

Metabolic and immunological approaches to assessing the characteristics of chronic periodontitis associated with diabetes mellitus type 2

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Applicant

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Abstract. Purpose: To develop a system of biomarkers for the comorbid course of chronic periodontitis and type 2 diabetes mellitus based on metabolic and immunological approaches to assessing the local pathological process. Materials and methods: a comparative analysis of indicators of molecular genetic research of 3 study groups was carried out: the main group consisted of 58 patients with an association of moderate chronic generalized periodontitis and compensated DM2; the comparison group consisted of 39 patients with moderate chronic periodontitis without concomitant somatic pathology; the control group consisted of 27 people without signs of chronic periodontitis with a sanitized mouth without clinically pronounced manifestations of somatic pathology. Results and conclusions: The lipid profile of saliva has a pronounced diagnostic value in chronic periodontitis in association with type 2 diabetes mellitus, with triglyceride levels > 6.5 mmol/l, and LDLP < 2.8 mmol/ml.

Keywords: type 2 diabetes mellitus, periodontitis, diagnosis.

Introduction

Periodontal diseases have a complex etiopathogenesis and arise as a result of a combination of a number of factors leading to the destruction of the periodontium, irreversible resorption of bone tissue and loss of teeth [1]. The prevalence of periodontitis among the adult, able-bodied population in the world reaches 75%. This nosology is considered the second most common in the world [2], while the severe form of periodontitis is the sixth most common disease [3]. Periodontitis has a great impact on the health of the population also for the reason that it is associated with a number of systemic diseases, including diabetes mellitus (DM), cardiovascular diseases, including atherosclerosis, and others [4, 5]. Today, the polymicrobial nature of periodontitis is recognized, and the inflammatory response of the human body is considered a decisive factor in the development and progression of the disease [6, 7].

Chronic periodontitis and DM are two comorbid diseases common throughout the world [8–10]. The International Diabetes Federation (IDF) has predicted that the number of patients with DM will grow steadily and will reach over 500 million by 2030 [11, 12]. Approximately 90% of all DM patients have the so-called DM2, due to a combination of a decrease in insulin secretion by β -cells of the pancreas and a general increase in the resistance of human somatic cells to insulin [13]. Many researchers have shown that metabolic changes associated with DM2 are systemic in nature, affect many organs and tissues and cause chronic periodontitis, among other consequences [14, 15].

According to modern researchers, the pathogenesis of DM is based on four leading mechanisms - hyperglycemia, cell resistance to insulin, hyperlipidemia, and immune dysfunctions [16]. The same mechanisms, manifested to varying degrees at the local level, underlie the pathogenesis and chronic periodontitis [17]. The existence of a relationship between the development of DM2 and infectious and inflammatory periodontal diseases is not in doubt among modern researchers [17]. At the same time, it is emphasized that against the background of DM, chronic periodontitis, as a rule, flows much more heavily [18]. At the same time, in recent years, quite a lot of evidence has appeared that not only DM affects the development of periodontitis, but also periodontal diseases can affect the development and course of DM [19]. The mechanisms of these mutual influences are not fully disclosed, and their study on the basis of modern biotechnologies, as well as the determination of biomarkers of such an interconnection, still remain an urgent medical problem.

Purpose of the study – development of a system of biomarkers for the comorbid course of chronic periodontitis and type 2 diabetes mellitus based on metabolic and immunological approaches to assessing the local pathological process.

Materials and methods

To achieve this goal, 124 patients were observed, which made up 3 study groups - the main, comparison and control groups. The main group consisted of 58 patients with the association of moderate chronic generalized periodontitis and compensated DM2; the comparison group consisted of 39 patients with moderate chronic periodontitis without concomitant somatic pathology; the control group consisted of 27 people without signs of chronic periodontitis with a sanitized mouth without clinically pronounced manifestations of somatic pathology.

Molecular genetic studies were carried out using PCR and metagenomic analysis. In the latter case, the methods of 16S sequencing and whole genome sequencing were used to decipher the taxonomic and metabolic profiles of the microbiome of periodontal pockets/periodontal sulcus in the study groups.

In parallel, the lipid profile of saliva was determined spectrophotometrically, as well as the metabolism of ceramides by the level of ceramide kinase and the cytokine profile in saliva by enzyme immunoassay.

Statistical data processing was carried out on the basis of the statistical software package IBM SPSS, version 24.

Results and discussion

The novelty of the conducted research is also associated with a unique phenomenon registered using 16S sequencing.

Four metabolic pathways were established, the reduction of which is characteristic of the group of chronic periodontitis in combination with DM2 and distinguishes this group from the control group and the group of chronic periodontitis only: pyrimidine metabolism (ko00240), methane metabolism (ko00680), sphingolipid metabolism (ko00600) and biosynthesis of fatty acids (ko00061). In other words, among these features, half is accounted for by lipid metabolism (figure 1).

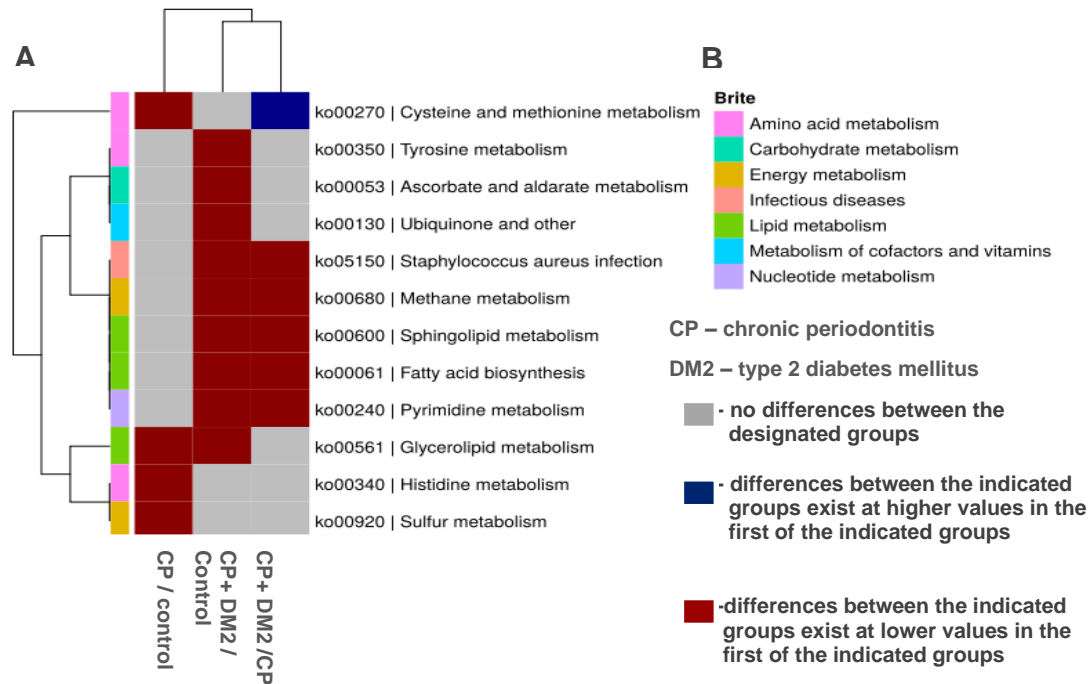


Fig. 1. A - a graph of intergroup differences in the functional profile of the microbiome of periodontal pockets/periodontal sulcus; B - the nature of metabolic reactions

Sources of scientific literature show that, in the oral microbiome, the most active producers of, in particular, sphingolipids/ceramides among periodontopathogens are representatives of the families Porphyromonadaceae and Prevotellaceae [20, 21]. In addition, sphingolipids in large quantities produce saprophytic microorganisms living in the oral cavity and belonging to the Sphingobacteriaceae family. With the help of additional studies, a low content of bacteria of the Sphingobacteriaceae family in the microbiome of patients with the association of chronic periodontitis and DM2 was confirmed, however, along with this, a rather high content of other sphingolipid-producing bacteria related to periodontopathogens was noted in the biofilm of patients in this group. To find out how this is combined with metabolic disorders that are characteristic separately for chronic periodontitis and for DM2, it is necessary to determine the effect of the lipid composition of the periodontal tissues themselves, regardless of the microbiome.

When assessing the characteristics of the general state of lipid metabolism in the oral cavity of patients with chronic periodontitis associated and not associated with DM2, the lipid profile of saliva in all groups was analyzed by spectrophotometry. The lipid profile included salivary cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, and the content of very low density lipoproteins in saliva was calculated (figure 2A).

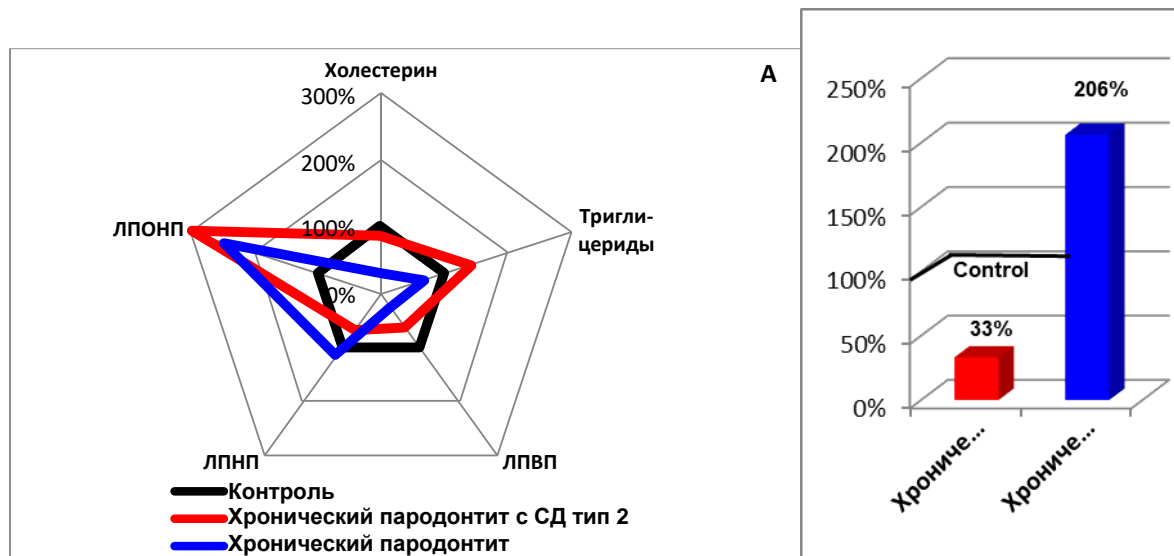


Fig. 2. Percentage of deviation from the control of lipid profile parameters (A) and ceramide kinase (B) in the saliva of patients in the study groups

It was determined that in chronic periodontitis associated with DM2, the level of triglycerides acquires a distinct tendency to increase in comparison with the control, and the content of low density lipoproteins, on the contrary, to a decrease. In chronic periodontitis without DM, the indicated tendencies acquire the opposite character, which makes the differences between the indicators in the comparison groups reliable. At the same time, the level of triglycerides with a value above 6.5 mmol/l has a high diagnostic value (AUC = 0.758), while the diagnostic value of LDLP below 3.4 mmol/l can be characterized as close to high (AUC = 0.691). These data do not contradict the literature data with the only difference that the authors, as a rule, determined the lipid profile according to the above parameters either only in chronic periodontitis, or only in type 2 diabetes mellitus [22].

In addition, one of the most important indicators of sphingolipid metabolism in the tissues of the oral cavity of patients with chronic periodontitis associated and not associated with DM2, as well as healthy individuals, may be the content of ceramide kinase in saliva, an enzyme that causes the breakdown of bioactive ceramides. The content of ceramide kinase in the saliva of patients with chronic periodontitis in combination with DM2 significantly decreases 3 times, while only in

chronic periodontitis it increases very significantly - 2.1 times (Figure 2B). With salivary ceramidase values below 28 ng/ml, it can be argued with a very high diagnostic value (AUC = 0.9) that chronic periodontitis is pathogenetically associated with DM2. We have not found a description of this phenomenon in the available literature.

For a more substantiated conclusion about the pathogenetic role of triglycerides, very low density lipids and ceramidase, it was decided to investigate the main cytokines in saliva that regulate inflammation and create conditions for the development of oxidative stress with its leading role in lipid peroxidation. For this purpose, the levels of the pro-inflammatory cytokines IL-1 β , IL-6, TNF α , IL-17A, as well as the anti-inflammatory cytokine IL-10 were determined (Figure 3).

Differences between the groups of chronic periodontitis associated and not associated with DM2 were identified by the content of 2 cytokines in saliva - IL-17A and IL-10. These cytokines have a multidirectional effect: IL-17A has a powerful pro-inflammatory effect, while IL-10, on the contrary, suppresses inflammatory reactions.

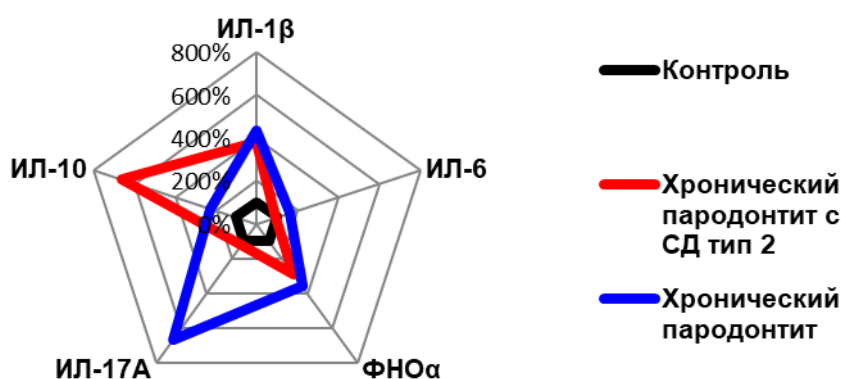


Fig. 3. Percentage of deviation from the control of indicators of the cytokine profile of patients in the study groups

In chronic periodontitis without concomitant disease, the level of IL-17A significantly prevailed, and in the association of chronic periodontitis with DM2, the level of IL-10 prevailed. Both indicators demonstrate high diagnostic significance and, at values below 28 pg/ml for IL-17A and above 13 pg/ml for IL-10, against the background of the existing clinical manifestations of chronic periodontitis, they characterize the presence of comorbidities in the form of DM2.

Conclusion

1. The lipid profile of saliva has a pronounced diagnostic significance in chronic periodontitis in association with type 2 diabetes mellitus, with triglyceride levels > 6.5 mmol/l, and LDLP <2.8 mmol/ml.

2. Metagenomic analysis showed disorders of sphingolipid metabolism, which is confirmed by the results of enzyme immunoassay to determine the level of ceramidase in saliva: at values below 28 ng/ml, it can be argued with high diagnostic significance that chronic periodontitis is pathogenetically associated with type 2 diabetes mellitus.

3. The pro-inflammatory cytokine IL-17A and anti-inflammatory IL-10 demonstrate a high diagnostic value, and at values below 28 pg/ml for IL-17A and above 13 pg/ml for IL-10 against the background of the existing clinical manifestations of chronic periodontitis, they characterize the presence of concomitant pathology in the form of sugar type 2 diabetes.

4. To assess the degree of influence of dyslipidemia on the course of chronic periodontitis, it is advisable to determine the lipid profile of saliva and the metabolism of ceramides by the content of ceramide kinase in saliva with the greatest diagnostic significance of assessing the metabolism of ceramides. When assessing the state of dyslipidemia, it should be borne in mind that for chronic periodontitis of moderate severity in combination with compensated type 2 diabetes mellitus, the content in saliva is characteristic: triglycerides > 6.5 mmol/l, low density lipoproteins <2.8 mmol/l, ceramide kinase < 28 ng/ml.

5. To assess the degree of immune dysfunction in chronic periodontitis, it is advisable to determine the level of proinflammatory IL-17A and immunosuppressive

IL-10 in saliva. When assessing the state of immune dysfunction, it should be borne in mind that for chronic periodontitis of moderate severity in combination with compensated type 2 diabetes mellitus, saliva content is characteristic: IL-17A <28 pg/ml, IL-10 > 13 pg/ml.

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