ORIGINAL ARTICLE

CONTENTS

The content of copper, iron and manganese in the saliva and blood of individuals with generalized periodontitis working in harmful industries

Zoryana B. Popovych, Volodymyr I. Sarapuk, Veronika Yu. Kateryniuk, Iryna Z. Chubiy, Iryna Z. Ostapyak, Natalia M. Pavelko, Oleksandra V. Bazalytska

IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: To investigate the content of Copper, Iron, and Manganese in the saliva and blood of patients affected by generalized periodontitis who have been working in harmful industries for an extended period.

Materials and Methods: The study enrolled 133 employees of the Burshtyn Thermal Power Plant (TPP). A comprehensive examination was conducted in 125 individuals with chronic generalized periodontitis of I and II degrees by clinical and biochemical methods. The study also enrolled 30 people not working at the Burshtyn TPP, examined with intact periodontium (controls). We investigated the content of Copper, Iron, and Manganese in biological materials using spectral analysis in 110 patients with chronic generalized periodontitis and 27 controls.

Results: The conducted examination indicated a disturbance in the content of Copper, Iron, and Manganese in the blood and mixed saliva of patients with generalized periodontitis who were employees of the Burshtyn TPP. We observed a decrease in the level of Manganese and Iron and an increase in Copper's content in patients with generalized periodontitis (vs. controls), which may indicate a violated metabolism of trace elements upon chronic exposure to small doses of heavy metal salts. The changes in the content of trace elements (particularly Copper and Manganese) were associated with the severity of the disease.

Conclusions: The changes in the level of particular trace elements in the saliva and blood of the examined individuals indicate their role in the development of generalized periodontitis.

KEY WORDS: periodontitis, trace elements, ecology, blood, saliva

Wiad Lek. 2025;78(6):1014-1018. doi: 10.36740/WLek/207358 DOI

INTRODUCTION

Over the period of the last few decades, the occurrence of dental diseases has been increasing. The latter is determined by many factors such as unfavorable ecology, deterioration of general health in adults and children, and worsening of living conditions due to the ongoing war. According to many observations, Ukraine is currently one of the most environmentally polluted countries in Europe and in the world due to the ongoing war. Environmental factors are considered one of the trigger factors which affect human health [1–7]. Therefore, studying the role of bad ecology regarding the occurrence and development of dental diseases remains relevant.

Numerous studies of the dento-maxillary system conducted in regions of intense technogenic pollution are gaining great importance; moreover, the question of allocating a special section — ecological dentistry — is being raised. In this aspect, the periodontium is regarded as an important informative structure that can be used in ecological monitoring.

Furthermore, the constant influence of man-made environmental pollution and psycho-emotional tension leads to disruption of adaptation processes and disease occurrence [2, 4, 5]. This problem is a part of extensive research directed towards early diagnosis of pre-pathological conditions, and justification of the principles and criteria concerning the real and maximum permissible load of adverse environmental factors on the population [8–10]. Such a system requires fundamental investigation, taking into account achievements in the field of medical and biological sciences in studying the mechanisms and patterns of the biological effects of the environment on the organism [10, 11].

In the process of the research, we examined children and adults from Burshtyn and surrounding villages (Ivano-Frankivsk region, Ukraine). There is a powerful thermal power plant here. According to the Ministry of Ecology and Natural Resources of Ukraine, by 2021 the Burshtyn Thermal Power Plant (TPP) ranked third in Ukraine in terms of emissions of pollutants into the atmosphere. About 965 thousand tons of waste were generated here during the year (pre-war period), of which about 158.6 thousand tons were emitted into the air. The Burshtyn TPP was repeatedly bombed during the war, as a result of which a large amount

of toxic substances found in slag ash dumps, as well as other toxic compounds, were released into the air.

The syndrome of environmentally induced lowered resistance leads to the development of disorders associated with the influence of eco-toxins and social factors, and causes elevated sensitivity of the body to adverse factors.

Copper is an element which is recommended for the assessment of human health in case of exposure to heavy metals [11]. This trace element enhances bone re-calcification, promotes the reversal of osteoporotic processes, and plays an important role in bone metabolism.

Cadmium ranks first in terms of toxicity among heavy metals and significantly affects both specific and nonspecific changes in the human body. We have already reported on the content of this trace element in our previous studies [6].

Iron is closely related to Cadmium. Its sufficient amount in food prevents the absorption of Cadmium. Iron also plays an important role in metabolic processes in areas of osteoporosis, in the formation of bone tissue, and has a positive effect on the formation of hydroxylapatites in the osteoid substance.

Manganese also has a protective effect. It is part of the active center in many enzymes, superoxide dismutase in particular, which are involved in protecting against peroxide radicals. Manganese affects the activity of phosphatases, which are involved in the metabolism of phosphorus-calcium compounds and influences the growth and maturation of cartilage and bone tissue.

We would like to report on the results regarding the content of trace elements (Copper, Iron, Manganese) in biological fluids — blood and mixed saliva — in people who have been employees of a thermal power plant for a long period of time and who live near this enterprise as well.

AIM

The aim of the research is to study the content of Copper, Iron**,** and Manganese in mixed saliva and blood in those affected with generalized periodontitis working in hazardous production for a long time.

MATERIALS AND METHODS

There were 163 people enrolled in this study, namely: 133 employees of the Burshtyn TPP and 30 people who do not work at the Burshtyn TPP. Among them were 109 men and 54 women aged from 20 to 49 years. One hundred and thirty-three employees of the Burshtyn TPP were diagnosed with generalized periodontitis of I and II degrees of severity, of whom 125 were diagnosed with chronic periodontitis and 8 were affected by acute periodontitis, which amounted to 93.98% and 6.02%, respectively.

A comprehensive examination was conducted in 125 patients with chronic generalized periodontitis of I and II degrees of severity (among them were 64 patients with generalized periodontitis of I degree and 61 with generalized periodontitis of II degree) using clinical (including periodontal pocket depth, Ramfjord Index [RI], and bleeding index by Mühlemann), biochemical, and radiological research methods; and there were also 30 people not working at the Burshtyn TPP examined with intact periodontium. The obtained results were recorded in the patients' outpatient charts. The examination consisted of complaints, and medical, social, and life history.

Biochemical studies were conducted at the Center for Bioelementology of Ivano-Frankivsk National Medical University (Head of the Department – Doctor of Biological Sciences, Professor G.M. Erstenyuk).

Quantitative determination of micro- and macro-elements (Iron, Copper, Manganese) in biological material was performed by the spectral method in 110 patients with generalized periodontitis and 27 people with intact periodontium. Biological fluids (blood and saliva) of patients were collected before breakfast, then dried at a temperature of 70–80°C. After drying, they were ashed in a muffle furnace at a temperature of 450–500°C.

Mineralization was carried out until the ash was free of coal impurities. Determination of Iron, Copper, and Manganese was carried out in ash solutions using an atomic absorption spectrophotometer C-115 "Saturn" in accordance with the requirements of the state standard and regulations.

All examinations were performed only with the voluntary written consent of the patients and in compliance with all bioethical norms, particularly the Declaration of Helsinki, and according to Ukrainian legislative and regulatory documents.

The data were analyzed using Statistica v. 14.0 (TIBCO Software Inc., USA) software. Quantitative variables were presented as mean ± standard error of the mean. We used the Student's t-test or ANOVA (with the Tukey's HSD post hoc test) for independent samples (to compare two or three independent groups of subjects, respectively). A p-value < 0.05 was considered statistically significant (considering the Bonferroni correction).

RESULTS

The data on the clinical characteristics of patients with chronic generalized periodontitis are presented in Table 1.

It was established that there was a significant increase in the depth of periodontal pockets in patients with generalized periodontitis of II degree by 25.16%, up to (3.83 ± 0.04) mm, while in patients with generalized periodontitis of I degree, this indicator was (3.06 ± 0.06) mm ($p < 0.001$).

The study of the RI also showed its notable increase depending on the severity of generalized periodontitis. The RI was (4.26 ± 0.14) points in the case of generalized periodontitis of I degree, increasing up to (4.63 ± 0.06) points in individuals with generalized periodontitis of II degree.

Table 1. Clinical characteristics of condition of periodontal tissues in patients with chronic generalized periodontitis

Indicators	Patients with chronic generalized periodontitis of I degree n=64	Patients with chronic generalized periodontitis of II degree n=61	p
Periodontal pocket depth, mm	3,06±0,06	3,83±0,04	<0,001
RI, points	4,26±0,14	4,63±0,07	<0,05
Bleeding index by Mühlemann, points	1,33±0,09	2,25±0,10	<0,001

Table 2. The content of trace elements in the blood and oral liquid of patients with generalized periodontitis and controls with intact periodontium

Indicators		People with intact periodontium n=27	Patients with chronic generalized periodontitis of I degree n=56	Patients with chronic generalized periodontitis of II degree n=54
Copper, mg% / raw sub.	Blood	0,17±0,01	0,19±0,01 p<0,01	0,22±0,01 p<0,001 p_1<0,001
	Oral liquid	17,19±0,92	20,34±0,99 p<0,05	21,43±0,96 p<0,05
Iron, mg% / raw sub.	Blood	79,68±2,93	67,70±2,96 p<0,05	63,75±3,00 p<0,001
	Oral liquid	0,79±0,03	0,67±0,03	0,59±0,04 p<0,05
Manganese, mcg% / raw sub.	Blood	3,90±0,06	3,45±0,06 p<0,001	3,00±0,06 p<0,001 p_1<0,001
	Oral liquid	0,03±0,002	0,02±0,002 p<0,05	0,01±0,003 p<0,001

Note: p – the significance of difference between the indicators of people with intact periodontium and patients with generalized periodontitis of I and II degrees, p1– the significance of difference between the indicators of patients with generalized periodontitis of I and II degrees

When studying the Mühlemann bleeding index, its growth was observed with the progression of the disease (see Table 1). In the group of patients with generalized periodontitis of I degree, this indicator was (1.33 ± 0.09) points, increasing by 69.17% to (2.25 ± 0.10) points in patients with generalized periodontitis of II degree ($p < 0.001$).

Thus, the study of the clinical condition of periodontal tissues in employees of the Burshtyn TPP suggests that there was a significant increase in all indicators in patients with generalized periodontitis as the pathological process in the periodontium progressed.

The results of our study, concerning the level of trace elements in the blood and oral fluid (mixed saliva) of Burshtyn TPP workers and controls (those not working at the Burshtyn TPP with intact periodontium), are illustrated in Table 2. The data obtained indicate a disruption of the trace element status, reflected in fluctuations of the statistical results. As can be seen from Table 2, the level of Copper, Iron, and Manganese in those diagnosed with generalized periodontitis is clearly associated with the severity of the disease.

In particular, there was an increase in Copper in the blood of patients with generalized periodontitis of degree I, with its level rising by 11.76% compared to (0.17 ± 0.01) mg%/raw sub. in subjects with intact periodontium ($p < 0.01$). In the case of generalized periodontitis of degree II, the concentration of Copper in the blood increased by 29.41%, reaching (0.22 ± 0.01) mg%/raw sub. ($p < 0.001$ vs. both periodontitis I degree and intact periodontium).

Similar changes were observed in the oral fluid (mixed saliva). The amount of Copper in the oral fluid of patients with generalized periodontitis of degree I was (20.34 ± 0.99) mg%/raw sub., and of degree II – (21.43 ± 0.96) mg%/raw sub., exceeding the corresponding indicator of people with intact periodontium (17.19 ± 0.92) mg%/raw sub. by 18.32% and 24.66%, respectively ($p < 0.05$ for both comparisons).

When studying the amount of Iron in patients with generalized periodontitis, it was found that its level decreased with the progression of the pathological process (see Table 2). If in the blood of patients with generalized periodontitis of degree I, its concentration decreased by 15.04%, then in patients with generalized periodontitis of degree II, it also declined by 20.00%, compared to the indicator in people with intact periodontium.

A significant reduction of Iron was detected in the oral fluid of patients with generalized periodontitis of degree II, down to (0.59 ± 0.04) mg%/raw sub., which differed from

the indicator in controls (0.79 ± 0.03) mg%/raw sub. by 25.32% ($p < 0.05$).

The study demonstrates that the amount of Manganese also decreased in patients with generalized periodontitis, namely: in the case of degree I, it lowered significantly by 11.54% in the blood and by 29.04% in the oral fluid; in the case of degree II, its level reduced by 33.33% in the blood and by 66.67% in the oral fluid (data compared with the corresponding indicators of individuals with intact periodontium). Additionally, the patients with generalized periodontitis of degree II demonstrated a lower level of Manganese, as opposed to those with degree I (Table 2).

Therefore, the results of the study show disturbances in the levels of Copper, Iron, and Manganese in the biological fluids of patients affected by generalized periodontitis, who have lived nearby and worked at the Burshtyn TPP for an extended period of time. There is a significant rise in Copper and a decline in Iron and Manganese levels in those affected by generalized periodontitis.

DISCUSSION

There are notable changes in the microelement spectrum found in the blood and mixed saliva of the examined patients, namely: a decreased amount of Manganese and Iron and an increased amount of Copper, which may indicate a disturbed metabolism of microelements under conditions of chronic exposure to small doses of heavy metal salts. Therefore, it can be concluded that the severity of generalized periodontitis is caused by changes in the content of trace elements that affect the permeability of cell membranes, the activity of enzymes responsible for compensatory mechanisms in tissues, including the periodontium. The latter is confirmed by the disordered microelement spectrum of blood and saliva in cases of worsening of the disease. The results of our studies are consistent with the studies of other scientists [12-15]. It has also been proved that the imbalance of microelements affects all organs and systems of the human body, causing various pathological conditions such as diabetes mellitus [13] and cardiovascular diseases [14]. The authors also indicate that Copper is an essential trace element for normal cell functioning; its deficiency has been reported to impair the function of vital copper-binding enzymes, while its excess can lead to cell death [13]. The precise and tight regulation of Copper homeostasis is summarized, and recent advances regarding the relationship between diabetes and plasma Copper are discussed.

The influence of heavy metals on the course of cancer has also been proven [9], as well as their effect on the development of Alzheimer's disease [15]. The authors admit that the disease is a complex multi-factorial disorder in which the convergence of polygenic, epigenetic, environmental, vascular, and metabolic factors tend to enhance susceptibility to the disease and shape its course. One of the cofactors converging with Alzheimer's disease is brain metal dysregulation.

There is a correlation reflected in the results of our study, which evidences the disturbances in Copper, Iron, and Manganese content in biological fluids of patients with generalized periodontitis working in poor environmental conditions.

CONCLUSIONS

The analysis of the gained results and the data of scientific research allow us to suggest that generalized periodontitis found in workers employed in hazardous production and living in adverse environmental conditions may be caused by technogenic microelementosis, which is characterized by an increased content of Copper in biological fluids and a decreased level of essential microelements (namely Iron and Manganese). The established changes in the microelement spectrum could lead to a disruption in the ratio of osteogenesis and osteolysis, decreased synthesis, and increased collagen breakdown, which weakens the mineralization of the organic matrix of periodontal bone tissue and enhances its resorption.

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The study was carried out within the framework of two researches: the research «Clinical effectiveness of complex treatment of diseases of hard dental tissues and periodontium in the population from ecologically bad regions», which was financed from the state budget of Ukraine (state registration number 0118U004144, implementation period 2018-2020); and the research «Complex morpho-functional study and substantiation of the use of modern technologies for the treatment and prevention of dental diseases», which is financed from the state budget of Ukraine (state registration number: 0121U109242, implementation period: 2021 – 2023).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Zoryana B. Popovych
Ivano-Frankivsk National Medical University
2 Galytska St., 76018 Ivano-Frankivsk, Ukraine
e-mail: zpopovych@ifnmu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Zoryana B. Popovych: 0000-0003-4472-3748 A D F
Volodymyr I. Sarapuk: 0000-0001-6986-7911 B C
Veronica Yu. Katerynyuk: 0000-0003-3256-3599 B E
Iryna Z. Chubij: 0009-0001-0826-5500 B C
Iryna Z.Ostapyak: 0000-0002-3042-7019 B C F
Natalia M. Pavelko: 0000-0002-5727-7399 B C
Oleksandra V. Bazalytska: 0009-0007-3842-6663 A B

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 10.02.2025
ACCEPTED: 26.05.2025