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The state of the antimicrobial system of the oral cavity in patients with insulin resistance

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ABSTRACT

The aim of this study was to evaluate the antimicrobial activity of oral fluid peptides in patients with insulin resistance. The objects of the study were 32 patients who were divided into the main study group and the control group. Of these, 18 patients were included in the main group at the stages of treatment for insulin resistance, in which a high level of C-peptide in the blood was detected by enzyme immunoassay. In the oral fluid, the amount of the antimicrobial peptide, cathelicidin LL-37, alpha-defensin 1-3, was assessed by enzyme immunoassay. In studies in the oral fluid of patients with IR, instead of a pronounced increase in the antimicrobial peptide, a decrease in its concentration was revealed, which requires additional studies.

Introduction

At present, the actual problem is the knowledge of the oral fluid as a biologically significant microenvironment of the body from the standpoint of a multidisciplinary approach, its metabolic and immunological profile. Taking into account the availability of oral fluid, the non-invasiveness of obtaining it, the possibility of multiple dynamic research, this bioenvironment is increasingly of interest as an object of study in fundamental and clinical practice as an alternative to blood in the diagnosis of many diseases.

It should be noted that the arguments for choosing the oral fluid as an object for studying the body are the following: being a complex filtrate of blood plasma, it reflects the state of dynamic constancy of the internal environment of the body; is an indicator of the reactivity of the organism, as it can change in composition, physico-chemical and biological properties under the influence of various factors.

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As is known, blood components enter the oral cavity through the fluid of the periodontal sulcus, mucous and mucous transudate and through bleeding that occurs in the oral cavity. As a result, a huge molecular diversity is found in the oral cavity, often referred to as "mixed saliva". Mixed saliva plays an important role for both physicochemical and immune protection of the oral mucosa through direct antimicrobial activity and agglutination of microorganisms. At the same time, saliva proteins are multifunctional in nature to protect the oral mucosa, as well as soft and hard tissues of the oral cavity. Antimicrobial peptides (AMPs) occupy a special place among the protective factors of the oral cavity. These are small molecules containing from 12 to 50 amino acid residues that can kill microbial cells. Most currently known AMPs. These are small molecules containing from 12 to 50 amino acid residues that can kill microbial cells. Most currently known AMPs have a broad spectrum of antimicrobial activity, acting against gram-positive and gram-negative bacteria, as well as yeasts and some viruses. In addition, convincing evidence has been obtained that a number of AMPs have anticarcinogenic activity and are also immunomodulators. Mixed saliva proteins provide antimicrobial protection by binding to bacteria and lysing microbial walls. They may also be responsible for the antifungal and antiviral properties of saliva. It has now become clear that AMPs in the oral cavity not only destroy pathogenic microorganisms, but also participate in the maintenance of normal microflora.

Currently, the following types of AMPs have been found in the oral cavity: a- and p-defensins, histatins, adrenomedullin and human cathelicidins, the sources of which are the oral mucosa, salivary glands and neutrophils protein calprotectin complement the protective function of antimicrobial factors of the salivary glands, lysozyme, immunoglobulins and histatins.

It should be noted that the microflora of the human oral cavity is extremely diverse and is normally represented by several hundred species of microorganisms. In periodontal diseases, as a rule, the quantitative ratio of microbes changes, and their species composition remains constant. This circumstance suggests that the cause of diseases of the tissues of the oral cavity is not actually a bacterial infection, but a violation of the adequate interaction of the macroorganism with the microflora. In this regard, researchers are of particular interest in the study of the protective systems of the oral cavity, in particular, antimicrobial peptides of the oral fluid.

The aim of this study was to evaluate the antimicrobial activity of oral fluid peptides in patients with insulin resistance.

Material and research methods

The objects of the study were 32 patients who were divided into the main study group and the control group. Of these, group I included 18 patients (12 men and 6

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women) aged 18 to 65 years (average age 47.6 years), who were at the stages of treatment for insulin resistance in whom the enzyme immunoassay revealed a high level of C-peptide in the blood, group 2 (14) consisted of healthy individuals aged 18 to 65 years (9 men and 5 women) without concomitant pathology. A comprehensive dental and laboratory study of patients in groups 1 and 2 was carried out at the clinical bases of TSDI and TMA.

To assess the state of insulin resistance, blood was taken in the morning, on an empty stomach. The content of insulin and the level of C-peptide in the blood serum were determined by enzyme immunoassay using firm from HUMAN. The concentration of insulin on an empty stomach was taken as the norm of C-peptide, which amounted to 0.78 -1.89 ng / ml (SI: 0.26-0.63 mmol / l). HOMA insulin resistance index (HOMA IR) = (fasting blood insulin x fasting blood glucose) / 22.5; (norm < 2.5). In our opinion, the determination of C-peptide is preferred in the diagnosis of insulin resistance in patients, since the concentration of insulin in the blood is about 5 times less than that of C-peptide, while the duration of the "life" of the insulin molecule is 4 minutes, C-peptide - 20 minutes.

The intake of oral fluid was carried out on an empty stomach from 8 to 9 hours and was carried out by spitting into a glass, sterile test tube for 5 minutes, without its preliminary stimulation. The volume of oral fluid averaged 7 ml. The samples were then purified by centrifugation at 10,000 × g for 5 min from cells, frozen and stored at -30 C. On the day of analysis, the samples were thawed and further purified of mucin by centrifugation at 3000 × g for 5 min. In the oral fluid, the amount of antimicrobial peptide - cathelicidin LL-37, alpha-defensin 1-3, was assessed by enzyme immunoassay using a set of reagents from «ООО БиоХимМак” Russia. Statistical processing of the material was carried out using standard software packages (statistics 6 0, Excel 2003). To determine the statistical significance of differences in continuous values depending on the distribution parameters Styudenta t-tests or the Mann-Whitney test were used. Differences were considered significant for all analyzes at a significance level of $p<0.05$.

Results and its discussion

Our data indicate that patients with IR have characteristic basal hyperinsulinemia, i.e. decreased sensitivity of peripheral tissues to insulin in the observation group. Apparently, the prescription of the disease and age affect the insulin receptors in peripheral tissues to the action of insulin. An indirect method for detecting IR also includes the determination of C-peptide on an empty stomach. The study of the level of C-peptide in the blood can serve as a more accurate confirmation of insulin hypersecretion in IR, since there are some known methodological limitations in the laboratory determination of insulin in the blood, which is 50% bound in the liver and has a half-life in the peripheral blood of about

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4 minutes. The C-peptide is cleaved from the proinsulin molecule when it is converted into insulin, does not bind to cell receptors on the periphery, has a half-life of about 30 minutes, and is not extracted from the blood plasma by the liver. According to our studies, in the group of patients with IR, the level of C-peptide was significantly higher in relation to patients in the control group. Therefore, our results of the study indicate a direct relationship between the level of C-peptide in the blood of patients with IR.

Table 1

Criteria for insulin resistance and blood levels of adiponectin in examined patients

Index	Patients with IR n=18	Healthy person n=14
Insulin (on an empty stomach) (mkED/l)	15,78±0,62*	12,61±0,47
C-peptide (ng/ml)	5,85±0,39*	1,51±0,16
HOMA index (kg/m2)	4,01±0,25*	3,02±0,23
Adiponectin (mkg/ml)	4,12 ±0,41*	7,22±0,44

Notes: * - significance of differences P<0.05

As you know, adipose tissue appears as a hormonally active organ, which is credited with the production of leptin, adiponectin, and resistin. Studies by numerous authors have shown that the secretion of adiponectin by adipocytes is stimulated by insulin. Adiponectin regulates energy homeostasis and has an anti-atherogenic and anti-inflammatory effect by inhibiting the adhesion of monocytes to vascular endothelial cells and exerting an inhibitory effect on growth factor-induced proliferation of smooth muscle cells in the vascular wall. As shown in our studies, the low content of adiponectin in the blood serum of patients of group 1 is an independent factor in the prognosis of the development of IR, since the expression of adiponectin does not correlate with blood C-peptide values.

Recently, scientists from the University of California, San Diego School of Medicine, USA, in a new study found that adipocytes, fat cells, are able to produce antimicrobial peptides that prevent the introduction of bacteria and pathogens of various origin. To confirm this version, we studied some antimicrobial peptides in patients with IR. At the same time, it was found that in persons with insulin resistance, the concentration of antimicrobial peptides in the blood was significantly lower relative to the comparison groups, which, in our opinion, is due to the low level of synthesis of complete antimicrobial peptides by adipocytes.

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Recently, it has been proven that a-defensins have a unique spectrum of antimicrobial activity, in particular, they are highly effective against Porphyromonas gingivalis, which cause damage to periodontal tissues. Also, a-defensins have a pronounced antiviral activity against herpes, influenza, hepatitis C viruses, human immunodeficiency virus (HIV)-1, cytomegaloviruses, papillomaviruses, adenoviruses. They are present in the epithelium of the gums, tongue, salivary glands and mucous membranes. Modern literature reports that defensins directly affect the adhesion of microorganisms to periodontal tissues and oral mucosa, and hence the development of periodontitis and diseases of the oral mucosa.

As can be seen from the presented research results, the content of a-defensins 1-3 in the oral fluid in patients of the main group and in healthy individuals, presented in Table 2, indicates a reduced secretion of a-defensins 1-3 in the oral fluid of the main group relative to the comparison group. The revealed factual material indicates the inactivation of β-defensins, which can lead to an increase in microbial colonization and increases the risk of viral and bacterial infections in the oral cavity.

The antimicrobial peptide LL-37 has antibacterial properties and is basically. expressed in neutrophil granules and epithelial cells. But at the same time, it can increase in biological media under stress, cell damage, contributing to wound healing and repair. The characteristics of the content of cathelicidin LL-37 in the oral fluid in patients of the main and control groups are presented in Table 2. The decrease in cathelicidin KL-37 in the oral fluid in patients of the main group can be explained by the following circumstance. As is known, cathelicidin LL-37 is synthesized in increased amounts in various lesions of the oral mucosa, which contributes to its faster healing. In our studies in the oral fluid, instead of a pronounced increase in the antimicrobial peptide, a decrease in its concentration in patients with IR was noted. The revealed factual material requires additional studies to explain the results of studies in patients with IR…

Table 2

Indicators of the content of antimicrobial peptides in the oral fluid in the examined patients with IR.

Indicators	Healthy person (n=14)	Patients with IR n=18
Cathelicidin LL-37 (ng/ml)	48,17±3,72	31,26±2,81*
Alpha defensins 1-3 (ng/ml)	984,2±±16,71	697,2±13,86*

Notes: * - significance of differences P<0.05

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Thus, despite the fact that antimicrobial peptides, being a component of innate immunity, perform the function of the body's natural defense against a wide range of microbes in many human organs and systems, their multidirectional changes in patients with IR undoubtedly arouse interest in studying the pathogenetic mechanisms of their role. in the implementation of antimicrobial protection in IR. In addition, in our opinion, the study of antimicrobial peptides of oral fluid is the fact that peptides produced in response to bacterial invasion of the oral cavity can be identified and used as biomarkers for early diagnosis of the disease and its prevention.

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