NAO "Toregeldi Sharmanov National Medical University"

Annotation
dissertation work

for соискание the academic degree of Doctor PhD
Educational programm: 8D10102 - "Medicine"

Topic**: " "Assessment of the Clinical and Immunological Characteristics and Oral Microbiome in Patients with Ankylosing Spondylitis "**

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Relevance

Ankylosing spondylitis (AS) is a chronic, progressive inflammatory rheumatic disease that mainly affects the axial skeleton with frequent involvement of the entheses and peripheral joints, as well as other organs and systems.

It was found that the global incidence of ankylosing spondylitis is 0.1-0.5% [Ward M. M. et al., 2019]. AS is mainly detected in males and affects the axial skeleton andileosacral joints [Garcia-Montoya L. et al., 2022]. The disease most often makes its debut before the age of 45, when patients are at the most able-bodied age, leading to disability and a significant decrease in the quality of life [Hwang M. C. et al., 2021].

The etiology and pathogenesis of ankylosing spondylitis at the present stage are sufficiently studied. The main risk factors for AS include genetic predisposition, infectious agents, and traumatic exposures [Voruganti A. et al., 2020]. Currently, in addition to these three factors, the etiology of AS has also begun to consider the state микробиомаof the intestinal microbiome, the relationship of which with autoimmune pathology is the object of close attention of researchers at this time [Wang Y. et al., 2022].

An important place in the immunopathogenesis of AS is occupied by an imbalance of innate and adaptive immune responses with activation of the IL-23/IL-17 axis, increased production of TNF-α, IL-6, IL-17, and IL-22, which supports chronic inflammation in the entheses and contributes to pathological osteoproliferation. Evidence is accumulating that these cytokine shifts are closely associated with microbial signals from mucosal barriers [Chen T. et al., 2024].

According to the data of Tavasolian F. et al, the connection between the microbiome and immune cells plays an important role in the pathogenesis of AS, which can be used as a new approach to the diagnosis and therapy of the disease.

A systematic review of 29 studies on changes микробиомain the gut microbiome in patients with AS performed by Wang L. et al in 2022 анкилозирующимrevealed significant differences in the composition of the gut microbiome in patients with ankylosing spondylitis compared with healthy individuals. These data are also supported by the results of a meta-analysis of 92 studies микробиомаof the intestinal microbiome of patients with rheumatic diseases, in which the greatest difference in the state микробиомаof the intestinal microbiome was noted in patients with a diagnosis of ankylosing spondylitis, in particular according to the indicators of beta-diversity [Wang Y. et al., 2022].

The gut, however, is not the only mucosal barrier involved in the immunopathogenesis of AS. The oral cavity is another important microbiota reservoir that can influence systemic inflammation and cytokine response. To date, studies микробиома ротовой of the oral microbiome have revealed significant biomarkers for the development of diseases such as rheumatoid arthritis, cardiovascular diseases, Alzheimer's disease, colorectal cancer, and others [Rajasekaran J. et al., 2024]. At that time, only 4 publications on the studyмикробиомаof the oral microbiome in patients with AS were known: Bisanz

J. et al (Australia, 17 patients), Lv L. et al (China, 37 patients), Gill T. et al (USA, 14 patients), Stoll M. et al (USA, 5 patients). However, we have not found any similar studies on the territory of the Central Asian region, which indicates the relevance of this topic.

Thus, research in this area is of undoubted scientific and practical interest, as it will provide objective information about the nature of immunopathological disorders in AS and can become an important tool for early diagnosis, assessment of activity, severity of the course, prognosis of the disease and the effectiveness of therapy.

Objective: to evaluate the impact of микробиоматhe oral microbiome on the clinical course and immunological profile of patients with ankylosing spondylitis.

Research objectives:

1. To analyze the features of the clinical course of ankylosing spondylitis depending on the HLA B27 status and gender of patients.
2. To assess the state of the immunological profile and the relationship of immunological mediators with clinical and anamnestic parameters in patients of the study group.
3. To study the state микробиомаof the oral microbiome in patients with ankylosing spondylitis.
4. To study the relationship of the immunological profile of patients with AS with clinical and laboratory parameters and the composition микробиомаof the oral microbiome
5. To analyze the clinical, immunological and microbiomic features of the studied patients depending on the therapy performed.
6. To develop prognostic models of possible complications of ankylosing spondylitis in the studied patients

Материалы Research materials:

The study included 140 people: 70 patients with a verified diagnosis of ankylosing spondylitis and a control group of healthy individuals in the amount of 70 people.

Study design: Observational analytical one-step cross-sectional study.

Research methods:

1. General clinical methods: collection of complaints, anamnesis of the disease and life, objective examination of organs and systems.
2. Functional disorders of the spine and joints were evaluated using the BASFI (the Bath Ankylosing Spondylitis Functional Index) and BASMI (Bath Ankylosing Spondylitis Metrology Index) indices.
3. Assessment of the degree of disease activity using the BASDAI calculator, (Bath Ankylosing Spondylitis Disease Activity Index), ASDAS (Ankylosing Spondylitis Disease Activity Score)

4. Assessment of quality of life using the SF-36 (Short Form-36 Health Survey) and ASAS HI (ASAS Health Index) questionnaires.
5. General clinical laboratory research methods: general blood analysis, biochemical blood analysis (ALT, AST, creatinine, total protein, CRP).
6. Immunological study: determination of cytokines and chemokines sCD40L, EGF, Eotoxin, FGF2, FLT3L, Fractalkine, G-CSF, GM-CSF, GROa, IFNa2, IFNy, IL1a, IL1b, IL1RA, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12(p40), IL12 (p70), IL13, IL15, IL17A. IL17/IL25, IL17F, IL18, IL22, IL27, IP10, MCP1, MCP3, M-CSF, MDC, MIG, MIP1a, MIP1b, PDGF-AA, PDGF-AB/BB, TGFa, TNFa, TNFb, VEGF-A.
7. Molecular genetic study: determination of HLA B27.
8. Microbiological study микробиомаof the oral microbiome by 16s sequencing.
9. Statistical method for processing the received data.

Inclusion criteria for the main study group:
- age 18-62 years
- established diagnosis of AS according to the criteria modified NewYork criteria and ASAS criteria
- availability of informed consent

Exclusion criteria:
-age under 18 and over 62
- insufficient number of criteria for the diagnosis of AS
- severe concomitant somatic diseases and / or mental illnesses
- pregnancy or lactation
- taking antibiotics and probiotics in the last 6 months before taking the study material
- inflammatory diseases of the oral cavity

Scientific novelty

The state микробиомаof the oral microbiome of patients with ankylosing spondylitis in the Central Asian region was studied for the first time.

For the first time in the world, the features микробиомаof the oral microbiome in patients with AS were studied depending on gender, HLA B27 status, and clinical course of the disease.

For the first time in the world, a study of the relationshipbetween the immunological profile andмикробиомаthe oral microbiome was conducted in patients with ankylosing spondylitis.

Practical significance of the results obtained.

The obtained data supplemented the knowledge about the phenotypic features of ankylosing spondylitis and the role of immunological markers in the etiopathogenesis of AS (author's certificate No. 43489 dated 05.03.24). The results of the study revealed new microbiome markers of ankylosing

spondylitis and associated metabolic pathways, and were registered in the NCBI Sequence Read Archive (SRA) international database under the access number BioProject PRJNA1358013. The results obtained on predicting the course of AS, taking into account microbiome and immunological markers, made it possible to calculate the probability of outcome in the studied patients (author's certificate No. 43485 dated 05.03.24).

Main provisions of the dissertation submitted for defense

1. In patients with ankylosing spondylitis with a positive HLA B27 status and in male patients, regardless of the HLA B27 status, the clinical course of the disease was characterized by early onset, manifestations of sacroileitis stage 4 and NFS stage 3. When studying the immune status of patients with ankylosing spondylitis with HLA-B27-positive status, there was a significant increase in the level of cytokine IL-17A, in the HLA-B27-negative group - the GM-CSF factor, in men (regardless of the HLA B27 status) - a significant increase in EGF and M-CSF factors.
2. The intersample diversity микробиомаof the oral microbiome in patients with ankylosing spondylitis significantly differs by the unweighted UniFrac index and is characterized by an increase in the number of Actinobacteria by more than 2 times at all taxonomic levels up to the species level. The microbiome of HLA-B27 positive patients, both male and female, is also characterized by a significant enrichment of bacteria of the Stomatobaculum and Neisseria oralisgenera, in male patients there is a statistically significant increase in the abundance of bacteria of the Propionibacteriales and Absconditabacteriales orders (SR1), and in female patients - bacteria Rothiaof the Rothia and Actinobacillus genera (LEfSe, LDA > 2.0, $p < 0.05$).
3. In patients with ankylosing spondylitis taking synthetic basic anti-inflammatory drugs, there is a significant increase in the relative number of bacteria of the species Oribacterium sinus and Kingella sp. and an increase in pro-inflammatory markers G-CSF and IL-17F. In patients receiving genetically engineered biological therapy, a significant increase in Coriobacteriales bacteria Coriobacterialesand an increase in the cytokine IL-5 are observed.

CONCLUSIONS:

1. In patients with ankylosing spondylitis with positive HLA B27 and male patients (regardless of HLA B27 status), the clinical course was characterized by early onset, manifestations of sacroileitis stage 4 and NFS stage 3.
2. The following proinflammatory immunomediators were significantly higher in the studied patientsпровоспалительные иммуномедиаторы: the level of IL-17A was 2.46 times higher compared to patients with negative HLA-B27 status (2.95 vs. 1.2, respectively; U = 144; Z = -2.004; p = 0.045), in the HLA-B27-negative group - GM factor-CSF was 9.31 times higher compared to the HLA-B27-positive group (24.2 vs. 2.6, respectively; U = 180; Z = -2.236; p = 0.025; In men, regardless of HLA-B27-status, the EGF level was 3.76 times higher compared to the comparison group (109 [Q1–Q3: 38-148] vs. 29 [Q1–Q3: 6.5-

55]), and the M-CSF level is 2.77 times higher (61 [Q1–Q3: 26-98] vs. 22 [Q1-Q3: 9-49]).

3. In the study of correlations: хемокинMIG chemokine had a positive correlation of weak strength with age of debut in HLA-B27-positive patients ($r = 0.381$, $p = 0.010$) and a negative correlation of high strength in HLA-B27-negative patients ($r = -0.781$, $p = 0.013$). In female patients, positive correlations of high strength of proinflammatory chemokines MIP1Aand cytokines IFNy, IP10 with disease duration were found ($r = 0.605$, $p = 0.037$, $r = 0.599$, $p = 0.040$ and $r = 0.577$, $p = 0.049$, respectively).
4. When comparing микробиоматhe oral microbiome by beta-diversity, a significant difference was found with the control group by the unweighted UniFrac index (PERMANOVA: $F = 7.61$, $p = 0.001$; ANOSIM $R = 0.24$, $p = 0.001$). In patients with ankylosing spondylitis, Actinobacteria was significantly higher at all taxonomic levels up to the species (the median proportion in the main group was 2 times higher than in the control group: 9.46 % (Q1-Q3: 5.37–14.27) and 4.67 % (Q1-Q3: 2.92-9.68) with a very high positive correlation with metabolism of inositol phosphates (ko00562).
5. Особенности Features of microbiome diversity in HLA-B27–positive patients showed a significant increase in the number of bacteria of the Stomatobaculum genus Stomatobaculum(5.00 % (Q1–Q3: 1.00–12.00) versus 1.00 % (Q1–Q3: 0.00-2.00) in HLA-B27-negative patients), including Stomatobaculum the Stomatobaculum longum species (2.00 % (Q1–Q3: 0.00–6.00) vs. 0.00 % (Q1–Q3: 0.00–0.00)), as well as Neisseria oralis (22.00 % (Q1–Q3: 4.00–44.00) vs. 2.00 % (Q1–Q3: 0.00–18.00)) ($LDA > 2$, $p < 0.05$). Men had significantly higher numbers of Propionibacteriales Propionibacterialesand (4.00 % (Q1–Q3: 2.00–8.00) vs. 2.00 % (Q1–Q3: 1.00–4.00) in women) and Absconditabacteriales (SR1) (1.00 % (Q1–Q3: 0.00–5.00) vs. 0.00 % (Q1–Q3: 0.00-5.00) in women).Q3: 0.00–1.00)), Propionibacteriaceae (4.00 % (Q1–Q3: 2.00–8.00) vs. 2.00 % (Q1–Q3: 1.00–4.00)) and Porphyromonadaceae (53.00 % (Q1–Q3: 26.00–160.00) vs. 27.00 % (Q1-Q3: 10.00–75.00)), as well as the genus Porphyromonas (53.00 % (Q1–Q3: 26.00-160.00) vs. 27.00 % (Q1-Q3: 10.00-75.00)). Women had higher relative abundance of the genera Rothia (212.00 % (Q1–Q3: 66.00–464.00) vs. 106.00 % (Q1–Q3: 49.00–224.00)) and Actinobacillus (10.00 % (Q1–Q3: 1.00–90.00) vs. 0.00 % (Q1–Q3: 0.00–1.00)).
6. An increase in the abundance of Porphyromonadaceae and Saccharimonadaceae is associated with an increase in the concentration of key pro-inflammatory mediators. An increase in the abundance of the Porphyromonadaceae family Porphyromonadaceaewas associated with an increase in the concentration of IL-9 ($r = 0.363$; $p = 0.006$), MIP-1a ($r = 0.345$; $p = 0.009$), IL-8 ($r = 0.325$; $p = 0.013$), and MIP-1b ($r = 0.290$; $p = 0.029$)., EGF ($r = 0.288$; $p = 0.030$), IL-15 ($r = 0.288$; $p = 0.030$), IFN-γ ($r = 0.267$; $p = 0.044$), and IL-17F ($r = 0.266$; $p = 0.045$). An increase in Saccharimonadaceae abundance Saccharimonadaceaecorrelated with an increase in IL-9 ($r = 0.394$; $p = 0.002$), IP-10 ($r = 0.321$; $p = 0.015$), IL-8 ($r = 0.277$; $p = 0.037$), IL-6 ($r = 0.272$; $p = 0.041$), and GRO- α ($r = 0.265$; $p = 0.047$).

7. Comparative analysis, depending on the type of therapy received, showed that in patients with AS on basic therapy, the levels of pro-inflammatory markers G-CSF and IL-17F were higher than in patients receiving GIBT (45 (Q1-Q3: 30-58) versus 35 (Q1-Q3: 22-53) and 10.5 (Q1-Q3: 8.8-15.7) vs. 9.4 (Q1–Q3: 8.4–10.4), respectively), and in patients receiving genetically engineered biological therapy-a high level of IL-52.4 (4.37 (Q1–Q3: 1.9-12.0) vs. 2.4 (Q1–Q3: 0.9–3.0) vs.).
8. МикробиомныйMicrobiome analysis showed that the relative abundance of Oribacterium sinus (1.8 % (Q1–Q3: 0.4–4.2) versus 0.3 % (Q1–Q3: 0.0–0.9) in patients on GIBT) and Kingella sp. (0.9 % (Q1-Q3: 0.2-2.1) vs. 0.1 % (Q1-Q3: 0.0–0.4)). Patients receiving genetically engineered biological therapy had significantly more Coriobacteriales bacteria Coriobacteriales(2.4 % (Q1–Q3: 0.8-5.6) vs. 0.6 % (Q1-Q3: 0.1-1.5) on basic therapy) (LDA > 2, $p < 0.05$).
9. The methods used to analyze the prediction of complications in patients with ankylosing spondylitis made it possible to build models of the probability of developing sacroileitis and ankylosis of the lumbar spine, taking into account such factors as certain microbial taxa, cytokines, duration of the disease, occupational hazards and smoking.

Testing the work. The dissertation work was tested at the meeting of the Department of Internal Diseases No. 2 of NAO "Astana Medical University", Protocol No. 12A of June 23, 2025.

The results of the work are presented:

1) National Forum of Laboratory Medicine and scientific and practical conference with international participation "Modern trends in laboratory medicine for clinical practice", Astana, September 21-22, 2023.

Publications on the topic of the dissertation. The main results of the study are published in eight publications, including 3 publications recommended by the Committee for Quality Assurance in Education and Science of the Republic of Kazakhstan, 3 (three) in international scientific publications included in the international peer-reviewed database with quartile Q1 (93 percentile), Q3 (41 percentile, Q3 (37 percentile), 1 abstract in conference proceedings. 2 author's certificates were obtained.

Implementation of research results in healthcare practice. The results of this study were tested and implemented in the medical and diagnostic center of the State Enterprise for PCV of the State Medical Hospital No. 2,Astana.

Scope and structure of work.

The dissertation consists of 150 pages, including an introduction; a literature review; sections on materials and methods; the main part containing three sections from the results of their own research; conclusions; conclusions; a list of references, including 150 sources:143 sources in English and 7 sources in Russian. The text of the dissertation is illustrated with 15 tables and 57 figures.