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EXPLORING THE ROLE OF GUT MICROBIOTA IN PATIENTS WITH MULTIPLE SCLEROSIS IN UKRAINE: A CROSS-SECTIONAL STUDY

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Abstract

Introduction. Multiple sclerosis (MS) is an inflammatory disorder characterized by demyelination in the central nervous system (CNS), linked to both genetic predispositions and environmental influences. Gut microbiota can be considered as an environmental factor that plays a role in MS disease.
Aim. The study aims to assess enterotype and microbiota composition in multiple sclerosis patients and a control group in the Ukrainian population, as well as to identify factors influencing their formation and role in disease pathogenesis.
Materials and methods. A total of 33 subjects, from which 28 diagnosed with multiple sclerosis (MS) and 5 healthy volunteers participated in this single-center cross-sectional study. Data were collected from stool samples obtained from participants, medical records and neurological exam during 2025. Microbiome analysis was performed via 16S rRNA gene sequencing on the Illumina MiSeq platform.
Results. The MS and control cohorts had comparable demographic characteristics. The median age was 33 (IQR: 31-37). In this study we investigated the impact of gut microbiota on adults with multiple sclerosis in Ukraine and found that enterotype has potential moderate-strong association with MS, and were significantly related to treatment status. The association among enterotype distribution and treatment status was with large effect (Cramér's V = 0.41), indicating relationship between microbiome changes and DMT therapy. In our study MS patients also had an increased level of the phylum Proteobacteria (d= –0,36) and decreased levels for Bacteroidetes (d=0.27) and Firmicutes (d=0.44) compared to healthy controls. Kruskal-Wallis H test showed that Firmicutes (H = 12.262, p = 0.016) and Proteobacteria (H = 10.18, p = 0.037) differ significantly between control group, group without treatment and preventive therapy groups. Other phyla do not show statistically significant differences.
Conclusions. This study demonstrates that gut microbiota composition in MS patients differs from that of healthy controls, with enterotype distribution potentially influenced by disease-modifying therapies. Increased levels of proinflammatory phylum have been identified in the MS cohort, so further studies on genus and species level is needed.

Keywords: **gut microbiota, enterotype, EDSS, multiple sclerosis, 16S rRNA gene sequencing**

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disorder characterized by demyelination in the central nervous system (CNS), linked to both genetic predispositions and environmental influences [1]. The gut microbiome has emerged as a significant factor potentially influencing MS pathology [2]. The gut microbiota in MS patients shows distinct differences from healthy controls, particularly in terms of microbial diversity and composition [3]. These differences are believed to play a role in immune system regulation, possibly exacerbating or ameliorating MS symptoms [4]. Microbiota describes the living microorganisms found in a defined environment, such as oral and gut microbiota. Microbiome refers to the collection of genomes from all the microorganisms in the environment, which includes not only the community of the microorganisms, but also the microbial structural elements, metabolites, and the environmental conditions [5].

Enterotypes is a classification of the gut microbiota of different populations, indicating that variation in gut

microbiota is stratified among individuals. It has no direct relationship with gender, age, geography and cultural background [6].

Each of the three enterotypes is identifiable by variation in the levels of one of three main genera: Bacteroidetes (enterotype 1), Prevotella (enterotype 2) and Ruminococcus (enterotype 3) [7].

Enterotype 1 is characterized by a Bacteroides predominance and the ability to recover maximum energy from carbohydrate and protein fermentation. Additionally, people who have this enterotype produce more biotin, vitamin B2, B5, C. This enterotype has been linked to a higher level of intestinal inflammation and, as a result, a higher level of overall inflammation (46) [6]. Prevotella bacteria are preponderant in Enterotype 2, which is linked to a carbohydrate-rich diet in vegetarian individuals [8]. Produce high levels of vitamin B1 and vitamin B9 [6]. Ruminococcus enrichment is a characteristic of enterotype 3, specific to the resistant starch diet and it consists of bacteria able to degrade mucin [8]. Furthermore, the bacteria that make up this enterotype can absorb simple sugars, suggesting that they can play a role in immune system modulation. Enterotypes are stable, which is mainly affected by long-term dietary habits [6].

Diets high in fermentable fibers and polyunsaturated fats are linked to increased microbial diversity, production of anti-inflammatory short-chain fatty acids, and an enhanced gut barrier function, potentially reducing MS activity [9]. In contrast, diets high in processed foods can promote an inflammatory microbial environment [10].

The role of probiotics in MS management is also under investigation. Preclinical studies suggest that certain probiotics can modulate T-cell responses, crucial in MS's autoimmune pathology [11]. However, while some human studies show promise, the overall evidence remains mixed regarding probiotics' efficacy in altering MS progression [12]. Further, the gut-brain axis, where gut microbiota influence brain function, is considered a pathway through which the microbiome might affect MS [13]. Studies have explored how gut microbial metabolites interact with the CNS, potentially influencing neuroinflammation or neuroprotection. L. Rothhammer et al. demonstrated that type I interferons and microbial metabolites of tryptophan can modulate astrocyte activity and CNS inflammation via the aryl hydrocarbon receptor [14]. Research suggests gut microbiota may influence MS treatment outcomes by modulating drug metabolism and efficacy. A. J. Kostic et al. explored the long-term dynamics of the human gut microbiome in inflammatory conditions, highlighting its potential role in immune-mediated diseases [15]. This underscores the potential for personalized therapeutic strategies, wherein targeted modulation of the gut microbiome may enhance treatment efficacy [16].

Several associations between enterotypes and disease phenotypes in humans have been reported by Yang et al. (2019) [17]. However, the interplay between MS and the gut microbiome is complex, involving potential influences on immune function, inflammation, and disease progression. The emerging field holds promise for developing targeted interventions, but further research is needed to solidify these connections and translate them into practical treatments [1].

While the exact mechanisms and causal links between the gut microbiome and MS are still under scrutiny, the correlation is evident enough to warrant continued research. This encompasses investigations into the role of dietary interventions and microbial modulation as potential therapeutic strategies for MS management [2].

Although previous studies have investigated the gut microbiota in multiple sclerosis, data specific to the Ukrainian population remain scarce. Given the distinct dietary habits and socio-economic factors in the region, our study aims to provide novel insights into the microbiome's influence on MS. These findings may help identify microbiota-related characteristics and contribute to more personalized therapeutic approaches for patients.

MATERIALS AND METHODS

Study population

A single-center, non-interventional, cross-sectional study was conducted to assess and analyze the relationship between the gut microbiota profile, disease activity and course type in patients with multiple sclerosis (MS) in the Ukrainian population. The study included a total of 33 participants, including 28 patients undergoing inpatient and outpatient treatment from June 2024 to January 2025 at the Multiple Sclerosis Department of Kyiv City Clinical Hospital No. 4, Kyiv, Ukraine and 5 healthy volunteers.

Initially, 50 adults aged 20-40 years old were randomly enrolled from the indicated health care unit. Twenty individuals were excluded from the study due to disease exacerbation, receipt of pulse-therapy, use of antibiotics, pregnancy, or relocation to another country. The criterion for the purposeful sampling was having patients with all types of disease modifying therapy with the aim to have all segments represented in the study.

Participants were recruited based on established diagnosis of MS (ICD-10 code G35), both types of MS: relapsing-remitting MS (RRMS) or progressive MS, willingness to participate in a microbiome study, and meeting the inclusion/exclusion criteria.

Inclusion criteria for subjects with MS included a diagnosis of MS according to the latest McDonald criteria. Disease subtypes were further classified as RRMS or primary progressive MS, gender: male/female, age: 20-40 years, consent for stool sample sequencing, ability to maintain consistent contact, signed informed consent after full explanation of the research method, the Expanded Disability Status Scale (EDSS) 1-8.

Exclusion criteria for both MS subjects and healthy control subjects were as follows: antibiotic use in the prior 6 months; probiotic use; corticosteroids within the past 3 months; history of gastroenteritis; or travel outside of the country in the prior month, pregnancy. None of the MS patients had an active relapse at the time of sampling. Treated patients in the cohort were those who had received interferon beta 1b, glatiramer acetate, ocrelizumab, teriflunomide, dimethyl fumarate for at least 3 months. Untreated patients were treatment naive or with no steroid treatment in the previous month, no disease-modifying therapy treatment in the previous 3 months, and no other treatments over the prior 6 months.

Healthy subjects n=5 were individuals who are self-reported to be free of chronic infectious and inflammatory diseases and recallable by demographic or genotypic feature for biosampling, were approached to provide a stool sample. Collection and processing procedures were identical to the ones used for the MS patients.

A survey about satisfaction of dietary recommendations was administered to subjects with MS before collection of samples.

The protocol for this study received prior approval from the Bioethics and Research Ethics Committee of Bogomolets National Medical University during its meeting on November 07, 2018, as documented in Protocol No. 115., and informed consent was obtained from each subject. All the participants provided sociodemographic and other clinical data, which included age, sex, body mass index (BMI).

Sample collection

Each patient was sent instructions by the researcher on the rules for sample collection. We used a consistent methodology for processing and storage of all samples. Subjects collected a single-sample produced at any time of day with no specific dietary restrictions. Study subjects collected a stool sample at home. The sample was collected in a sterile container with a spoon designed for biological material (stool) collection. Among the recommendations, there was information stating that if the sample was collected after a radiological examination with contrast agents, stool could only be collected if more than 2 days had passed. The stool sample was delivered by the patient to the clinic within 3-24 hours, where the samples were numbered and placed in a box with an ice packs before being transported to the laboratory, where they were placed in a freezer and frozen at -18°C upon receipt. Samples were only subjected to a single free-thaw cycle.

Microbiome Analysis

Microbial DNA was extracted from faecal samples and 16S rRNA gene sequencing was performed on the Illumina MiSeq platform using primers targeting the V3 and V4 variable regions, respectively.

DNA was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo research, USA). Quantification of DNA was performed via a spectrofluorometric DeNovix dsDNA Broad Range Assay on a Denovix QFX fluorometer (DeNovix, USA). Optimal input range of 2.5-25 ng and concentration of 0.5-5 ng/µl was met in all samples.

NGS Library preparation was performed using the ViennaLab 16S Microbiome NGS Assay (ViennaLab Diagnostics GmbH, Austria). After DNA extraction, variable bacterial V3-V4 regions of 16S rRNA gene were amplified with target specific primers. This process was followed by magnetic bead clean-up of PCR products. Afterwards, an indexing PCR was performed. For each sample, a unique combination of indexing forward and reverse primers had been selected. After the second clean-up with magnetic beads, we set up a capillary gel electrophoresis via Agilent TapeStation (Agilent, USA) to assess library size distribution. Library quantification was performed via both dsDNA High Sensitivity Assay on a Denovix QFX fluorometer (Denovix, USA) and KAPA Library Quantification Kit (Roche, Switzerland) on a Bio-Rad CFX96 qPCR system (Bio-Rad, USA). Library normalization, dilution and pooling was performed according to quantification data. Sequencing was set up on the Illumina MiSeq system and NGS data analysis was performed via ViennaLab Microbiome Analysis Webtool (ViennaLab Diagnostics GmbH, Austria). Microbiome sequencing and NGS data analysis were performed at CSD LAB, Kyiv, Ukraine.

Data Analysis

Statistical analysis was performed using IBM SPSS Statistics software (Version 23.0; Armonk, NY: IBM Corporation; for identification only). Exploratory analysis using Chi-square and the Fisher-Freeman-Halton test compared results across Firmicutes/Bacteroidetes ratio, Shannon index, Enterotype in control group and patients with MS. Microbiome diversity was discovered with the Shannon index. For evaluation of the strength of association and size effects we calculated Cramér's V.

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Continuous variables were reported as mean (SD) or median (IQR), while categorical variables were presented as frequencies and percentages.

To investigate overall differences in microbial community structure at the phylum level and to identify MS-associated microbiota changes between groups of MS patients receiving disease-modifying therapy, those without preventive treatment, and the control group Kruskal-Wallis tests and Fisher exact test were performed. Statistical significance was set as $p<0.05$.

RESULTS

Study population

MS patients were recruited from the MS Center, Kyiv City Clinical Hospital No. 4 and healthy subjects

were those who had previously expressed interest in participating in microbiome research and provided informed consent in response to an email invitation.

The study encompassed a cohort of 33 subjects, consisting of 34.6% (n=12) men and 65.4% (n=21) women, among whom 28 were diagnosed with Multiple Sclerosis (MS) and 5 healthy volunteers. The MS and control cohorts had comparable demographic characteristics. The median age was 33 (IQR: 31-37). Details of the study population are provided in Table 1.

The distribution by body mass index (BMI) is presented in Table 2.

Table 1

Age distribution of study participants

Min	Max	Mean	StDev	Percentile						
				0.05	0.10	0.25	0.50 Median	0.75	0.90	0.95
23.00	40.00	32.50	4.59	23.65	24.30	31.00	33.00	35.75	37.00	38.00

Table 2

BMI breakdown of the research cohort

Min	Max	Mean	StDev	Percentile						
				0.05	0.10	0.25	0.50 Median	0.75	0.90	0.95
17.00	35.00	22.76	4.77	17.65	18.00	19.00	22.50	24.75	29.00	33.00

The majority of patients were diagnosed with relapsing-remitting MS, accounting for 93.1% (n=27) of the sample, followed by 6.9% (n=2) with primary-progressive MS.

The majority of patients had EDSS score 2 (n=7), score 4.5 (n=5), score 3.5 (n=4), score 4 (n=4), score 3 (n=3), 2.5 (n=2), and score 1, 5, 5.5 and 6.5 had n=1 as indicated in Figure 1.

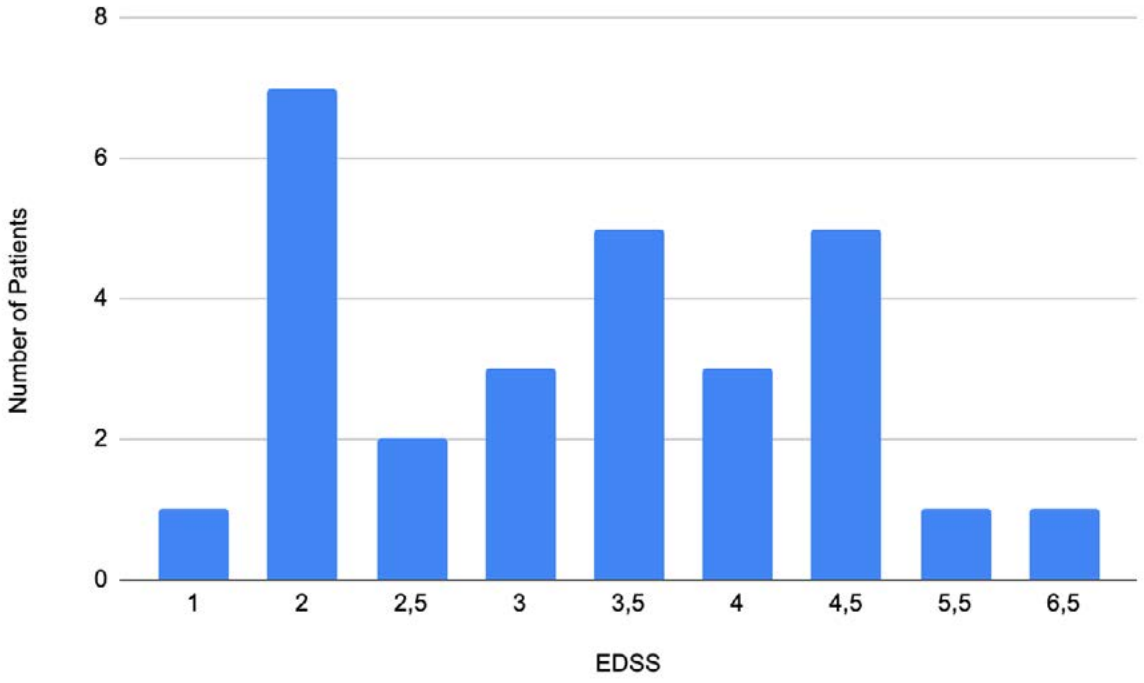

Figure 1. Disability status: EDSS score distribution.

A significant proportion of patients (67.9%, n=19) reported being on disease-modifying therapies (DMTs). The most commonly used DMTs included Ocrelizumab (n= 5), Interferon 1b (n=4), Glatiramer acetate (n=3), Dimethyl fumarate (n=3), and Teriflunomide (n=2), reflecting the diversity of treatment regimens within the cohort. Also there were 2 patients (7.1%) that received Bioven as part of MS therapy. In the sample, 32.1% (n=9) of participants had not received preventive therapy, allowing for the investigation of the direct relationship between the disease and gut microbiome structure while minimizing the influence of therapy. Participation in the study did not affect patients' decisions regarding their choice of therapy, nor did it interfere with their treatment.

Enterotypes between the control group and MS patients

For enterotype interpretation, we used the categorization described by Arumugam M. et al. (2011) [8]. In this study we found that the majority of participants from the MS group had Enterotype 1 (n=17). However, a subset of samples (15%, n=5) could not be assigned to any predefined enterotype due to ambiguous microbial profiles. Given that sequencing depth was sufficient (mean reads per sample: 392,786.8), these samples were categorized as «Unclassified».

Table 3

Enterotype distribution across study groups

Enterotype	Control (N)	Control (%)	Disease (N)	Disease (%)
Unclassified	0	0.0%	5	17,85714286
1	1	20.0%	17	60,71428571
2	2	40.0%	2	7,142857143
3	2	40.0%	4	14,28571429
Total	5	100.0%	28	100.0%

Since 75% of the cells had expected counts below 5, Fisher-Freeman-Halton Exact Test was used, yielding a p = 0.052, which indicates a trend toward significance. Although the p-value does not reach the conventional significance level, the observed trend suggests a potential association that may warrant further investigation with a larger sample size. To evaluate the strength of association, we calculated Cramér's V, which resulted in a value of 0.477. This indicates a moderate-to-strong association between the variables, suggesting a meaningful relationship between variables.

Enterotypes between the control group and different disease-modifying therapy (DMT) groups

Given that enterotype is potentially associated with MS (p=0.052, Cramer's V = 0.477) and depends on the diet and other environmental factors, such as treatment, we decided to assess the influence of the treatment on the subjects' enterotypes.

We clusterized subjects into 5 main groups: Control, Untreated, Glatiramer Acetate/ Interferon beta-1b, Ocrelizumab, Dimethyl fumarate/ Teriflunomide /Bioven. Patient distribution across enterotypes in both treatment and control groups can be found in Table 4.

The data in Table 5 present the chi-square test results assessing the association between enterotype distribution and treatment status. Pearson Chi-Square p=0.05 confirms the existence of a statistically significant relationship between enterotypes and groups. Given that all expected frequencies are below 5, the Fisher-Freeman-Halton Exact Test was calculated. The result is p = 0.010 also confirms statistically significant relationships between measured values.

Table 4

Patient distribution by enterotype and treatment group

Enterotype	Control N (%)	Untreated N (%)	Glatiramer acetate or Interferon beta-1b N (%)	Ocrelizumab N (%)	Dimethyl fumarate/ Teriflunomide/Bioven N (%)
Not identified	0 (0.0%)	1 (11.1%)	4 (57.1%)	0 (0.0%)	0 (0.0%)
1	1 (20.0%)	4 (44.4%)	2 (28.6%)	4 (80.0%)	7 (100.0%)
2	2 (40.0%)	1 (11.1%)	0 (0.0%)	1 (20.0%)	0 (0.0%)
3	2 (40.0%)	3 (33.3%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Total	5 (100.0%)	9 (100.0%)	7 (100.0%)	5 (100.0%)	7 (100.0%)

Table 5

Statistical assessment of enterotype distribution across study groups

Test	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	26.045	12	0.011	0.007	-	-
Likelihood Ratio	27.229	12	0.007	0.011	-	-
Fisher-Freeman-Halton Exact Test	18.979	-	-	0.010	-	-
Linear-by-Linear Association	5.085	1	0.024	0.026	0.013	0.004
N of Valid Cases	33	-	-	-	-	-

The degree of association was measured using Cramér's V, which resulted in 0.513. This indicates a large effect size.

We also analysed the relationships between enterotypes and EDSS scores, where EDSS scores were divided into 4 groups (0-1; 1,5-3; 3,5-5; 5-7) no significant correlation was found p = 0.877 (Fisher Exact test).

After we explored relationships between enterotypes and numbers of relapses for the last year, where the maximum relapses number is 5 and minimum is 0, no

significant correlation was found p = 0.691 (Fisher Exact test).

MS-associated microbiota changes at the phylum level

At the phylum level, the faecal microbiota of both groups was dominated by Firmicutes and Bacteroidetes, with smaller contributions of Proteobacteria, Euryarchaeota and Verrucomicrobia. MS patients had an increased level of the phylum Proteobacteria, Verrucomicrobia and Actinobacteria and decreased levels for Bacteroidetes and Firmicutes compared to healthy controls. We received trend to moderate effects for Proteobacteria (d= 0,36), Bacteroidetes (d=0.27) and Firmicutes (d=0.44). For others phylum size effects were small, which is indicated in Table 6.

Table 6

Comparison of bacterial abundance with effect size

Phylum	Control Mean	MS Mean	Cohen's d	Difference (MS-Control)	Change	Effect Interpretation
Bacteroidetes	30,25	27,54	0,2673340787	-2,71	Lower in MS	Moderate effect
Firmicutes	65	60,55	0,4389803138	-4,45	Lower in MS	Moderate effect
Proteobacteria	2,54	6,16	-0,3571030867	3,62	Higher in MS	Moderate effect
Verrucomicrobia	0,87	1,66	-0,0779313366	0,79	Higher in MS	Small effect
Actinobacteria	0,7	2,65	-0,19236216	1,95	Higher in MS	Small effect
Tenericutes	0,26	0,63	-0,03649948676	0,37	Higher in MS	Small effect
Euryarchaeota	0	0	0	0	No change	Small effect
Fusobacteria	0	0,21	-0,02071592492	0,21	Higher in MS	Small effect
Other	0,38	0,61	-0,02268887015	0,23	Higher in MS	Small effect

Treatment-associated microbiota changes at the phylum level

Because MS therapy may skew microbiota composition, we separately analysed changes in the microbiota in untreated patients and between different groups. For this reason we performed the Kruskal-Wallis H test. We found that Firmicutes (H = 12.262, p = 0.016) and Proteobacteria (H = 10.18, p = 0.037) differ significantly between control group, group without treatment and preventive therapy groups. Other phyla do not show statistically significant differences, which is indicated in Table 7.

Table 7

Distribution of phylum depending on control and disease-modifying therapy groups

Category	Bacteroidetes	Firmicutes	Proteobacteria	Verrucomicrobia	Actinobacteria	Tenericutes	Fusobacteria	Other
Kruskal-Wallis H	9,198	12,262	10,18	5,563	1,255	8,637	4,543	4,345
df	4	4	4	4	4	4	4	4
Asymp. Sig.	0,056	0,016	0,037	0,234	0,869	0,071	0,338	0,361

DISCUSSION

In this study we investigated the impact of gut microbiota on adults with multiple sclerosis in Ukraine and found that enterotype has potential moderate-strong association with MS, and were significantly related to treatment status. The association among enterotype distribution and treatment status was with large effect (Cramér's V = 0.41), indicating relationship between microbiome changes and DMT therapy.

Consistent with other microbiota studies in humans, we detected interindividual variability within control and MS patients. Cekanaviciute et al. have documented significant alterations in the gut microbiome of MS patients, characterized by an increased abundance of potentially pathogenic Proteobacteria [18]. In our study MS patients also had an increased level of the phylum Proteobacteria (d= –0,36) and decreased levels for Bacteroidetes (d=0.27) and Firmicutes (d=0.44) compared to healthy controls. Proteobacteria are gram-negative and produce lipopolysaccharides (LPS), which induce strong proinflammatory immune responses. These findings emphasize the importance of continuing research and deepening at the genus and species levels with the search for possible connections between the microbiome and the pathogenesis of MS.

Based on our results, a prevalent number of patients had enterotype 1, (n=17). However, in the study group, despite the qualitative depth of reading (mean reads per sample: 392,786.8) and no errors from the technical-analytical part of the study, in 5 samples, enterotype was not classified into the existing 3 enterotypes

described firstly by Arumugam M. et al. (2011) [8]. These «unclassified» samples don't reach the standard enterotyping criteria that is why they are important for further analysis at the species level, which we intend to do next.

The results of Reynoso-García J. et al. (2022) [19] indicate that the gut microbiome is involved in metabolizing drugs and is related to therapy efficiency. Our findings reveal that Firmicutes ($H = 12.262$, $p = 0.016$) and Proteobacteria ($H = 10.18$, $p = 0.037$) differ significantly between control group, group without treatment and preventive therapy groups as well as enterotypes showed statistically significant relationship between the control and different treatment group. Among patients who received Interferon beta and Glatiramer Acetate in 57.1% (n=4) enterotypes can't be identified, which may show potential influence on gut microbiota on patients who receive such type of treatment. All patients (n=7, 100%) who received Dymethyl Fumarat or Teriflunomide or Bioven and a meaningful proportion of patients with Ocrelizumab therapy (n=4, 80%) had enterotype 1. Enterotype 3 is represented in a control group (n=2, 40.0%) and untreated group (n=3, 33.3%) with only one representation in the Interferon beta and Glatiramer Acetate group which can be interpreted as potential influence of immunomodulation therapy on microbiome. There is no significant correlation between enterotypes and EDSS score or numbers of relapses in this study, however further research is needed with a larger study group.

As for our knowledge, we first in Ukraine analyzed the community structure of the faecal microbiome in MS patients using 16S rRNA gene sequencing on the Illumina MiSeq platform. Performing next-generation sequencing of the 16S rRNA gene inside the country made it possible to avoid the transportation of biological samples (extracted DNA) abroad during Russian-Ukrainian war, reduced the cost of research, and also opens up new opportunities for further scientific activity in this direction due to the increased availability of the method in the region. 16S rRNA gene sequencing compared to qPCR or traditional microbiological methods, offers the following advantages: identification of a broader range of bacterial taxa, including unculturable and low-abundance species, unbiased overview of the entire bacterial community in a sample, elimination of culture biases, which is selective and does not reflect the true diversity, cost-effective compared to metagenomic sequencing.

Although our study provides new insights into the role of gut microbiota in MS patients, it is not without limitations. The observed trend between enterotypes, control and MS groups suggests a borderline significance and may warrant further investigation with a larger sample size with more participants in the control group. However, this bias is unlikely to have affected our main conclusions because the size effect is moderate to strong. Also, the epidemiological distribution of this disease coincides with our group, but further studies should include more participants with progressive forms of the course.

Previous research highlights that enterotype 1 is more prevalent in individuals eating a protein and animal fat-rich Western diet, enterotype 2 is linked to vegetarian dietary pattern, enterotype 3 is specific to the resistant starch diet [8], so further investigation of the dietary pattern with subjects from our study group should be performed to deeply understand the role of gut microbiome and enterotypes in MS pathogenesis.

Our findings highlighted changes of gut microbiota in people with MS compared to control group. Also we found enterotype shifts in response to environmental factors such as treatment with no significant clinical outcomes (disability status, relapse rates during last year) in this study. The absence of a significant correlation between gut microbiota composition, relapse frequency or EDSS may be partly attributed to the effects of immunomodulatory therapy, which modulates disease activity independently of microbial composition. Possibly, according to these findings, shifting the enterotype profile with nutrition recommendation for people on DMT may lead to increasing therapy effect. Prospective cohort study or longitudinal study may help to determine whether a specific enterotype influences therapy effectiveness or not and vice versa.

CONCLUSIONS

This is the first microbiome study about the possible relationships between the change of the intestinal microbiota and MS disease in Ukraine. We undertook studies to define the community structure of the faecal microbiome in MS patients using 16S rRNA gene sequencing.

This study demonstrates that gut microbiota composition in MS patients differs from that of healthy controls, with enterotype distribution potentially influenced by disease-modifying therapies. According to the study's findings, enterotype 1 is associated with MS diseases. Increased levels of proinflammatory phylum have been identified in the MS cohort, so further studies on genus and species level is needed. Taking into account that enterotype 1 is associated with the consumption of high amounts of animal proteins and fatty foods it is recommended further investigation of the dietary pattern for personalized recommendations.

These findings highlight the importance of further research into microbiome-based biomarkers for MS management.

Perspectives for further research include larger cohorts and longitudinal collection of samples will

be required to investigate these clinical associations, including subjects with progressive forms of the disease.

COMPLIANCE WITH ETHICAL REQUIREMENTS

This study, as part of the MS microbiome investigation project, was reviewed and approved by the Bioethics and Research Ethics Committee of Bogomolets National Medical University during its meeting on November 07, 2018, as documented in Protocol No. 115. The research complies with all applicable ethical standards and guidelines.

FUNDING AND CONFLICT OF INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no conflicts of interest regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

Potapova K. P.[A, B, C, D, E, F]

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Резюме

ВИВЧЕННЯ РОЛІ МІКРОБІОТИ КИШКІВНИКА У ПАЦІЄНТІВ З РОЗСІЯНИМ СКЛЕРОЗОМ В УКРАЇНСЬКІЙ ПОПУЛЯЦІЇ: ПОПЕРЕЧНЕ ДОСЛІДЖЕННЯ

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Вступ. Розсіяний склероз (РС) – це аутоімунне демієлінізуюче захворювання центральної нервової системи (ЦНС) розвиток якого пов'язаний як з генетичною схильністю, так і з впливом навколишнього середовища. Мікробіоту кишечника можна розглядати як фактор навколишнього середовища, який може відігравати важливу роль у патогенезі розсіяного склерозу.
Мета. Дослідження спрямоване на визначення ентеротипу та складу мікробіоти у дорослих пацієнтів з розсіяним склерозом та групі контролю в українській популяції з визначенням факторів, що впливають на їх формування та ролі в патогенезі захворювання.
Матеріали та методи. В одноцентровому поперечному дослідженні взяло участь 33 учасники, серед яких 28 пацієнтів з діагнозом розсіяний склероз (РС) і 5 здорових добровольців. Дані були зібрані зі зразків стулу, наданих учасниками, і медичних записів та неврологічного огляду протягом 2025 року. Аналіз мікробіоти кишківника проводився за допомогою секвенування гена 16S рРНК на платформі Illumina MiSeq.
Результати. Група дослідження та група контролю мали порівнянні демографічні характеристики. Середній вік становив 33 роки (IQR: 31-37). У цьому дослідженні ми вивчали вплив кишкової мікробіоти на дорослих із розсіяним склерозом в Україні та виявили, що ентеротип має потенційний помірний або сильний зв'язок із РС і достовірно пов'язаний з хворобо-модифікуючою терапією (ХМТ). Виявлено великий ефект (V Крамера = 0,41) зв'язку між ентеротипом та ХМТ. У нашому дослідженні виявлено, що пацієнти з РС мали підвищений рівень бактерій типу Proteobacteria (d= –0,36) і знижений рівень Bacteroidetes (d=0,27) і Firmicutes (0,44) порівняно із групою контролю. Критерій Kruskal-Wallis H показав, що Firmicutes (H = 12,262, p = 0,016) і Proteobacteria (H = 10,18, p = 0,037) значно відрізняються між контрольною групою, групою без лікування та групами профілактичної терапії. Інші типи не виявляють статистично значущих відмінностей.
Висновки. Це дослідження демонструє, що склад мікробіоти кишечника у хворих на РС відрізняється від складу здорової контрольної групи, причому на розподіл ентеротипів потенційно може впливати хворобо-модифікуюча терапія. Підвищені рівні прозапального типу Proteobacteria були виявлені в групі РС, тому необхідні подальші дослідження на рівні роду та виду.

Ключові слова: **кишкова мікробіота, ентеротип, EDSS, розсіяний склероз, секвенування гена 16S рРНК**

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