DOI: 10.1002/JPER.21-0144.
15. Zhao D, Wang Y, Wong ND, Wang J. Impact of Aging on Cardiovascular Diseases. JACC Asia. 2024; 4 (5–6); 345–358. DOI: 10.1016/j.jacasi.2024.02.002.

Стаття надійшла 02.06.2024 р.

DOI 10.26724/2079-8334-2025-2-92-24-29
UDC 617.7-001.4+617.7-003.6

H.O. Bayramova, E.M. Gasimov
National Ophthalmology Center named after Academician Z. Aliyeva, Baku, Azerbaijan

ANALYSIS OF EYE AND ITS ADNEXA INJURIES INVOLVING INTRAOCULAR FOREIGN BODIES

e-mail: med_avtor@mail.ru

An analysis was conducted on the structure of injuries of eye and its adnexa involving foreign bodies among patients hospitalized at the National Ophthalmology Center named after Academician Zarifa Aliyeva. A total of 2,225 inpatient records from 2019 to 2023 were reviewed, among which 394 cases involved the presence of superficial or intraocular foreign bodies. In cases of closed globe injuries, superficial foreign bodies were identified in 29.3 % of cases (n=194), with the cornea being the most frequent site of localization (57.7 % of all such cases). In open globe injuries – a statistically significant portion of all injuries – 64.8 % of cases involved foreign bodies localized in the vitreous body. Among all injuries affecting the ocular adnexa, foreign bodies were noted in the eyelid region in 12 cases (6.9 %). The composition of foreign bodies varied depending on the site where the injury occurred. The predominantly organic nature of the foreign bodies highlights the importance of assessing the timeliness of patient presentation to prevent bacterial complications in the injured eyes.

Key words: eye injuries, penetrating, nonpenetrating, intraocular foreign bodies.

Х.О. Байрамова, Е.М. Гасимов

АНАЛІЗ ТРАВМ ОКА ТА ЙОГО ПРИДАТКОВОГО АПАРАТУ З НАЯВНІСТЮ СТОРОННІХ ТІЛ

Проведено аналіз структури травм ока та його придаткового апарату з наявністю чужорідних тіл у пацієнтів, госпіталізованих до Національного центру офтальмології імені академіка Заріфи Алієвої. Було вивчено 2 225 історій хвороби за період з 2019 по 2023 рік, з яких у 394 випадках відзначалася наявність поверхневих або внутрішньоочних чужорідних тіл. За закритих травм ока поверхневі чужорідні тіла виявляли в 29,3 % випадків (n=194), причому найчастішою їх локалізацією була рогівка (57,7 % від усіх вивчених закритих травм). За відкритих травм очного яблука, які склали статистично значущу частку всіх травм, у 64.8 % випадків чужорідні тіла локалізувалися в склоподібному тілі. Серед усіх травм, що зачіпають придатковий апарат ока, у ділянці повік чужорідні тіла відмічено в 12 випадках (6.9 %). Склад чужорідних тіл варіював залежно від місця отримання травми. Переважно органічний характер чужорідних тіл підкреслює важливість своєчасного звернення пацієнтів за медичною допомогою з метою запобігання бактеріальних ускладнень пошкодженого ока.

Ключові слова: травми ока, проникаючі, непроникаючі, внутрішньоочні чужорідні тіла.

Ocular trauma with the presence of foreign bodies in open and closed globe injuries is one of the most complex and dangerous forms of eye injuries that require immediate specialized care [3, 8]. Nowadays, the relevance of this problem throughout the world and in Azerbaijan is due to both the high level of ocular trauma and the complexity of treatment, which requires a highly qualified approach and the use of advanced diagnostic technologies. According to various authors, there is currently an increase in the number of cases of traumatic eye injuries, which is associated with the expansion of industrial activity, an increase in road accidents and an increased interest in sports [1, 7]. In addition, in various branches of agriculture, there has also been an increase in the number of cases associated with the penetration of foreign bodies into the eye area. An increase in the frequency of eye injuries among the elderly is also of great