It is known that minerals play an important role in the regulation of various functions of the body and each cell separately, their optimal content is necessary for the formation of the body’s adaptive response, for maintaining human health, while some of them are involved in the development of pathological processes. The imbalance of trace elements is one of the causes of membranotoxic enzymatic effects of cell structure and function disorders, imbalance of the body’s microflora, increased lipid peroxidation, activation of adhesion processes, disruption of intercellular homeostasis, etc. Changes in the elemental composition of the oral fluid play an important role in the development of dental pathology, such as dental caries and periodontal tissue diseases. Oral fluid is highly sensitive to the effects of adverse factors, is a convenient informative non-invasive medium for clinical, including early, diagnosis of human health, and can show macro- and microelemental imbalances, which can be used as an additional marker for assessing adverse effects, such as tobacco smoke.

The aim of the study was to investigate the effect of smoking on the macro- and microelement composition of oral fluid in teenagers and young adults.

Materials and methods of the study. To achieve this goal, we studied the state of macro- and microelement composition of oral fluid (calcium, iron, copper, zinc and manganese using atomic absorption spectrophotometry) in 114 teenagers and young adults aged 15 to 24 years (50 teenagers (15 to 18 years) and 64 young adults (18 to 24 years), who were divided into groups: Group I included 26 people who regularly smoke traditional cigarettes; Group II - 22 people who regularly smoke electronic cigarettes (vapes); Group III - 23 people who regularly smoke tobacco heating devices (IQOS); Group IV - 43 people without a smoking habit.

Results of the study. It was found that the amount of copper in the oral fluid of the participants of the study of group I increased by 2.6 times (p<0.05) compared to the values in group IV. In teenagers and young adults subjects of groups II and III, an increase in copper was also recorded, but much less - by 1.3 times (p<0.05). The decrease in the content of iron in the oral fluid in the subjects of group I, compared with the reference values in group IV, was 2.2 times (p<0.05). In teenagers and young adults subjects of groups II and III, a slight decrease in the trace element was also recorded - by 1.4 times (p<0.05). It was found that in the subjects of group I there was a decrease in the content of zinc in the oral fluid, compared with the reference values in group IV - by 2.7 times (p<0.05). In teenagers and youthful subjects of groups II and III, a decrease in the trace element was also recorded - by 1.4 and 1.3 times, respectively (p<0.05). The study of the amount of manganese in the oral fluid in teenagers and young people of group I, compared with the values in group IV, decreased by 2.8 times (p<0.05). In teenagers and young adults subjects of groups II and III, a decrease in the trace element was also recorded - by 1.5 and 1.3 times, respectively (p<0.05). The analysis of the data shows that in the participants of the study of group I, an increase in the amount of calcium in the oral fluid was recorded compared to the values in group IV - by 1.5 times (p<0.05). In teenagers and young adults of groups II and III, an increase in calcium was also recorded - by 1.3 times (p<0.05).

Conclusion. The obtained results of the study of the state of macro- and microelement composition of oral fluid in teenagers and young adults indicate the presence of an imbalance, the severity of which depended on the presence of a bad habit and type of smoking: an increase in the amount of copper and calcium and a decrease in the amount of iron, zinc and manganese were found, indicating a decrease in the activity of the antioxidant system. Indicators of the state of macro- and microelement composition of oral fluid can be an early prognostic test for assessing the state of the oral cavity in teenagers and young adults smokers.

Key words: teenagers and young adults, macro- and microelements, oral fluid, smoking.

Problem statement and analysis of the latest research. Macro- and microelements play a significant role in many physiological processes of the body (redox reactions, protein synthesis, tissue differentiation and growth, interaction with nucleic acids, etc.), in the regulation of various functions of the body and each cell separately, their optimal content is necessary for the formation of the body’s adaptive response, for maintaining human health, and also participate in the development of pathological processes. For example, trace elements are structural elements of tissues (Ca, P, S), they play an important role in the processes of haematopoiesis (Fe, Co, Cu), tissue respiration, intracellular metabolism, and are part of metalloproteins, hormones (I, Zn, Cu, Fe, Se,
V, Mo), and enzymes (Fe, Zn, Mg, Mn, Cu, K). Todate, modern methods have identified 81 elements in the human body, of which 32 are clinically important, and their deficiency contributes to the development of various diseases and irreversible changes. Thus, the deficiency of certain trace elements, such as Cu, Fe, Mo, Se, Zn, Ca, disrupts the balance of most metabolic processes in the body, while even a slight increase in the level of essential trace elements has a toxic effect. In addition, different elements interact with each other, and this interaction can be both antagonistic and synergistic. It has been established that there are 105 two-way and 455 three-way relationships between the 15 vital elements. For example, there is a physiological antagonism between copper, on the one hand, and manganese, zinc, and calcium, on the other. Therefore, it is not so much the average concentrations of trace elements that should be taken into account as the nature of the relationship between them. Trace element imbalance is one of the causes of membranotoxic enzymatic effects of cell structure and function disorders, imbalance of the body’s microflora, increased lipid peroxidation, and activation of adhesion processes [1, 2, 11, 13, 19, 20]. In addition, micronutrient imbalance initiates a cascade of pathological reactions, such as activation of subclinical chronic mild inflammation and contributes to the development of oxidative stress; physiological transformations of adipokines change, intercellular homeostasis is disturbed and disorders occur at the cellular and microvascular levels, epithelial-mesenchymal interaction changes and endoplasmic reticulum stress develops, progenitor cell migration is disturbed, etc [16, 18].

Oral fluid is highly sensitive to the effects of adverse factors, is a convenient informative non-invasive medium for clinical, including early, diagnostics of human health, and can show macro- and microelement imbalances, which can be used as an additional marker for assessing adverse effects, such as tobacco smoke. Normally, the oral fluid is characterised by various properties and a certain constancy of composition, ensuring the body’s interaction with the external environment through the intake of macro- and micronutrients, water, and inhaled air, and with the internal environment through the intake of bioregulators, intermediates, and end products of metabolism. It is known that the elemental status of oral fluid depends on genetic characteristics and is formed under the influence of a number of factors (diet, place of residence, profession), and also reflects the quantitative intake of pollutants. Changes in the elemental composition of the oral fluid play an important role in the development of dental pathology, such as dental caries and periodontal tissue diseases. Analysis of elemental imbalances can also be a reliable criterion for assessing the effectiveness of treatment and the formation of risk groups for hypo- and hyperemesis [1, 2, 3, 12, 17, 21].

The results of studies of micro- and macroelements of oral fluid in dental diseases have been reported in the literature, but the effect of different types of smoking on mineral indices in teenagers and young adults has not been sufficiently studied. It is known that trace elements, acting as antioxidants and prooxidants, play an important role in the formation and maintenance of equilibrium in the system «free radical generation - free radical detoxification» [4, 10, 15]. Therefore, in order to assess the level of antioxidant protection, it would be advisable to analyse the content of essential trace elements in the oral fluid, which are cofactors of enzymes – zinc, copper, iron, magnesium and calcium, a decrease in the concentration of which can lead to a decrease in enzyme activity. Thus, the issue of studying the impact of smoking on the mineral parameters of oral fluid in teenagers and young adults remains relevant.

The aim of the study was to investigate the effect of smoking on the macro- and microelement composition of oral fluid in teenagers and young adults.

Materials and methods of the study

To achieve this goal, we’ve studied the condition of macro- and microelements in the oral fluid of 114 teenagers and young adults aged 15 to 24 years (50 teenagers (15 to 18 years) and 64 young adults (18 to 24 years), who were divided into groups: Group I – included 26 people who regularly smoke traditional cigarettes; Group II – 22 people who regularly smoke electronic cigarettes (vapes); Group III – 23 people who regularly smoke tobacco heating devices (IQOS); Group IV – 43 people without a smoking habit. All participants in the study did not complain of somatic health disorders and were not registered with related specialists.

Oral fluid for the study was collected in the morning, on an empty stomach, without stimulation, after preliminary rinsing the mouth with distilled water, by spitting it out 3 minutes after rinsing into sterile measuring containers. In order to assess the impact of different types of smoking, the content of biometals in the oral fluid: calcium, iron, copper, zinc and manganese, taking into account their antioxidant-prooxidant, coenzyme and osteotropic functions, was studied using atomic absorption spectrophotometry. To determine the concentration of these biometals, the oral fluid was prepared for determination according to the method of G.O. Babenko: dried at a temperature of 100-200°C, and then ash was isolated in a muffle furnace at a temperature of 450-500°C. Mineralization was carried out until the ash was free of coal impurities. The method is based on spraying a solution of mineralate in an air- acetylene flame and measuring the resonant absorption of atoms of the element of interest using an atomic absorption spectrophotometer (ATOMIC ABSORPTION SPECTROPHOTOMETER AA-7000 SHIMADZU).

For the statistical processing of the material during the study, computer programs based on Microsoft Excel
were used, in which the materials were grouped by the study population (calculation of relative and average values, their errors, t-test). Some of the data development tasks were performed using licensed statistical analysis packages “Microsoft Excel” and “Statistica 12.0”; in particular, descriptive statistics, pairwise and multiple correlation and regression analysis, and graphical representation.

The study was performed in compliance with the main provisions of GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (from 04.04.1997), the Helsinki Declaration of the World Medical Association on Ethical Principles of Scientific Medical Research with Human Participation (1964-2013), orders of the Ministry of Health of Ukraine dated 09.23.2009 No. 690, dated 08.03.2012 No. 616. The clinical and laboratory research protocol was approved by the ethics committee of the Ivano-Frankivsk National Medical University (protocol No. 119/21 dated 02.24.2021).

Research results and their discussion

Oral fluid is a multicomponent complex structure formed by the secretion of large and small salivary glands, which includes inorganic (macro- and microelements such as calcium, magnesium, phosphorus, potassium, sodium, copper, zinc, silicon, iron, manganese, etc.) and organic components, as well as microorganisms and their waste products, gingival fluid, periodontal pocket contents, desquamated epithelium, migrating leukocytes, food residues, etc. The examination of oral fluid is characterized by simplicity and convenience, the absence of infection and the possibility of multiple sampling, informativeness and high sensitivity, which allows for early diagnosis of diseases, as well as evaluation of the effectiveness of treatment [1, 3, 9, 12].

The results of the study of the concentration of macro- and microelements in the oral fluid of teenagers and young adults revealed a difference between the reference values in non-smokers and study participants with a bad habit (Fig. 1, Fig. 2).

Copper as an essential trace element, is a component of many enzymes (about 25), participates in metabolic processes, plays a major role in maintaining the structure of bones, cartilage, tendons, elasticity of blood vessel walls, skin, etc. In addition, the element is one of the main antioxidants in the blood, a catalyst for the oxidation of ascorbic acid, adrenaline, serotonin, and ensures the balance of the concentration of biogenic amines in the blood [5, 8, 13, 21]. The results of the study indicate an increase in the amount of copper in the oral fluid of the participants of the study group I compared to the values in group IV – 2.6-fold (p<0.05). In teenagers and young adults of groups II and III, an increase in copper was also recorded, but much less – 1.3-fold (p<0.05).

Iron as one of the priority trace elements – plays an important role in energy metabolism and redox processes: it blocks toxic hydrogen peroxides by neutralising them with catalase, participates in haematopoiesis; promotes full functioning of components of nonspecific defence, cellular and local immunity, phagocytosis, sufficient activity of natural killer cells, synthesis of lysozyme, interferon. It has been established that iron metabolism is closely related to the metabolism of other trace elements. Deficiency of the element can occur in case of disorders of copper, zinc, and manganese metabolism [7, 8, 13, 16]. Thus, the results obtained indicate a decrease in the content of iron in the oral fluid in the subjects of group I, compared with the reference values in group IV – 2.2-fold (p<0.05). In teenagers and young adults of groups II and III, a decrease in iron was also recorded, but much less – 1.3-fold (p<0.05).

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![Fig. 1. Indicators of the level of trace elements Cu, Fe, Zn, Mn in the oral fluid of participants in the comparison groups (mg/kg).](image-url)
adults from groups II and III, a slight decrease in the trace element was also recorded – 1.4-fold (p<0.05).

Zinc is an important trace element, without which the normal functioning of the human body is impossible, and performs various functions: the trace element is a co-factor of a large group of enzymes (about 300, for example, DNA and RNA polymerases, phosphatases, dehydrogenases, carboxypeptidases, etc.) involved in protein, carbohydrate and other types of metabolism. In addition, zinc takes an active part in the regulation of free radical reactions and cellular protection against damage by oxygen metabolites, as it is the active centre of the cytosolic enzyme superoxide dismutase (Cu/Zn-SOD) and acts as an antioxidant [6, 8]. It has been found that in the subjects of group I there is a decrease in the content of zinc in the oral fluid, compared with the reference values in group IV – 2.7-fold (p<0.05). In teenagers and young adults from groups II and III, a decrease in the trace element was also recorded – 1.4-fold and 1.3-fold, respectively (p<0.05).

Manganese is also included into the list of essential trace elements, which is involved in the vital process of energy accumulation and transfer. At the initial stages of immune defence, the element plays the role of an intermediary between inflammation and antioxidant systems. In addition, manganese is one of the essential components for Mn superoxide dismutase (MnSOD), which is primarily responsible for the scavenging of reactive oxygen species (ROS) in mitochondrial oxidative stress [16, 19, 20]. The study of the amount of manganese in the oral fluid in teenagers and young adults of group I, compared with the values in group IV, decreased 2.8-fold (p<0.05). In teenagers and young adults from groups II and III, a decrease in the trace element was also recorded – 1.5-fold and 1.3-fold, respectively (p<0.05).

Calcium is the most abundant and important mineral in the human body, helps regulate the metabolism of nutrients between the cell and intercellular space; promotes the elimination of a number of metals and radionuclides; is a powerful antioxidant and antistressor, etc. It is known that 99% of the macronutrient is concentrated in bones and teeth. At the same time, it has been determined that excess calcium in the oral fluid can be deposited in the form of mineralised dental plaque, which plays an important role in the pathogenesis of periodontal tissue diseases [14, 17].

The analysis of the data shows that in the participants of the study group I, an increase in the amount of calcium in the oral fluid was recorded compared to the values in group IV – 1.5-fold (p<0.05). In teenagers and young adults from groups II and III, an increase in calcium was also recorded – 1.3-fold (p<0.05) (Fig. 2).

Conclusions
The results of the study of the macro- and microelemental composition of oral fluid condition in teenagers and young adults indicate the presence of an imbalance, the severity of which depended on the presence of a bad habit and type of smoking:
1. an increase in the amount of copper and calcium and a decrease in the amount of iron, zinc and manganese were found;
2. the results indicate a decrease in the activity of the antioxidant system;
3. indicators of the state of macro- and microelement composition of oral fluid can be an early prognostic test for assessing the state of the oral cavity in teenagers and young adult smokers.

Prospects for further research in this area are to study changes in the state of macro- and microelement composition of oral fluid depending on the age subgroup,
smoking duration and treatment measures in teenagers and young adult smokers.

Ethical standards (See Statement of Human and Animal Right).

Conflict of Interest. The authors declare no conflict of interest.

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