ORIGINAL ARTICLE

CONTENTS

Biochemical changes in oral fluid in individuals with generalized periodontitis living in different environmental conditions

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ABSTRACT

Aim: To study the biochemical changes in the oral fluid of individuals with generalized periodontitis living in different environmental conditions.

Materials and Methods: In order to investigate biochemical changes in the oral liquid of people diagnosed with generalized periodontitis associated with the impact of Cd, we examined 113 people aged from 20 to 59 years. Generalized periodontitis was detected in 83 (73,45%) patients: initial – 19 (22,9%) cases, stage I – 32 (38,55%), and stage II – 32 (38,55%). All the examined individuals were further divided into two groups. The first group (n=52) consisted of people from certain areas in the Ivano-Frankivsk region of Ukraine with elevated Cd content. The second group (n=61) included individuals who lived in areas of the Ivano-Frankivsk region where the Cd content in the environment (soil) did not exceed the maximum permissible concentration. We estimated the content of calcium (Ca), inorganic phosphorus (Pi), and the hydroxyproline level in the oral liquid of the enrolled subjects.

Results: A decrease in Ca content in the oral liquid of patients with generalized periodontitis was found, which was accompanied by an increase in Pi and hydroxyproline, observed in both study groups as compared to controls. These changes were associated with the progression of generalized periodontitis severity.

Conclusions: The obtained results indicate a certain similarity in the biochemical changes of oral fluid among individuals residing in areas with different environmental conditions, thus requiring further research.

KEY WORDS: oral liquid, biochemical studies, cadmium

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INTRODUCTION

Many studies have proven that environmental conditions have a significant impact on human health. Numerous studies demonstrate that the prevalence of somatic and dental diseases in the population is on the rise in ecologically polluted areas [1–11]. Particularly, they indicate that residents of environmentally polluted regions belong to risk groups and have a high incidence of diseases of the digestive, endocrine, cardiovascular, and hematopoietic systems [10-13].

An environmental increase in toxic compounds, heavy metals in particular, leads to deterioration in the health of adults and children [1, 3, 4, 9, 12–16]. We focused on studying cadmium (Cd) regarding its impact on the periodontal tissues. Cd has the ability to accumulate in the human body, creating toxic concentrations and causing long-term negative health consequences. Moreover, Cd intoxication causes various health effects, leading to the development of malignant tumours, cardiovascular diseases, and diabetes mellitus [12–14]. Furthermore, not only employees of enterprises that have direct contact with xenobiotics are in the risk group, but also other people living in areas contaminated with heavy metals. It was found that the content of Cd exceeded the maximum permissible concentration by 1.2 times in some areas of the Ivano-Frankivsk region of Ukraine (in particular, the village of Horokholyno, Bohorodchany district) [4].

Interest in Cd is due to its toxic properties and ability to interact with vital elements such as calcium (Ca) and zinc, and also to influence phosphate and Ca metabolism. Recent epidemiological studies show that dental [1–3, 5, 7, 9, 14, 17–19] and somatic diseases [10–13] significantly increased in areas contaminated with salts of heavy metals.

Although the role of toxic compounds is of great importance for many countries nowadays, it is especially vital for Ukraine because the ecological situation in the country during the ongoing war has become extremely difficult due to air, water, and soil pollution and the

damage done to industrial facilities. The population and ecosystems of Ukraine feel the terrible consequences of missile attacks on factories and thermal power plants.

AIM

The aim of our research was to study the biochemical changes in oral fluid of individuals with generalized periodontitis living in different environmental conditions.

MATERIALS AND METHODS

In order to study the effect of Cd on biochemical changes that occur in the oral liquid in generalized periodontitis, we examined 113 people aged from 20 to 59 years. Among them were 54 (47,79%) men and 59 (52,21%) women. Generalized periodontitis was detected in 83 (73,45%) patients: initial – 19 (22,9%) cases, stage I – 32 (38,55%), and stage II – 32 (38,55%). Consequently, 30 (26,55%) people were free from generalized periodontitis (relatively healthy controls).

All the examined individuals were divided into two groups. The first group (n=52) consisted of people from areas with elevated Cd content (the village of Horokholyno, Bohorodchany district, and the city of Ivano-Frankivsk, Ukraine), where a local increase in Cd levels was detected (conditionally contaminated areas): healthy controls – 15 cases; initial periodontitis – 7; stage I – 15; and stage II – 15.

The second group (n=61) consisted of individuals who lived in areas where the Cd content in the environment (soil) did not exceed the maximum permissible concentration (Bohorodchany and Ivano-Frankivsk districts of the Ivano-Frankivsk region, Ukraine) (conditionally clean areas): healthy controls – 15 cases; initial periodontitis – 12; stage I – 17; and stage II – 17.

The clinical examination was conducted at the Department of Dentistry of the Educational and Scientific Institute of Postgraduate Education, Ivano-Frankivsk National Medical University (head of the department: Prof. Ivan V. Paliichuk). The investigation also included a complete clinical examination and biochemical analysis of oral fluid.

Biochemical analyses of oral fluid were conducted spectrophotometrically at the G. Babenko Bioelementology Center of Ivano-Frankivsk National Medical University (head: Prof. Hanna M. Ersteniuk). The key indicators that characterize the depth of destructive processes in periodontal tissues and phosphate-Ca metabolism were studied, in particular: the content of Ca, inorganic phosphorus (Pi), as well as the determination of hydroxyproline, which reflects the state of collagen fibres.

The determination of Ca concentration in oral fluid is based on the ability of Ca to form a blue complex with Arsenazo III dye in a neutral medium, which has an absorption maximum at 590–650 nm.

The concentration of Pi was determined using a standard reagent kit («Simko Ltd», Ukraine). The principle of the method is based on the interaction of Pi with ammonium molybdate to form a phosphorus–molybdate complex, which has an absorption maximum at a wavelength of 340 nm, the value of which is proportional to the concentration of Pi.

The determination of hydroxyproline was conducted in order to assess the state of collagen fibres and bone tissue. The principle of the method is based on the oxidation of hydroxyproline by hydrogen peroxide to pyrrole in an alkaline solution in the presence of copper ions, removal of excess hydrogen peroxide, and the formation of a pink colour with paradimethylaminobenzaldehyde in an acidic medium. The intensity of the colour is proportional to the concentration of hydroxyproline.

The data were analysed using Statistica v. 14.0 (TIBCO Software Inc., USA) and EZR v. 1.68 software. Quantitative variables were presented as mean ± standard error of the mean. We used the ANOVA test (with Tukey's HSD post hoc test) for independent samples (to compare four independent groups of subjects). An adjusted p-value <0,05 was considered statistically significant.

ETHICS

All studies were conducted in accordance with the requirements of the Declaration of Helsinki, the Convention on Human Rights and Biomedicine, and Ukrainian regulatory documents. The research protocols were approved by the Bioethics Commission of Ivano-Frankivsk National Medical University.

FRAMEWORK

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 by the state budget of Ukraine (state registration number 0118U004144, implementation period 2018-2020); and the research «Complex morpho-functional study and substantiation 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).

RESULTS

The obtained data demonstrated a decrease in Ca content in the oral fluid of individuals in both research groups who were diagnosed with different stages of generalized periodontitis, which was also accompanied by a statistically significant increase in Pi and hydroxyproline levels (Table 1).

Table 1. The biochemical indicators of oral liquid in the healthy controls and patients with generalized periodontitis from the studied ecological regions, M±m

Investigated indicator	Ecological region	Healthy (controls)	Patients			p
			Initial periodontitis	I stage of periodontitis	II stage of periodontitis	
Ca, mmol/l	Group I (N=52)	1.68 ± 0.06 n=15	1.39 ± 0.05 n=7	1.18 ± 0.02 n=15	1.05 ± 0.02 n=15	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}=0.025 p_{2-4}<0.001
	Group II (N=61)	1.63 ± 0.06 n=15	1.34 ± 0.03 n=12	1.19 ± 0.02 n=17	1.0 ± 0.02 n=17	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}=0.039 p_{2-4}<0.001 p_{3-4}=0.003
P_i, mmol/l	Group I (N=52)	5.97 ± 0.03 n=15	6.59 ± 0.09 n=7	6.86 ± 0.04 n=15	7.06 ± 0.03 n=15	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}=0.003 p_{2-4}<0.001 p_{3-4}=0.004
	Group II (N=61)	5.97 ± 0.04 n=15	6.48 ± 0.05 n=12	6.76 ± 0.04 n=17	7.02 ± 0.03 n=17	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}<0.001 p_{2-4}<0.001 p_{3-4}<0.001
Hydroxyproline, mmol/l	Group I (N=52)	4.55 ± 0.23 n=15	11.68 ± 0.15 n=7	14.97 ± 0.17 n=15	24.94 ± 0.32 n=15	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}<0.001 p_{2-4}<0.001 p_{3-4}<0.001
	Group II (N=61)	4.25 ± 0.27 n=15	11.46 ± 0.11 n=12	14.73 ± 0.19 n=17	24.20 ± 0.34 n=17	p_{1-2}<0.001 p_{1-3}<0.001 p_{1-4}<0.001 p_{2-3}<0.001 p_{2-4}<0.001 p_{3-4}<0.001

Notes: p_{1-2} – significance of difference between initial periodontitis and controls; p_{1-3} – significance of difference between stage I periodontitis and controls; p_{1-4} – significance of difference between stage II periodontitis and controls; p_{2-3} – significance of difference between initial and stage I periodontitis; p_{2-4} – significance of difference between initial and stage II periodontitis; p_{3-4} – significance of difference between stages I and II periodontitis
Source: compiled by the authors of this study

According to the study results, there was a 17,4% decrease in Ca content in the oral fluid of patients with the initial stage of generalized periodontitis compared to the control group in both investigated regions. In the first group, with generalized periodontitis of stage I, there was a 30% decrease in Ca level, and in the second group – a 27% decrease, as compared to the control group. In individuals with generalized periodontitis of stage II, these indicators were 37,5% and 38,6%, respectively (Table 1). Since Ca metabolism is closely related to phosphate metabolism, we also examined the level of Pi in the oral fluid of the examined individuals. The content of Pi increased by 10,4% in those with initial generalized periodontitis within the first group and by 8,6% within the second group compared to the control group (Table 1).

Considering generalized periodontitis of the first stage, the Pi level increased by 14,7% in the first group and by 13,2% in the second group, in contrast to the control group. In cases of generalized periodontitis of the second stage, the indicators increased by 18,25% and 17,6%, respectively (Table 1).

We also found a significant rise in hydroxyproline levels in individuals with initial generalized periodontitis: 2,6 times in the first group and 2,7 times in the second

group, as compared to the indicators of individuals from the control group. These changes in hydroxyproline are associated with the severity of generalized periodontitis. In particular, in generalized periodontitis of stage I, the hydroxyproline content exceeded the indicators in the control group by 3,3 and 3,5 times, respectively (Table 1).

There was also an increase in the concentration of hydroxyproline in individuals diagnosed with generalized periodontitis of stage II, which confirmed the tendency to rise with the worsening of the pathological process. Indeed, in the first group, the amount of hydroxyproline increased 5,5 times, and in the second group – 5,7 times, as compared to the corresponding indicators of the control group (Table 1).

DISCUSSION

Our research revealed a certain similarity in the biochemical changes observed among individuals residing in areas with different environmental conditions. We assume that this may indicate that, in addition to Cd, a combination of various factors influences these biochemical alterations – for instance, stress-related factors that may be associated with cadmium exposure. In our study, the Cd concentration in the examined area was 1,2 times higher than in the environmentally clean region, which may explain the observed similarity of changes. Some inconsistency in the results has also been reported by other researchers; however, they emphasize that a high concentration of Cd plays a decisive role in the development of pathology. In particular, scientists [1] have found that periodontitis, a microbiome-driven chronic inflammatory disease that destroys the supporting apparatus of the teeth, is influenced by various environmental factors, including exposure to heavy metals such as lead and Cd. The authors indicate that the results highlight the importance of reduced environmental exposure to these heavy metals as part of preventive strategies for periodontal diseases. Further research is needed to explore the underlying biological mechanisms and to evaluate potential interventions in order to reduce exposure-associated periodontitis [1].

Scientists [2] conducted a study aimed at examining the relationship between internal exposure to Cd and lead and periodontitis in a representative sample of adults who participated in the Fourth Korea National Health and Nutrition Examination Survey (KNHANES). A total of 1966 individuals aged over 19 years who took part in the survey were examined. A multivariate logistic regression analysis revealed an association between Cd and periodontitis [2].

It was confirmed that there is a connection between the effects of heavy metals, in particular Cd, and somatic health [10, 12]. It has been shown that an omnipresent environmental metal, cadmium, can cause oxidative stress in diabetic cardiomyocytes, leading to iron accumulation, glutathione depletion, lipid peroxidation, and, ultimately, exacerbation of ferroptosis and disruption of cardiac function. Moreover, Cd-induced hyperglycaemia can enhance the circulation of advanced glycation end products [12].

CONCLUSIONS

Over the last decades, the number of eco-dependent diseases has been on the rise, as evidenced by research around the world. Our biochemical study of oral liquid showed a decrease in Ca content in individuals with generalized periodontitis compared to individuals with healthy periodontium, which correlates with the data of other researchers. The results demonstrated that the content of Pi in individuals with generalized periodontitis also increased, as did the level of hydroxyproline, compared with the indicators of individuals from the control group. However, the results obtained in our study indicate a certain similarity in biochemical changes, which may be attributed to stress-related factors; therefore, this issue requires further research. Since generalized periodontitis is one of the main problems of modern dentistry, the obtained data can be used in treatment planning and prophylaxis of periodontal disorders.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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