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(SHORT COMMUNICATION)

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Diagnostic molecular genetic test-systems in the practice of a dentist

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Abstract

Chronic periodontitis is a disease accompanied by destruction of dental tissues and resorption of the alveolar process.

It is a widespread and serious public health problem. Currently, epigenetic risk factors for the development of this disease play a major role. However, the clinical practitioner needs to make a thorough diagnosis, to identify the risk factors for the development of the disease.

Diagnostic test systems are one of the main tools for identifying risk factors for the development of this or that disease at the practical reception. They allow the practitioner to make the right clinical decisions, assess the prognosis of further development of the disease.

This article is devoted to the long-term experience of using molecular genetic tests at clinical appointments to determine the genetic predisposition to the development of inflammatory diseases and to draw up an individual programme of disease prevention.

Keywords: Chronic Periodontitis; Risk Factors; Molecular Genetic Tests; Personalized Medicine; Clinical Decision Support

1. Introduction

Chronic periodontitis, a disease accompanied by supporting tissues of the teeth destruction and resorption of the alveolar process, it has a multifactorial etiopathogenesis. The prevalence of inflammatory periodontal diseases in both developed and developing countries in recent decades has reached such a scale that, according to statistical criteria, it already fits the concept of a pandemic [18,20,22,].

Genetic, environmental and microbiological factors are the three main parameters that can determine the course of the disease [10,16,17,19,21].

There are many diagnostic test systems that allow the physician to diagnose the presence of periodontopathogenic complexes and genetic predisposition to inflammatory processes in a patient.

The relevance of diagnosing risk factors for periodontal disease is beyond doubt. Identification of periodontopathogens is crucial for development of a proper plan of antibacterial therapy.

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In turn, there is a body immune response to periodontopathogen and its endotoxins invasion, expressed in a cascade of innate and acquired immune system responses [1,2,3,4].

Of a particular interest among cytokines was interleukin-1 (IL-1), which is encoded by the IL-1 gene cluster at position 2q13-21. This cytokine, produced by monocytes, macrophages and dendritic cells, induces a complex network of pro-inflammatory cytokines and plays an important role in the regulation of the immune and inflammatory response to infections. IL-1 induces the migration of immune cells to sites of infection through the expression of adhesion factors on endothelial cells [5,6,7,8,26,27,28].

Kornman K.C. et al (1997) first reported SNPs IL-1-889 and IL-1B+3953 linkage with disease severity in patients with periodontitis [11]. When they identified a polymorphism that showed a 4-fold increase in IL-1B production in people with the combined IL1-A allele 2 -889 plus IL-1B allele 2 +3953 genotype, the odds ratio of developing severe periodontitis to mild periodontitis was 6.8. This study showed that the association with increased IL-1 production may be a strong indicator of a predisposition to severe periodontal disease in the adult population.

In 1999, McGuire et al. looked at IL-1 genotype assessment as a means of determining prognosis and predicting tooth loss. This study involved 42 patients who had been in treatment for 14 years. Sixteen of these patients tested positive for a gene associated with increased IL-1 production [29].

In 2012, Karimbux et al presented a review of 27 studies, which concluded a significant clinical effect based on the differences observed in IL-1A and IL-1B production [9].

Finally, Socransky et al (2000) evaluated the microbiological parameters associated with the IL-1 gene polymorphism in patients with periodontitis. In this study, a finger blood sample was taken from 108 subjects to evaluate IL-1A (+4845) and IL-1B (+3954) genotyping by PCR. All subjects were sampled up to 28 teeth for the presence of bacterial deposits, which were assessed by DNA-DNA hybridisation. In the test population, 28 subjects were genotype positive. Among these genotype-positive subjects, red and orange complex species as well as S. intermedius and S. gordonii were significantly higher than those with a negative genotype. It has been suggested that elevated cytokine levels in subjects with a positive genotype may create a favourable environment for bacterial growth [25].

In other words, cytokines contribute to the predisposition to periodontal disease. It is possible that disease may be caused by inadequate production of cytokines against inflammatory activity. Some studies have shown that there is an association between cytokine SNPs and periodontal disease. Such variations can alter cytokine production levels, which in turn can alter the immune response and cause prolonged inflammation. Consequently, studying the relationship between the frequency of genotypes and/or alleles and susceptibility to disease may identify certain genotypes and alleles that may increase the risk of inflammatory diseases [8,10,12,1314,15,23,24].

This article focuses on 8 years of experience in the use of molecular diagnostic tests from HAIN-lifescience (Germany) in the clinical practice of a dentist. Their use enabled patients with an increased tendency to inflammatory reactions and unfavourable periodontopathogenic complexes to adequately treat and prevent premature tooth loss.

The aim of the study was to examine the effectiveness of identifying risk factors for periodontal disease, such as microbial and genetic predisposition factors for inflammatory processes, in order to develop an individual treatment plan for the patient.

2. Materials and Methods of Study

In a prospective study of 98 people, we selected 42 patients with mild to moderate cases of chronic periodontitis treated at the University Dental Clinic. Periodontal disease was initiated by periodontopathogenic bacterial complexes. The patients ranged in age from 28 to 56 years, general somatic status was not compromised, and there were no bad habits (smoking).

The dental diagnosis was based on physical and X-ray examination, according to the American Academy of Periodontology classification adopted in 2018. The periodontal status was assessed using the RBL, CAL index and the bone destruction evaluation index according to R.A.Attstrom (1998).

Two groups of patients were formed:

- Group 1 (24 people) - patients with periodontitis Stage I Grade B
- Group 2 (18 patients): patients with periodontitis Stage II (III) Grade C.

Since patients did not have five-year panoramic radiographs, we used indirect signs of disease determination based on fresh radiographs. We determined the ratio of the percentage of bone loss from the root length in the area of the most affected teeth to the patient's age.

Informed voluntary consent was obtained from the patients who took part in the study. The study was approved by the ethical committee of Pavlov First St. Petersburg Medical University (№05/2023 dated 28.04.2023) and was conducted in accordance with the Declaration of Helsinki.

2.1. Patient samples and cell lines.

Two types of molecular genetic tests were used (HAIN-lifescience, Germany):

- "Micro-IDent plus", a molecular genetic test for the detection of 11 periodontopathogenic strains.
- "GenoType IL-1", a test to determine genetic predisposition to the development of periodontal inflammatory diseases.

For the "Micro-IDent plus" test, biomaterial was collected with a sterile paper absorber from the periodontal sulcus, packed by eppendorf and sent to the University laboratory.

For the GenoType IL-1 test, a scraped gingival mucosa was taken, packed by eppendorf and sent to the University laboratory.

2.2. Micro-IDent plus11 Protocol (DNA-STRIP Technology)

2.2.1. Preparation of the amplification mixture

- One sample was taken: Sterile PCR tubes: per 0.5 ml - 2
- One of the two 0.5 ml tubes and added reagents:
- Amplification mixture no. 1: AM-A1 5 µl,
- AM-B 17.5 µl;
- Total volume 22.5 µl. Mixing by pipetting.
- In a second 0.5 ml test tube and reagents added:
- Amplification Mix №2:
- AM-A2 5 µl, AM-B 17.5 µl; Total volume 22.5 µl.
- Mixing by pipetting.

2.2.2. DNA extraction (corrected 2.2.2)

- Filling an ultrasound bath with water and detergent solution - 1ml of «Tween" 20 per 1 liter of water, warming up.
- Add 400 µl of 5% "Chelex" solution to a 1.5 ml tube with a screw-cap sample.
- Centrifuge for 1 min at full speed in a benchtop centrifuge (15000 rpm).
- Soaking in an ultrasound bath for 15 min at 60-65 oC.
- "Vortex" shaking of specimens for 10 sec, incubation for 15 min in heating block at 105oC.
- Shaking the samples on a vortex for 30 sec.
- Centrifuge sedimentation for 1 minute at full speed (15,000 rpm).
- Collect 5 µl of natant and add to PCR tube with PCR mixture.
- Add 20 µl of oil to the PCR tube to prevent evaporation during amplification.

2.2.3. Amplification (corrected 2.2.3)

Place the tube with the sample in the amplifier unit. Amplification program: 5 min - 95°C x 1 cycle; 30 sec - 95°C, 2 min - 58°C x 10 cycles; 25 sec - 95°C, 40 sec - 53°C, 40 sec - 70°C x 20 cycles; 8 min - 70°C x 1 cycle.

The amplification process takes 2h 24min 4sec.

2.2.4. Hybridization (corrected 2.2.4)

- Mixing solutions for hybridization (Con and Sub). One strip was taken. Per strip mixed: 10 µl of "Con C" concentrate (orange) and 990 µl of "Con D" buffer; 10 µl of "Sub C" concentrate (yellow) and 990 µl of "Sub D" buffer.
- Twenty µl of Denaturing Solution (DEN, blue) was added to the cell without mixing.
- 20 µl of amplification product was added to the Denaturing Solution (DEN) cell from each of the two tubes. Stirring by pipetting. Incubation for 5 min at room temperature. Preheat 1 ml of HYB solution (1 PCR tube per 1.5 ml) and STR hard-wash solution (1 PCR tube per 1.5 ml) to 45°C in a Thermoblock.
- 1 mL of prewarmed hybridization buffer (HYB solution, green) was added to the cell. The strip is placed in the cell.
- Incubation for 30 min at 45°C ("TwinCubator®" "Thermoshaker").
- Complete aspiration of Hybridization Buffer (HYB solution).
- 1 ml of Hard Wash Solution (STR, red) was added to the strip cell. Incubation for 15 min at 45°C ("TwinCubator®", Step 2 S2).
- Complete aspiration of the hard-washing solution (STR).
- Washing the strip in 1 ml of Rinsing Solution (RIN) ("TwinCubator®", Step 3 S3). Removal of the RIN solution.
- Add 1 ml of freshly diluted Conjugate (Con C + Con D) to the cell. Incubation for 30 min ("TwinCubator®", Step 4 S4).
- Remove the solution (see step 11) and wash the strip twice ("TwinCubator®" 1 min Steps 5, 6, 7): - first1min in 1 ml of "Rinsing Solution" (RIN) - Repeat for 1 minute in 1 ml of RIN Solution - and 1 min in 1 ml of distilled water.
- Add 1 ml of freshly diluted substrate (Sub C + Sub D) to the strip. Incubate in a heat shaker for 5 min (Step 8 S8).
- Double rinsing with distilled water (S 9,10).
- Dry the strip between two layers of filter paper.

2.2.5. Interpretation of the results with the template (corrected 2.2.5)

2.3. GenoType IL-1 Protocol (DNA-STRIP Technology) (corrected 2.3)

2.3.1. Preparation of the amplification mixture **(corrected 2.3.1)**

- One sample was taken.
- Number of sterile PCR tubes:
- per 0.5 ml - 1 for 1.5 ml - 1 for sampling
- Reagents added to 0.5 ml tube:
- AM-A - per sample - 5 µl.
- AM-B - per sample - 17.5 µl

2.3.2. DNA extraction (corrected 2.3.2)

- 200 µl of 50 mM NaOH was added to a 1.5 ml test tube containing the sample. Shaking on a vortex for 30 seconds.
- Incubation for 5 min at 95C in a Thermoblock.
- Shaking on a Vortex for 15 sec. Precipitation by centrifugation for a short period of time. Tweezers squeezed the swab and removed from the tube.
- 20 µl of 1M «Tris", pH 8.0 was added.
- Shaking on a "Vortex" for 15 sec. Centrifugation for 1 minute at maximum speed (15,000 rpm) in a benchtop centrifuge.
- Transfer 5 µl of supernatant to a PCR tube with a ready-made PCR mixture. A new tip is used each time.
- Adding Vaseline oil to the 20 µl PCR tube to prevent evaporation during amplification. A new tip is used each time.

2.3.3. Amplification (corrected 2.3.3)

1) The sample tube is placed in the amplifier unit.

Amplification program: 15 min - 95°C x 1 cycle; 30 sec - 95°C, 2 min - 58°C x 10 cycles; 25 sec - 95°C, 40 sec - 53°C, 40 sec - 70°C x 20 cycles; 8 min - 70°C x 1 cycle.

The amplification process takes 2 h 33 min 50 sec.

2.3.4. Hybridization (Corrected 2.3.4)

- Mixing solutions for hybridization (Con and Sub). One strip was taken. Per strip mixed: 10 µl of Con C concentrate (orange) and 990 µl of Con D buffer; 10 µl of Sub C concentrate (yellow) and 990 µl of Sub D buffer.
- 20 µl of Denaturing Solution (DEN, blue) was added to the cell.
- 20 µl of amplification product was added to the cell with a drop of Denaturing Solution (DEN). Stirring by pipetting. Incubated for 5 min at room temperature. Preheat 1 ml of HYB solution (1 PCR tube per 1.5 ml) and STR hardwash solution (1 PCR tube per 1.5 ml) to 45°C in a thermoblock.
- 1 mL of prewarmed hybridization buffer (HYB solution, green) was added to the cell. The strip is placed in the cell.
- Incubation for 30 min at 45°C («TwinCubator®" "Thermoshaker").
- Complete aspiration of Hybridization Buffer (HYB solution).
- 1 ml of Hard Wash Solution (STR, red) was added to the strip cell. Incubation for 15 min at 45°C ("TwinCubator®", Step 2 S2).
- Complete aspiration of the rigid wash solution (STR).
- Washing the strip in 1 ml of Rinsing Solution (RIN) ("TwinCubator®", Step 3 S3). Removal of the RIN solution.
- Add 1 ml of freshly diluted Conjugate (Con C + Con D) to the cell. Incubation for 30 min ("TwinCubator®", Step 4 S4).
- Remove the solution (see step 11) and wash the strip twice ("TwinCubator®" 1 min Steps 5, 6, 7): - first1min in 1 ml of Rinsing Solution (RIN) - Repeat for 1 minute in 1 ml of RIN Solution - and 1 min in 1 ml of distilled water.
- Add 1 ml of freshly diluted substrate (Sub C + Sub D) to the strip. Incubate in a heat shaker for 5 min (Step 8 S8).
- Double rinsing with distilled water (S 9,10).
- Dry the strip between two layers of filter paper.

2.3.5. Interpretation of the results using a template (corrected 2.3.5)

The diagnostic kits and Protocols of analysis were provided by HAIN-lifescience (Germany).

The genotype types are presented in Table 1.

3. Statistical Research Methods

The Mann–Whitney U-test was used to reliably compare and verify differences between the control groups ($p < 0.05$).

4. Results and Discussion

DNA-based disease risk assessment, the use of molecular signatures for prediction, and the choice of therapeutic agents are currently important aspects of personalized medicine.

Patients in the Group 1 had an average value of CAL - 1.94+0.24 (corresponding to stage 1), RBL - 14.7%+0.22 with no signs of tooth mobility. The general somatic status is not compromised, the patients denied the presence of bad habits. The main criterion - the ratio of the percentage of loss of bone tissue (the most affected area) by patients age was 0.86 +0.48. There was no loss of teeth due to periodontitis.

In the Group 2, the average value of the bone resorption index was 33.2%+0.56. In addition, 11 out of 18 patients (61%) had degree I-II tooth mobility. The main criterion - the ratio of the percentage of loss of bone tissue (the most affected area) by patients age was 1.6 + 0.62. Which corresponds to grade C according to the AAP Classification (2018).

The "Micro-IDent plus" test in patients of the 1st group revealed a red complex in 6 patients (25%), an orange complex in 14 patients. Analysis of the "GenoType-IL1" test showed that among 24 patients of group 1, genotype A (normal inflammatory response) was diagnosed in 15 patients, genotype B (impaired inflammatory response) in 9 patients.

At the same time, in patients of the 2nd group, representatives of the "red complex" were found in 13 out of 18 patients (72.2%), "violet complex" in 3 patients (16.6%), "orange complex" in 4 patients. (22.2%). Analysis of the results of the GenoType IL-1 test showed that 10 patients were diagnosed with the "D" genotype, 5 with the "C" genotype, and only 3 with the "B" genotype.

Thus, in patients with stage II (III) Grade C, representatives of the most dangerous periodontopathogens were most often encountered. At the same time, in patients with stage I Grade B, against the background of biofilm deposition, a low level of destruction of periodontal tissues was noted.

The use of molecular genetic tests at a practical appointment with a dentist is relevant for determining the risk of developing a disease, prognosis and an individual plan for the treatment of periodontal diseases.

The presence of "red" and "pink" complexes in the oral cavity with the patient's tendency to an intense inflammatory reaction (genotypes C and D) allows us to conclude that the prognosis of the disease is unfavorable. Elimination of the microbial risk factor in the oral cavity and constant monitoring of unfavorable genotypes will stabilize the course of periodontal disease.

Traditionally, clinicians focus on the patient's clinical signs and symptoms. However, traditional methods are not always effective because each person has a different genetic architecture.

Figures 1,2,3 show the patient's clinical case.

Abbreviations used in this paper:

- SNP – Single Nucleotide Polymorphism
- RBL - radiological bone loss (the primary criteria for periodontitis grading are direct or indirect evidence of disease progression. Direct evidence consists of longitudinal documentation of progressive attachment loss and/or radiographic bone loss).
- CAL - clinical attachment loss (CAL represents the extent of periodontal support that has been lost around a tooth and is measured with the periodontal probe as the distance from the cemento-enamel junction (CEJ) to the base of the pocket).
- CP – Chronic periodontitis
- AAP-American Academy of Periodontology

5. Conclusions

Over the past two decades, the medical community worldwide has been an active participant in the global transformation of healthcare, which is taking place against a backdrop of rapid developments in biomedical technologies.

Personalized medicine is associated with the sequencing of the human genome. As commonly used, this term predicts a leap forward in disease prevention and drug treatment based on knowledge of individual genetic predisposition. biological individual that he or she is, thereby radically changing our paradigms and increasing efficiency.

Physicians in the clinical setting need a tool to determine the risk of disease development.

Molecular genetic tests developed by «HAIN-lifescience» (Germany) for dental practices are certainly effective in determining risk factors for periodontal disease and understanding prognosis. Nevertheless, test administration requires considerable financial resources, availability of PCR-laboratory.

At the same time, more extensive use of genetic testing is expected. But the main requirements for the test systems are ease of implementation and visibility of amplification results. Loop Isothermal Amplification (LAMP) is a simple, fast, specific and cost-effective method of nucleic acid amplification compared to PCR, nucleic acid sequence-based amplification, self-sustaining sequence replication and chain substitution amplification.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of informed consent

Informed consent was obtained from all subjects participating in the study.

Author Contributions

E.A.O. and N.G.M. contributed substantially to the conception, design, literature analysis, workshop discussion, drafting, and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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