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Evaluation of the concentration of short-chain fatty acids in the oral fluid in patients with chronic periodontitis

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Abstract: To develop a method for preparing analysis samples by gas chromatography with mass-selective detection was the purpose of this work, which makes it possible to determine SCFA in oral fluid in healthy individuals and patients with chronic generalized periodontitis (CGP). The research included 18 patients with an average degree of chronic generalized periodontitis aged 28 to 61 years. An Agilent 5977B GC/MSD gas chromatograph with mass spectrometry an Agilent 8890 GC mass detector were used. HP-5MS Ultra inert 30 m × 250 mkm × 0.25 mkm columns were used. During the study of the oral fluid in patients with CGP using GC-MS, a change in the absolute content of short chain fatty acids was revealed. A decrease in the level of acetic acid to 0.26 ± 0.02 mg/g, propionic acid to 0.07 ± 0.006 mg/g, and butyric acid to 0.013 ± 0.002 mg/g was revealed in the oral fluid of patients with moderate CGP.

Keywords: free fatty acids, chronic generalized periodontitis (CGP), gas chromatography with mass spectrometry (GC-MSD).

Introduction

Currently, the oral cavity is considered as a complex ecological system in which external factors closely interact with internal factors (mucous membrane, periodontium, bacterial community, local immune system, saliva). Therefore, the study of the properties of the oral fluid (OM) is of interest not only to dentists, but also to doctors of other specialties, since the oral fluid is an environment in which the oral organs are throughout life and which are factors in maintaining the homeostasis of the body [1,5,7].

The study of the profile of the oral fluid is one of the key areas in the early diagnosis of diseases of the dentoalveolar system of the body. The oral fluid can serve as biomarkers of the early stages of development of various pathologies of the oral cavity. A detailed study of the status of oral fluid in dynamics can be very useful not only for diagnosing diseases, but also for evaluating the effectiveness of drug therapy [4,14].

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The main method for analyzing the composition of the oral fluid is gas chromatography (GC) with flame ionization detection, however, in combination with mass spectrometry, the GC method has a number of undeniable advantages both in terms of the reliability of analyte identification and the sensitivity and selectivity of their determination. At the same time, the determination of free fatty acids in the oral fluid by GC after interesterification into methyl esters can be considered as a classical approach [6, 8].

At present, generalized periodontitis can be attributed not only to an important medical, but also to a social problem due to its negative impact not only on the organs of the oral cavity, but also on the whole organism [2]. Numerous researchers have revealed the fact that one of the main roles in the occurrence of periodontal inflammation is played by an infectious factor, which should include pathogenic microflora that vegetates on the teeth and gums, its waste products, toxins and endotoxins, microbial enzymes [3]. Among other biologically active substances, short-chain fatty acids (SCFA) can be considered the most controversial product of the vital activity of anaerobic bacteria, which not only reflect the activity of microflora in the oral cavity, but also have an independent pro-inflammatory effect [4,19]. SCFA are involved in microcirculation, regulation of ion exchange, mucus secretion, affect the viscosity and reproduction of pathogenic and opportunistic flora, activate local immunity, phagocytosis, replenish the energy needs of various tissues, primarily epithelium, affect the proliferation and differentiation of epitheliocytes [9,10]. It is also known that various SCFAs are produced by microflora of certain genera. Aerobic microorganisms (Escherichia coli, strepto- and staphylococci) are producers of acetic acid and isoacids; anaerobic microorganisms - bacteria of the genus Bacteroides and others - propionic acid; bacteria of the genus Clostridium and Fusobacterium, etc. - butyric acid. It becomes obvious that these microbial metabolites have a certain diagnostic value, making it possible to judge the qualitative and quantitative nature of the microflora, the functional state of the system (organ) and can serve as a reflection of various processes occurring in the oral cavity [16].

The aim of this work was to develop a method for preparing samples for analysis by gas chromatography with mass-selective detection, which makes it possible to determine SCFA in oral fluid in healthy individuals and patients with chronic generalized periodontitis [11].

Material and research methods

The study included 18 patients with an average degree of chronic generalized periodontitis, aged 28 to 61 years, who applied to the TSDI clinic. Diagnosis of periodontal diseases was based on generally accepted clinical, index criteria and included: determination of the depth of periodontal pockets, the nature of exudate, pathological tooth mobility, dental plaque, the degree of bleeding, hygiene index (GI,

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Green J.C., Vermillion J.R., 1960), papillary-marginal -alveolar index (PMA, Parma C., 1960), periodontal index (PI, Russel A., 1956) [17]. Verification of the diagnosis of chronic generalized periodontitis was carried out on the basis of clinical (anamnesis data, complaints and dental examination) and instrumental research methods with the participation of Kamilov Kh.P., professor of the Department of Therapeutic Dentistry, TSDI. In this situation, hyperemia, swelling of the gums, bleeding during probing were noted in patients with CGP, periodontal pockets were found, the average depth was 5 mm [18]. The teeth had the first or second degree of mobility. There was an abundance of supra- and subgingival mineralized dental deposits. The mean values of IG were 1.83 ± 0.11, RMA index - 44.65 ± 2.37, PI - 2.27 ± 0.19. The comparison group included 12 patients without periodontal pathology. The study did not include the following criteria; if the study participant has diseases of the liver, cardiovascular system, diseases of the urinary system, endocrine system, pregnancy, taking drugs that affect lipid metabolism [15]. The study of short-chain fatty acids in the oral fluid was carried out by gas chromatography. The method for determining short-chain fatty acids (acetic C2, propionic C3, butyric C4, with isomers) included: sample preparation and gas chromatographic analysis [20]. The work standards were commercial sets of acids-C2, C3, C4, iso-butyric, isovaleric, caproic and iso-caproic acids. The composition of SCFA of the oral fluid was determined once, on an empty stomach, during the initial examination. The oral fluid was collected in eppendorfs and stored until analysis in a freezer at -80°C. Before the study, the oral fluid was centrifuged at 14,000 rpm. Within 20 minutes. To isolate lipids, a 0.1 ml lyophilized lipid fraction was added to a mixture consisting of 0.5 ml of 0.9% sodium chloride solution and 2.5 ml of a mixture of chloroform/methanol (2:1). Then 10 µl of fatty acid internal standards consisting of 17:0 (2 µg/ml), 19:0 (0.5 µg/ml) and 23:0 (0.25 µg/ml) were added to this mixture. After 5 minutes, the obtained the mixture was centrifuged at 16,000 rpm for 3 minutes. Then 10 ml of the lower FA layer is separated and transferred to Eppendorf tubes, evaporated under nitrogen flow. 1 ml of a 0.4 M NaOH solution in methanol was added to the resulting solution, thoroughly mixed in a vortex for 10 min, then the mixture was heated for 30 min in a water bath at 70C, 55 ml of a 32% hydrochloric acid solution and 1.5 ml of hexane were added. 3 ml of 3 M sodium chloride solution is added to the resulting mixture, and the hexane layer is separated. The solution is added in a ratio of 1:3 sodium sulfate solution and left for a day, filtered and the filtrate is introduced into a gas chromatograph [12]. The analysis was carried out by gas chromatography, mass spectrometry on an Agilent 5977B GC/MSD instrument, an Agilent 8890 GC mass detector. HP-5MS Ultra inert 30 m × 250 µm × 0.25 µm columns were used. (Agilent catalog number 19091S-433UI). Thermostat program: 40°C for 1 minute, then 25°C/min up to 220°C, then 10°C/min up to 240°C. Hydrogen (H2) 1 ml/min was used as the carrier gas, Split injection

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mode (20:1), source temperature 250°C, temperature in the transport line 280°C. Delay to eliminate solvent effects - 3.5 min, SIM data acquisition mode. Chromatogram data for each sample were presented numerically as the value of the detector signal intensity at the corresponding time. Statistical processing of the material was carried out using standard software packages (Statistica 6 0, Excel 2003). To determine the statistical significance of differences in continuous values depending on the distribution parameters, Student's t-tests or the Mann Whitney test were used. Differences were considered significant for all analyzes at a significance level of $p<0.05$.Результаты исследований и их обсуждение [13].

A study was made of the absolute content of SCFA in the oral fluid in apparently healthy patients and patients with chronic generalized periodontitis. The results of studying the absolute concentration of SCFA in the oral fluid in patients with chronic generalized periodontitis are presented in Table. 1, which shows that in inflammatory periodontal diseases there is a decrease in the absolute content of SCFA compared with practically healthy patients.

Table. 1

SCFA content in the oral fluid in patients with chronic generalized periodontitis

Monocarboxylic acids	Absolute content, mg/g	
	Healthy faces n= 12	Patients with CGP n=18
C2 (acetic)	0,87 ± 0,062	0,26 ± 0,02*
C3 (propionic)	0,18 ± 0,015	0,07 ± 0,006*
C4 (oil)	0,05 ± 0,003	0,013 ± 0,002*

Note: *-significance of differences $P < 0.05$

In the study of the relative content of individual SCFAs in the oral fluid in patients with CGP, a decrease in the relative content of the proportion of acetic acid (C2), the proportion of propionic acid (C3) and the proportion of oil (C4) was revealed, which characterizes the decrease in the metabolic activity of the lactic acid flora (bifidus and lactobacilli) . Analysis of SCFA profiles with the number of C2-C4 carbon atoms, which make the main contribution to the total pool of acids in the oral fluid in patients with CGP, indicates a 2.5-fold decrease relative to the content of propionic and acetic acids, which indicates a decrease in the activity of the aerobic link of microorganisms - E. coli, strepto- and staphylococci with an increase in the activity of the anaerobic link, in particular, the genera of propionibacteria, bacteroids (to a greater extent), the genera Clostridium, Fusobacterium, etc. (to a lesser extent). This is consistent with the literature data of microbiological studies that have established similar changes in the oral cavity in patients with inflammatory periodontal diseases. At the same time, the redox potential of the oral fluid in

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patients with moderate inflammatory CGP is sharply shifted towards more negative values, which contributes to the activation of facultative and residual anaerobic microorganisms. In this way, with CGP, a change in the quantitative and qualitative compositions of SCFA was noted, which indicates diverse changes in the microbiocenosis. When evaluating the indicators, it should be noted that the concentrations of SCFAs in the oral fluid depend not only on the amount of acids produced by the microbiota, but also on other factors, such as the activity of secretion of the salivary glands, which leads to a high dispersion. At present, the interpretation presents certain difficulties due to the small amount of accumulated clinical data, but taking into account the non-invasiveness of the method and the multidimensionality of the possible analysis, the use of assessing the content of short-chain fatty acids in the oral fluid is of great diagnostic importance in periodontology.

Conclusion

During the study of the oral fluid in patients with CGP using GC-MSD, a change in the absolute content of short-chain fatty acids was revealed. A decrease in the level of acetic acid to 0.26 ± 0.02 mg/g, propionic acid to 0.07 ± 0.006 mg/g, and butyric acid to 0.013 ± 0.002 mg/g was revealed. in the oral fluid of patients with moderate CGP.

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