The analysis of gut microbiome in patients with myalgic encephalomyelitis/ chronic fatigue syndrome, ME/CFS

Introduction:

Myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) is a serious, debilitating disease, with periods of remission and exacerbation, that significantly impairs daily activity and reduces the quality of life of patients. The primary symptoms of ME/CFS include: chronic fatigue, post-exertional malaise (PEM), unrefreshing sleep, memory and concentration disorders, cognitive dysfunction, and symptoms of dysautonomia. Despite conducting many scientific studies on ME/CFS, the etiology and pathogenesis have not been elucidated yet, and there is no biological marker or causative treatment of this disease. One of the suggested causative factors is intestinal dysbiosis and the systemic response of the host's organism to the abnormal composition and functioning of the gut microbiome. The development of research methods based on the recognition of 16S rRNA gene sequences has made it possible to learn more details about the human microbiome. Relatively few studies on the composition of the gut microbiome of people with ME/CFS have been conducted, and the correlation between alternations in microbiome homeostasis and the progression of ME/CFS has not been clearly confirmed. Therefore, it becomes crucial issue to extend the knowledge about gut microbiome alterations in ME/CFS patients, which is important for better understanding the ME/CFS etiology and pathogenesis, and can contribute to improve its diagnosis and also become a potential starting point in the treatment of this disease.

Aims:

The aim of the present research study is to analyse the qualitative and quantitative composition of the gut microbiome in patients who suffer from ME/CFS. The study also assess the relation between the gut microbiome and the age and sex of patients, as well as the intensity of selected ME/CFS symptoms, the functioning of the autonomic nervous system and the cognitive functioning of patients. On the basis of the network analysis of gut microbiome for the network created on grounds of ASV, a comparison between the test and control groups is also conducted.

Material and methods:

The study was conducted from January 2018 to March 2019 in Bydgoszcz. Adult volunteers aged between 25 and 65 took part in the research project. Recruitment for the

study was carried out in social media. Qualification was conducted among the volunteers and a group of 32 patients with confirmed ME/CFS was selected. 18 healthy people were included in the control group. Finally, 27 patients from the study group and 15 from the control group gave their consent for stool test and delivered stool samples for testing. In all included patients, the intensity of fatigue symptoms was assessed, moreover, functional assessment of autonomic nervous system was carried out and cognitive functioning was evaluated. In order to analyse the gut microbiome, method of fragment sequencing of 16S rRNA genes was used. Statistical analysis of obtained parameters was performed.

Results:

The analysis of the quantitative composition of stool microbiome between ME/CFS and the control group, shows no statistically significant differences in terms of Shannon's diversity and uniformity indexes. The study has shown that patients with ME/CFS are characterized by a significantly higher species richness of microbiome (understood as a higher number of OTU's units) compared to the control group. When it comes to the qualitative composition of the stool microbiome, the analysis at all taxonomic levels between the study and control groups were performed, and the results show that: at the phyla level - lower abundance of *Firmicutes, Actinobacteria* and *Proteobacteria* and rare phyla in the study group (patients with ME/CFS) compared to control group; at the classes level - higher abundance of *Bacteroidia* and lower abundance of *Negativicutes, Actinobacteria* and rare classes in the study group compared to the control group; at the orders level - higher abundance of *Bacteroidales*, and lower abundance of *Selenomonadales* and rare orders, in the ME/CFS patients compared to control group; at the families level - lower abundance of *Veillonellaceae* family and bacteria from rare families in ME/CFS patients than in control patients; at the genus level - greater abundance of *Bacteroides, Alistipes* and *Ruminococcaceae* in ME/CFS patients compared to control group. The qualitative composition of stool was also compared between the test and control groups on the basis of ASV sequences and the results show that the most characteristic for ME/CFS patients are the following variants of the ASV sequences: ASV121 (*Alistipes*), ASV147 (*Odoribacter*) and ASV180 (*Ruminococcaceae*), while for the control group: ASV 135 (*Lachnospiraceae*), ASV155 (*Christensenellaceae*) and ASV310 (*Lachnospira*). Significant differences demonstrated by using complexity reduction methods (sPLS-DA and NMDS) were observed between the stool microbiome in the group of ME/CFS patients compared to the control group. There were no statistically significant differences in the composition of the

stool microbiome depending on sex and age between the test and control groups. The stool microbiome of patients in the test and control groups was classified with the machine learning methods using for this purpose: neural network models, random classification tree and support vector machine, and the obtained results were below the clinically significant values. A network analysis was conducted for a network created on the basis of ASV sequences in the ME/CFS group compared to the control group, but no significant relations between individual ASVs were observed. Using the NMDS method, the microbiome in the group of patients with ME/CFS and in the control group was compared depending on the functioning of the autonomic nervous system, cognitive functioning and the intensity of ME/CFS symptoms, assessed on the basis of the FSS and FIS scales, and the correlation between these parameters and the stool microbiome of the compared groups was demonstrated.

Conclusions:

1. A statistically significant higher alpha-diversity of the stool microbiome was demonstrated in the group of patients with ME/CFS compared to the control group, understood as a higher number of OUT's units. There were no statistically significant differences in the Shannon's diversity and uniformity index of the stool microbiome between the study group and the control group.
2. Statistically significant differences in the beta-diversity of the stool microbiome between patients with ME/CFS and the control group using PERMANOVA were demonstrated.
3. There were no statistically significant differences in the composition of the stool microbiome depending on gender and age between the study group and the control group.
4. A significant correlation between the functioning of the autonomic nervous system, cognitive functioning and the level of fatigue with the composition of the stool microbiome of the compared groups was observed.