

ORIGINAL ARTICLE

PECULIARITIES OF THE GUT MICROBIOTA IN PATIENTS WITH MIGRAINE COMPARING TO HEALTHY INDIVIDUALS

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The aim: Analyze the gut microbiome state in patients with migraine (M) and healthy individuals, to assess possible correlations between the detected changes in patients with migraine and the frequency, intensity of headaches, psycho-emotional state of the patients, and their quality of life.

Materials and methods: 100 objects were enrolled, divided into 2 groups: main – patients with M and control – healthy volunteers. Investigation of the intestinal microbiome was performed by chromato-mass spectrometry. For M patients the following scales were used: Visual Analogue Scale (VAS), Migraine Disability Assessment (MIDAS), Back Depression Inventory (BDI).

Results: In main group increased amount of *Alcaligenes spp* ($p = 0.0061$), *Clostridium coccoides* ($p = 0.0021$), *Clostridium propionicum* ($p = 0.0287$), *Eggerthella lenta* ($p = 0.0138$), *Pseudonocardia spp* ($p = 0.0210$), *Rhodococcus spp* ($p = 0.0164$), *Candida spp* ($p = 0.0079$), *Micromycetes spp* (campesterol) ($p = 0.0011$) were found. Patients with M had a raised amount of *Herpes simplex* ($p = 0.0305$) and endotoxin level ($p = 0.0459$). Differences in gut microorganisms in both groups were significant. In patients with M negative correlations were observed between *Alcaligenes spp* amount and BDI score ($r = -0.6226$, $p = 0.007$), VAS score ($r = -0.489$, $p = 0.046$), headache frequency ($r = -0.487$, $p = 0.046$); between the levels of *Clostridium coccoides* and MIDAS score ($r = -0.51$, $p = 0.035$), BDI score ($r = -0.54$, $p = 0.025$) and positive correlation between *Eggerthella lenta* level and VAS score ($r = 0.4830$, $p = 0.049$).

Conclusions: Correlations between changes of gut microbiome and M are promising for further research.

KEY WORDS: migraine, gut microbiota, depression, influence

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INTRODUCTION

Migraine is the second leading cause of headache and illness in young people, has a significant negative impact on the daily life of patients and interferes with their work, leisure, and social activities [1]. The gut-brain axis, which refers to the bidirectional biochemical signaling pathways that connects the gastrointestinal tract with the central nervous system, and vice versa, plays a significant role in the pathophysiology of migraine [2]. There's data on the significant prevalence of migraine in patients with certain gastrointestinal diseases, such as *Helicobacter pylori* infection, irritable bowel syndrome, and celiac disease [3], as well as the coexistence of migraine with conditions such as constipation and dyspepsia [4].

It's believed that the effects on the gut microbiome can be used to treat a number of diseases of the nervous system [5]. However, the issue concerning the role of microbiota in the pathogenesis of migraine and the effectiveness and safety of probiotics in the treatment of migraine patients requires further investigation [6]. At the same time, chronic stress and mental disorders, including depression and anxiety, contribute to the release of proinflammatory neurotransmitters, which is a prerequisite for the formation of chronic inflammation of the intestinal wall, which, in turn, has a

potential negative impact on the gut microbiota [7]. Recent studies have shown significant progress in understanding the possible role of microbiota and their interaction with diet and lifestyle in patients with migraine [7]. It is known that changes in the microbiota can affect the normal absorption of nutrients, permeability of the intestinal wall, and immune function, disrupting the functioning of the gut-brain axis. This can affect the production of some important substances by gut microbiota such as calcitonin gene-related peptide (CGRP, an important mediator of migraines), IL-10 and serotonin [4].

THE AIM

The aim of the study was to analyze the gut microbiome state in patients with migraine and healthy individuals, to assess possible correlations between the detected changes in patients with migraine and the frequency, intensity of headaches, their psycho-emotional state, and quality of life.

MATERIALS AND METHODS

In total, 100 people aged from 18 to 55 years old (mean age 38.6 ± 8), among them 14.7% men, and 85.3% wom-

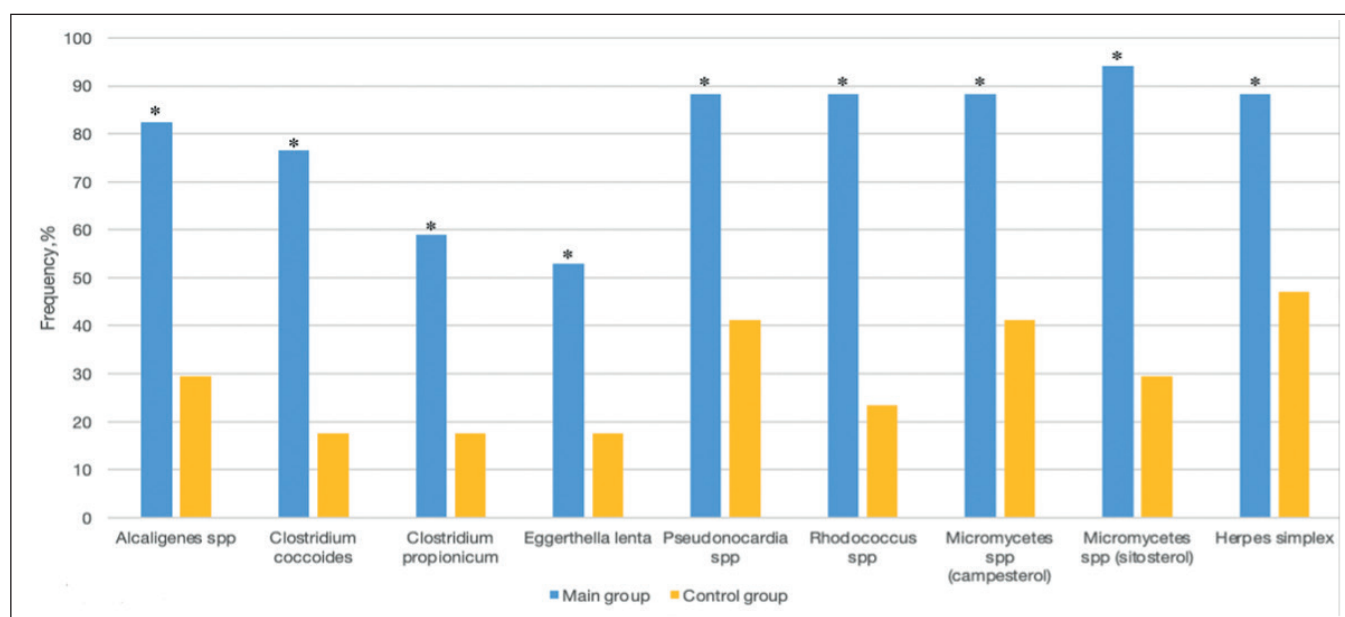


Fig. 1. Distribution of gut microbiota in both groups.

* $p < 0,05$

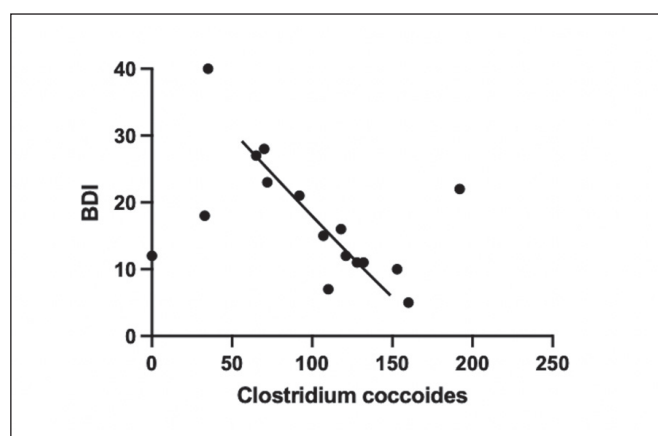


Fig. 2. The regression relationship between *Clostridium coccoides* level and BDI score in the patients with M.

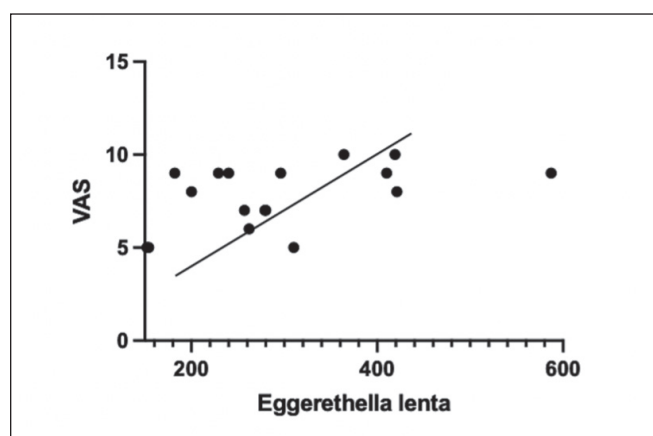


Fig. 3. The regression relationship between *Eggerthella lenta* level and VAS score.

en were enrolled. The study didn't include patients under the age of 18 and pregnant women. Subjects were divided into 2 groups depending on the presence of migraine: the main - patients with chronic and episodic forms of migraine, the control - healthy individuals. Qualitative and quantitative assessment of pain severity was performed using a visual-analog scale (VAS), psycho-emotional status was assessed using Hamilton's anxiety (HARS) and Beck's Depression Inventory (BDI). To establish the degree of social maladaptation, the Migraine Disability Assessment Scale (MIDAS) was used. The investigation of gut microbiota was performed by chromato-mass spectrometry, which determined not only the qualitative, quantitative composition of resident and transient microorganisms, but also microscopic fungi, chemical load of viruses. Statistical analysis of data obtained was performed using GraphPad Prism version 9.3.0. Student's t-test (t) was applied for evaluating credibility between mean quantitative positions of two samples. Proportions were compared using χ^2 .

Relationships between different indicators were assessed using the Pearson's correlation coefficient (r) according to statistical distribution.

RESULTS

In the main and control groups there was no significant difference in content of *Actinomyces* (*Actinomyces* spp, *Actinomyces viscosus*) ($p = 0.46$; $p = 0.45$). For *Alcaligenes* spp, which belong to resident microorganisms, substantial changes in their quantitative composition ($p = 0.006$) in patients with migraine were found. Also in the main group a notable increase in the quantitative composition of *Clostridium coccoides* ($p = 0.002$) and *Clostridium propionicum* ($p = 0.028$) was detected. While no significant differences between groups were found in the content of *Clostridium perfringens* ($p = 0.76$), *Clostridium ramosum* ($p = 0.12$), and *Clostridium tetani* ($p = 0.46$). A significant increase in the level of *Eggerthella lenta* ($p = 0.013$) was revealed

in the main group. Among resident microorganisms, the level of *Pseudonocardia spp* ($p = 0.02$) and *Rhodococcus spp* ($p = 0.016$) was increased in patients with migraine, compared to the control group. Regarding other microorganisms, no significant changes in their quantitative composition in the main group in comparison with the control were found. For the transient group, including *Bacillus cereus*, *Bacteroides fragilis*, *Bacteroides hypermegas*, *Campylobacter mucosalis*, *Clostridium difficile*, *Enterococcus spp*, *Flavobacterium spp*, *Helicobacter pylori*, *Kingella spp*, *Acinetobacter spp*, and *Peptostreobipostecoccinus E coli*, no qualitative changes were found in either the main or control groups. The number of microscopic fungi such as *Candida spp* ($p = 0.007$), *Micromyces spp* (campesterol) ($p = 0.001$), and *Micromyces spp* (sitosterol) ($p = 0.001$) were increased in patients with migraine compared to the control group. The study of the chemical load of viruses in a comparative aspect showed a significant increase in the number of *Herpes simplex* in patients with migraine (*Herpes simplex*) ($p = 0.03$). Changes in other viruses were not significant. In the main group, a valid increase in the amount of endotoxin ($p = 0.04$) was found.

Patients with migraine had a significantly higher frequency of elevated *Alcaligenes spp* ($\chi^2 = 9.66$, $p = 0.001$), *Clostridium coccoides* ($\chi^2 = 11.81$, $p = 0.006$), *Clostridium propionicum* ($\chi^2 = 6.1$, $p = 0.013$), *Eggerthella lenta* ($\chi^2 = 4.63$, $p = 0.03$), *Pseudonocardia spp* ($\chi^2 = 8.24$, $p = 0.004$), *Rhodococcus spp* ($\chi^2 = 14.44$, $p = 0.0001$), *Micromyces spp* (campesterol) ($\chi^2 = 8.24$, $p = 0.004$) and *Micromyces spp* (sitosterol) ($\chi^2 = 15.7$, $p = 0.0001$), and *Herpes simplex* ($\chi^2 = 6.58$, $p = 0.01$) compared to the control group (Fig.1).

In the main group, a negative correlation was found between the level of *Alcaligenes spp* and the score on the BDI score, HARS score (respectively: $r = -0.62$, $p = 0.007$; $r = -0.51$, $p = 0.03$), the frequency of attacks ($r = -0.49$, $p = 0.04$) and the VAS score ($r = -0.48$, $p = 0.04$). Significant negative correlation was found between elevated level of *Clostridium coccoides* and MIDAS score ($r = -0.51$, $p = 0.03$), BDI score ($r = -0.54$, $p = 0.02$) in the patients with migraine. (Fig.2).

At the same time, in the main group a positive correlation was detected between *Eggerthella lenta* level of and VAS score ($r = 0.48$, $p = 0.049$) (Fig.3).

In the main group negative correlations were found between slightly increased content of endotoxin in the main group, the BDI score, HARS score (respectively: $r = -0.69$, $p = 0.001$; $r = -0.56$, $p = 0.02$) the frequency of attacks ($r = -0.55$, $p = 0.023$), on the VAS score ($r = -0.53$, $p = 0.028$), as well as MIDAS score ($r = -0.56$, $p = 0.02$).

DISCUSSION

Among actinomycetes (*Actinomyces spp*, *Actinomyces viscosus*), which are common inhabitants of the gastrointestinal tract and oral cavity, no reliable changes were found in the main and control groups. For *Alcaligenes spp*, which belong to resident microorganisms (normally inhabit gastrointestinal tract), significant changes were

detected in the quantitative composition in the main group compared to the control one. *Alcaligenes* produce antibiotics and original antibacterial components that disrupt the growth of a wide range of bacteria, as well as initiate growth of B-lymphocytes and *Alcaligenes*-specific antibodies, to create their own "cloak" coating [8]. We found an elevated incidence of the increased number of these microorganisms in the main group and their negative relationship with the BDI and HARS score, the frequency of migraine headaches, and the VAS score. Thus, the higher amount of these microorganisms corresponds to the lower level of depression and anxiety, and the less severity and frequency of headache in the patients of the main group. *Clostridia* are normally present in the human intestine, on the skin, mucous membranes of the oral cavity, in the genital system, and in the respiratory tract [9]. Many clostridia are pathogens of dangerous diseases (*C. botulinum*, *C. tetani*, *C. perfringens*), but it should be noted that due to the regulatory effects of microbial biofilms and the immune system, these diseases are not caused. The peculiarity of the negative impact of clostridia in the case of their excessive growth is the production of powerful bacterial exotoxins, as well as a number of proteolytic enzymes, which lead to local tissue damage [9]. The microbiome study showed a significant increase in the quantitative composition of *Clostridium coccoides* and *Clostridium propionicum* in migraine patients, along with a rise in their frequency in migraine patients comparing to those from the control group. *Clostridium propionicum* produces propionic acid as the main product of fermentation. *Propiobacteria* are gram-positive, non-spore-forming, anaerobic and rod bacteria. *Propiobacteria* are involved in the synthesis of vitamin B12, probiotics and propionic acid [10]. Regarding the increased level of *Clostridium coccoides*, they have an important prebiotic effect. Their level is directly related to the improvement of intestinal function, and they can enhance the growth of lactobacilli and have an inhibitory effect on a number of harmful microorganisms [11]. We found a negative correlation between elevated *Clostridium coccoides*, MIDAS, and BDI scores in migraine patients, indicating their potential positive impact on depression severity and quality of life in migraine patients. A significant increase in *Eggerthella lenta* in the main group may indicate inflammatory changes in the gastrointestinal tract in patients with migraine [12]. At the same time, the elevated level of these microorganisms was associated with increased pain severity according to VAS score.

In the patients with migraine, compared to the control group, the number of microscopic fungi such as *Candida spp*, *Micromyces spp* (campesterol, sitosterol) was increased. *Candida* is a conditional pathogen that lives on mucous membranes. There are about 140 species of *Candida* (most of which are pathogenic), which under certain conditions lead to candidiasis and weaken the immune system. It is established that colonization of the gastrointestinal tract by *Candida* fungi is asymptomatic. Assessing the chemical load of viruses (*Herpes simplex*, *Epstein-Barr virus* and *cytomegalovirus*) in a comparative

aspect, we found a potential increase in the number of *Herpes simplex* in patients with migraine. In the main group, slightly increased amount of endotoxin was found. *Endotoxin*, or *bacterial lipopolysaccharide* (LPS), is considered to be the most potent mediator of microbial origin involved in the pathogenesis of sepsis and septic shock. Small doses of LPS in a limited tissue space help the host to organize effective antimicrobial protection, and facilitate the removal of pathogens to the environment. At the same time, the sudden release of large amounts of LPS, on the contrary, has a negative impact on the human body as far as it triggers uncontrolled and the life-threatening release of numerous inflammatory mediators and procoagulants into the systemic circulation [13]. The negative correlations between slightly elevated total endotoxin level and VAS, MIDAS, BDI, HARS score, frequency of migraine attacks, indicate its possible positive effects on the intensity, frequency of pain, quality of life of migraine patients, and their psycho-emotional state.

CONCLUSIONS

1. The significant difference in the composition of the gut microbiota in patients with migraine comparing to healthy individuals was found.
2. In patients with migraine a remarkable influence of gut microbiome changes on the characteristics of headache and indicators of psycho-emotional status was established.
3. The revealed peculiarities of gut microbiome changes in patients with migraines need further clarification in order to identify the possible role of the gut microbiome in the pathogenesis, clinical picture, and therapy of migraine, and is a promising area of further scientific research.

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The Authors declare no conflict of interest.

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