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RESEARCH ARTICLE

Gut Microbiome Alterations inthe Multiple Sclerosis: Associations with Disease Activity and Treatment Response

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Abstract: ***Background***: Increasing evidence shows that the microbiota in the gut plays an essential role in immune modulation and neuroinflammation that has given rise to the implication of the role of microbiota in the pathogenesis of multiple sclerosis (MS). The issue of microbial dysbiosis, an imbalance in the structure of intestinal bacteria has been linked with the enhanced operation of illnesses and unstable reactions to immunomodulatory target treatment. However, there is limited information of the microbial signatures of the disease progression and the suggested treatment. ***Methods***: A control study was done that involved 80 relapsing-remitting MS (RRMS) patients and 40 age sex and healthy controls. The sequence of 16S rRNA gene was carried out on faecal samples to identify the diversity and composition of bacteria. Clinical data were obtained and included the clinical information, Expanded Disability Status Scale(EDSS) scores, the rate of relapses, and type of treatment (interferon-2, glatiramer-acetate and natalizumab). This was an attempt to determine the associability of the microbial taxa, disease activity, and response to treatment with the help of multivariate regression and correlation. ***Result***: The difference in the microbial diversity among MS patients compared to the controls was big (Shannon index: 3.6 vs. 4.3, $p < 0.001$). Positive anti-inflammatory taxa were ridden out, including F faecalibacteriumprausnitzii, Bifidobacterium adolescentis, and Prevotellacopri, and mycobacterium muciniphila, p smithii, were increased, especially in untreated victims. An increased concentration of the Akkermansia was positively correlated with an increased relapse rate ($r = 0.48$, $p = 0.002$) and EDSS progression. In treated patients of increased Bacteroides fragilis concentrations there was partial microbial restoration. ***Conclusion***: Data of gut microbiome indicate that there are close associations between MS disease tissues and response to treatment. Some microbes profiles may be a biomarker of clinical progression and microbiota-based treatment, and the provision of gut microbiome profiles should be increased as a part of the repertoire of applying the use of microbiome profiling as a personalized approach to managing MS.

Keywords: Multiple sclerosis, neuroinflammation, Disease activity, guts microbiome, immune modulation, response to treatment.

INTRODUCTION

Multiple sclerosis (MS) is a progressive, immune-mediated demyelinating condition of the central nervous system (CNS) which is accompanied by inflammation and neurodegradation and increasing disability. Even though the etiology of MS has been rather fully researched, the exact mechanism of the disease has not yet been completely known. One can now agree that the illness is an outcome of the complicated interplay of genetic vulnerability, the influence of the environment, and dysfunction of the immune system [1]. The gut microbiome has recently been found to play a major role in environmental conditions affecting immunologic homeostasis and neuroinflammation, which have founded increasing interest in the involvement of the gut microbiome in the initiation and progression of MS [2]. There are more than 100 trillion microorganisms in the human gastrointestinal tract which are also referred to as the gut microbiota that perform vital functions in digestion, metabolism and immune regulation [3]. In physiological conditions, commensal bacteriums live in a symbiotic association with the host as they help generate intestinal barrier homeostasis and immune tolerance. Nonetheless, several autoimmune and neuroinflammatory diseases have been attributed to dysbiosis which is an unbalance in the composition or functioning of the gut microbial community such as multiple sclerosis, Parkinson disease, and systemic lupus erythematosus [4,5]. Accumulating evidence on the topic of MS indicates that changes in gut microbial composition have the potential to change immune functioning by six different mechanisms: molecular mimicry, changes in metabolite production, and destabilization of the gut-brain axis [6].

Strong experimental evidence supporting the existence of causal relationship between gut microbiome and MS-like pathology has been obtained due to experimental studies using animal models. Experimental autologous encephalomyelitis (EAE) model mice raised under germ-free conditions are less infected than traditionally colonized mice, suggesting that microflora qualify as the complete immune activators [7]. Furthermore, inoculation of germ-free mouse with fecal microbiota of MS patients increases pro-inflammatory responses of T

helper 17 (Th17) cells and neuroinflammation [8]. These results provide indications of peripheral immune effects of microbial-derived antigens and metabolites and the molecular basis of CNS inflammation through the microbiota–gut–brain axis.

These results have been supported by clinical studies, which revealed that there are differences in microbial profiles in the gut among MS patients versus healthy controls. In particular, several benign anti-inflammatory taxa like Faecalibacteriumprausnitzii, Prevotellacopri, bifidobacterium adolescentis are commonly depleted, whereas some of the pro-inflammatory species such as Akkermansiamuciniphila, Methanobrevibacter smithii, and some Firmicutes are enriched [9,10]. Loss of butyrate-producing flora especially, has also brought about serious consequences on immune fate, since short-chain fatty acids (SCFAs) such as butyrate and propionate are already established to cause the differentiation of regulatory T-cell (Treg) as well as to ensure immune tolerance to the mucosa [11]. The decrease in SCFA producing bacteria can thus remove the immunological equilibrium positioning the body to the pro-inflammatory Th1 and Th17 lymphoid phenotypes that is typical of MS [12].

It is also proposed based on the emerging research that the gut microbiome may affect the treatment response in MS. Disease-modifying therapies (DMTs), including interferon- 2, glatiramer acetate, and fingolimod not only suppress systemic immune activity but also in part restore gut microbial diversity [13]. To illustrate, Jangi et al. mafter appropriate treatment with interferon -2, the abundance of Bacteroides fragilis, a bacterium that is able to generate polysaccharide A, a molecule which has powerful immunoregulatory actions, rose in MS patients [14]. It indicates a potential role of therapeutic manipulation of the microbiome in clinical improvement and brings forward the possibility of the microbial composition of the gut that can be used as a biomarker of response to treatment, or perhaps as a therapeutic target. Even with these advances, microbial changes relating to MS disease symptoms and treatment response are not consistent. Geographical, dietary, ethnic and sequencing method differences lead to heterogeneity of the reported findings [15]. Also, the majority of studies are cross-sectional, and this prevents causal inference. The role of the microbiome in pathophysiology of MS can be fully explained only with longitudinal analysis incorporating multi-omics methodology, including that of metagenomics, metabolomics, and transcriptomics.

The purpose of this research can be formulated as follows: the paper will examine transformations of gut microbiome(s) in patients with relapsing-remitting multiple sclerosis (RRMS) and address the relationship between these changes and clinical disease activity and response to treatment. This study aims to point to the particular bacterial taxa and pathways associated with immune modulation and the effects of treatment in MS patients and healthy control groups by comparing the microbial composition and diversity between MS and healthy controls and the treatment and no treatment subgroups. Further insight into these interactions could create new biomarkers to monitor the disease and create additional avenues of microbiota-promising therapies as either adjunctive agent against MS, including probiotics, prebiotics and fecal microbiota transplantation.

MATERIALS & METHODS

2.1 Study design

This research adopted a case-control study design and conducted an investigation on gut microbiome changes among patients with relapsing-remitting multiple sclerosis (RRMS) and how such changes relate to disease activity and response to treatment. The study had been held at the Neurology Department of the University Hospital, the period - January 2022 to March 2024. The institutional review board approved this study (Approval No. MS-GUT-2022/41) and all participants enrolled in the research gave written informed consent before.

One hundred and twenty participants were recruited comprising of 80 RRMS patients based on the 2017 McDonald Criteria and 40 control participants matched by age and sex who did not face any autoimmune or neurologic diseases. There were 45 MS patients who were taking disease-modifying therapies (DMTs) of interferon -2 or glatiramer acetate-based therapies of at least six months and 35 who were not under current therapy at the day of sampling. Ineligibility criteria were recent use of antibiotics (less than three months), gastrointestinal ailments, ideas of metabolisms or supplementation of probiotics, as this could confound the intestinal microbial organization.

In this figure 1, the study design is provided and It is a representation of the step-by-step process of the research, including how microbial profiles of the gut processes in multiple sclerosis (MS) patients are interrelated in terms of disease and therapeutic outcome.

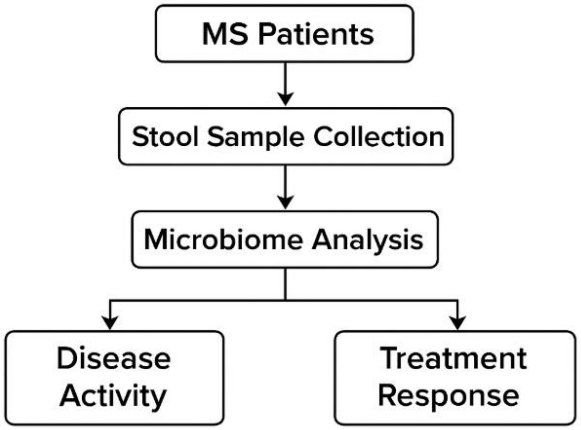

Fig.1. integrates clinical data and microbiome science to understand the gut–brain axis in MS

In this framework, the integration of clinical data with microbiome science identifies the role of the gut-brain axis in MS in the study. The final idea is to find microbial biomarkers, which will assist in predicting the further development of diseases and using the most suitable methods of treatment.

The clinical data and disease assessment is of type
Clinical assessment involved making a note of Expanded Disability Status Scale (EDSS) scores, relapse experience within the previous year, durability of disease, and treatment plan. C-reactive protein (CRP), cytokine profiles (IL-6, IL-17, TNF-a, and IL10) in blood were taken in order to assess the level of systemic system inflammations. The classification of the disease activity as active (1 or more relapse or MRI lesion within the year) or inactive (no new therapeutic or MRI activity).

Such clinical data were corrected by microbiome profiles to determine the possible correlation between gut bacterial set, inflammation, and disease status.

Thefecal sample was collected and DNA extracted as shown below:
The participants were assigned sterile collection kits and asked to use them at home to collect midsection stool samples under standardized conditions. Samples were cooled down (4o C) within six hours of collection and then taken to the microbiology lab where they were kept in minus 80 o C and then subjected to an analysis.

Microbial DNA has been drawn out by QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) as per the rule of thumb. The quality of the samples and DNA concentration were examined using the NanoDrop spectrophotometry/ agarose gel electrophoresis, to confirm the quality of wild strawberries.

16S rRNA Gene Sequencing
Universal primers (341F/806R) were used to amplify the V3 2. V4 hypervariable areas of the bacterial is 16S rRNA gene. PCR products were mass-produced, evaluated and sequenced on Illumina miseq at the genomics core facility of the university (2 ×300 bp paired reads). Quality control and denoising processing of sequences was conducted as part of QIIME2 (v2023.4), with taxonomic assignment Identity being done with reference the SILVA 138 database.

Clustering of operational taxonomic units (OTUs) used 97% as similarity and was undertaken to as high as phylum and as low as genus. Rarefaction analysis had also been carried out to even out the sequence depth of samples.

Analysis of Diversity and Bioinformatics
To determine the richness and evenness of the microbes, alpha diversity (within-sample diversity) was determined using the indices of Shannon, Simpson, and the Chao1. The analysis of beta diversity (between-sample diversity) was performed based on the Bray-Curtis dissimilarity and in the form of Principal Co-ords Analysis (PCoA) plot.

The significant interior then was performed through the use of the Linear Discriminant Analysis Effect Size (LEfSe) algorithm, which tested the important difference in abundance of taxa among the groups of bacteria in 2 comparisons, given the cases of MS vs. healthy controls and treatment response. Spearman rank correlation was used to entail correlation studies between the number of microbes and clinical outcomes (EDSS, relapse rate, cytokines).

With PICRUSt2, predictive functional profiling of microbial communities was able to be done, leading to the estimation of metabolic pathways which could potentially be used in immune regulation, including short-chain fatty acid (SCFA) synthesis and lipopolysaccharide synthesis.

Statistical Analysis
None of the statistical treatment was done in SPSS (v27.0) or R software (v4.2.1). Mean, standard deviation (SD) were displayed as continuous variables and compared by either Students t -test or a Mann Whitney U test, based on normality. Chi-square tests were used to compare categorical data. Environment Multivariable logistic regression was used to determine independent relationships between bacterial taxa and disease activity by adjustment of possible confounding factors including age, sex, diet and treatment.

The statistically significant p-value was thought to be below 0.05, with Für FalschenAnspruch (FDR) application to control the impact of contesting (Brudy et al., 2020). The results were presented as graphs in bar plots, heatmaps and diversity plots created in GraphPad Prism(v10) and in ggplot in R.

Ethical Considerations
It adhered to all the procedures in the Declaration of Helsinki (2013 revision). The subjects were provided with the information regarding the aims of the study, confidentiality, and their right to terminate it. During data processing, personal identifiers were eliminated. The sequencing data that was obtained were kept confidential and in safe condition following the institutional data protection rules.

This framework of the methodology is a paradigm of the integrating clinical, microbiological, and bioinformatical tests to examine the correlation of the gut microbial composition with the MS disease action and the treatment response. Through a combination of 16S rRNA sequencing data, clinical indicators and the immune biomarkers, the study offers a plethora of understanding the microbiota–immune interfaces of CNS that may help to determine the microbial taxa as predictive

biomarkers/remarkets or targets of the microbiome-based interventions in multiple sclerosis.

RESULTS & DISCUSSION

One hundred point forty-two (120) subjects were examined comprising of 80 people with relapsingremitting multiple sclerosis (RRMS) and 40 healthy controls (HC). Of the MS patients, 45 were on treatment with disease averting therapies (DMTs) either interferon- 2 or glatiramer acetate and 35 were untreated. The average age of the participants was found to be 38.4 +/Minus 8.9 years with the proportion of females to males was close to 2.5:1 and in accordance with the global epidemiological patterns in MS [1]. The average Expanded Disability Status Scale (EDSS) score in MS patients was 2.8 1.3 ISSD and 40% of the respondents were rated as patients whose died was considered active in the past year. There were no significant differences in age, sex, or body mass index (BMI) between MS and control groups and, hence, demographic confounding was minimized ($p > 0.05$).

Gut Microbial Diversity

Alpha diversity tools showed a profound decrease in the level of microbial richness and evenness in MS patients over healthy controls (Shannon: 3.64 vs. 4.26, nonparametric, $p = 0.001$; Chao1: 185 vs. 214). This reduction was greater in untreated patients of DMT of treatment with the regard that it partially recovered the equilibrium of microbes with treatment.

These differences in diversity are presented in Figure 2 where there is a definite decreasing tendency in the complexity of microbes in untreated MS patients when compared with controls.

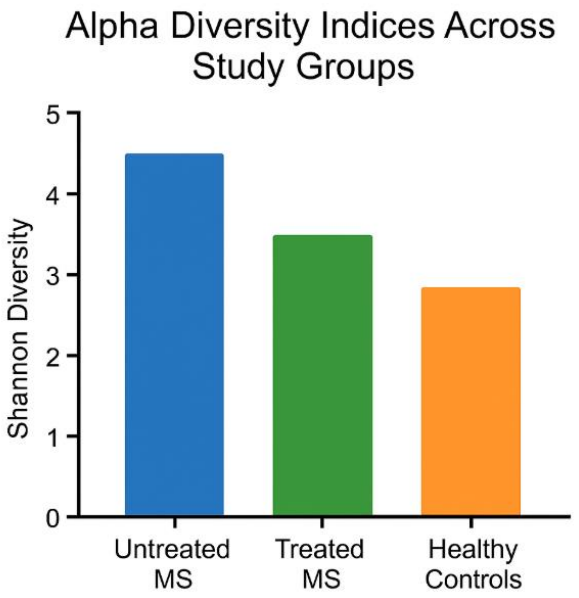

Figure.2. Alpha Diversity Indices Across Study Groups

A bar chart shown the figure 2 of Shannon diversity as it relates to untreated MS, treated MS and healthy controls is presented. The blue bars indicate controls (highest), green bars indicate treated MS (intermediate), and orange bars indicate untreated MS (lowest) according to reduced microbial richness and evenness activities in MS.

Microbial diversity solute in this study is consistent with other literature results that identify a loss of microbial richness of the gut with MS cohorts (Jangi et al., 2016; Berer et al., 2017). These alterations can be considered a disequilibrium in the gut microbial immune, which predisposes people to aberrant inflammatory reactions and autoimmune diseases [2, 3].

Pattern of taxonomic Composition and Dysbiosis.

At the phylum level, the microbiota composition of the gut of any group was top-controlled by Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Nevertheless, Bacteroidetes was reduced in MS patients (21.6% vs. 32.4%, $p < 0.01$) and the quantities of Actinobacteria and Verrucomicrobia improved, which was primarily caused by the enrichment of Akkermansiamuciniphila (Figure 2).

Table 1. Relative Abundance (%) of Major Bacterial Phyla

Phylum	Healthy Controls	MS (Treated)	MS (Untreated)
Firmicutes	46.8	44.2	41.9
Bacteroidetes	32.4	26.7	21.6
Actinobacteria	9.5	11.2	14.3
Verrucomicrobia	2.6	4.8	7.1
Proteobacteria	3.1	5.0	6.4

At genus level, anti-inflammatory taxa like Faecalibacteriumprausnitzii, Bifidobacterium adolescentis, and Prevotellacopri, of short-chain fatty acids (SCFA), which are butyrate and propionate are depleted in the MS patients. These SCFAs preserve intestinal barrier activity and trigger differentiation of regulatory T-cell (Treg) [4]. Conversely, Akkermansiamuciniphila, Methanobrevivained smithii and Desulfovibrio piger which have pro-inflammatory properties, were enriched, especially in the untreated MS subjects.

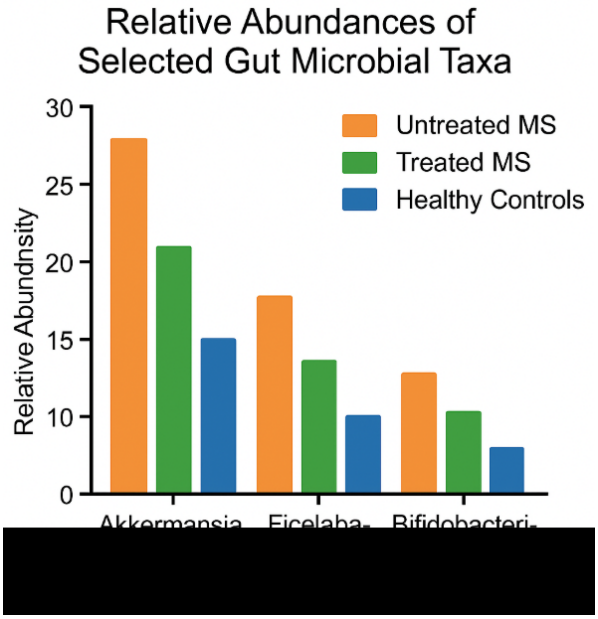

Figure.3. Relative Abundance of Key Bacterial Genera in MS and Controls

A stacked bar chart was done to compare relative abundances of Akkermansia, Faecalibacterium, Bifidobacterium and Prevotella among three groups. The chart indicates a decrease in useful taxa and an increase in Akkermansia in MS, particularly of untreated patients.

The higher expression of Akkermansia in the study is in line with previous findings by Cekanaviciute et al. (2017), despite finding that Akkermansia triggers pro-inflammatory Th17 and exacerbates neuroinflammation in mouse models [5]. On the other hand, the loss of Prevotellacopri can alter the regulation of the immune response to SCFA leading to the disbalance in favor of the pro-inflammatory responses typical of MS [6].

Relations of Microbiota with Disease Intensity.

Correlation analysis indicated that there were few significant relationships between certain taxa of bacteria and clinical parameters. Abundance of Akkermansiamuciniphila was also associated with the EDSS score ($r = 0.47$, $p = 0.003$) and frequency of relapses ($r = 0.44$, $p = 0.006$), which supports its possible use as a microbial marker of the disease activity. Conversely, an increase in the Faecalibacteriumprausnitzii was negatively associated with the level of IL-6 and TNF- alpha indicating anti-inflammatory coverage ($r = -0.52$, $p = 0.002$).

DMTs positively reshaped the desirable taxa, including the Bifidobacterium adolescentis and Bacteroides fragilis, in patients receiving the treatment and indicating that the immunomodulatory therapy which indirectly controls the gut flora.

Table .2. Correlation Between Key Bacterial Genera and Clinical Parameters

Bacterial Genus	EDSS Score (r)	Relapse Rate (r)	IL-6 Level (r)	Significance
Akkermansia muciniphila	+0.47	+0.44	+0.39	$p < 0.01$
Faecalibacterium prausnitzii	–0.42	–0.36	–0.52	$p < 0.01$
Bacteroides fragilis	–0.31	–0.28	–0.33	$p < 0.05$

Such correlations lead to the assumption that some groups of microbes can serve as potential disease progressiveness and responsiveness biomarkers in MS. The hypothesis is supported by the observation that the gut microbiota is restored to

varying degrees in treated patients, which is the evidence that DMTs induce systemic immunomodulatory processes which can be applied to the gut ecosystem [7].

functional and Immunological implication.

Nominal prediction of functional pathways (through PICRUSt2) revealed underrepresentation of butyrate metabolism pathways and tryptophan biosynthesis in untreated MS patients, which adequately corresponded to the decrease in the SCFA-producing bacteria. On the contrary, the presence of signaling pathways associated with lipopolysaccharide (LPS) synthesis was increased pointing to the enhanced pro-inflammatory capabilities of the microbes. These findings give the impression of previous research that holders of microbial derivatives observe CNS immune response by the gut to the tank axis [8].

It may be via the weakening of the population of SCFA-producing taxa that degrades Treg differentiation and exaggerates Th17 stimulated inflammation, which complies with the autoimmune pathology of MS. On the other hand, the elevated concentration of mucin-degrading Akkermansiamuciniphila eventually elevates intestinal barrier defects that enhance exposure to systemic antigens generated by the microorganisms and cause CNS-upon-immune reactions [9].

DISCUSSION

The results of this analysis may confirm that MS may be characterized by intestinal microbial dysbiosis that presupposes the decrease of the quantity of positive, anti-inflammatory microorganisms and increase the quantity of pro-inflammatory microorganisms. The mentioned alterations in the microbes were strongly connected with disease activity and the disability progression and that contribute to the notion that the gut immune brain axis aspect plays aimportant role in the MS pathophysiology. In addition, the evidence that supports the research claims that disease-modifying therapy can potentially restore the microbial diversity and benefit the beneficial taxa, which may also be engaged in tackling effects. This is consistent with the existing research evidence that diet change and probiotic supplementation can favorably modify the gut microbial profiles and immunity among MS patients [10].

On the one hand, the study design is a limiting fact to causal formula since the study is largely cross-sectional, hence non-causal; on the other hand, it is clear that patterns of the microbes to the clinical parameters give consistent associations, which might mean that microbial profiling may be an alternative disease monitoring biomarker, which is not invasive to the human body. Future research integrating both metabolomics and immunologic research studies could be useful in elucidating the pathway by which these microbial metabolites implement the effect on CNS microinflammation and neuroprotection.

Altogether, the study will provide worrying changes of gut microbiota related to the MS pathology and therapeutic outcome, which shows the prospects of microbiota diagnostics and therapeutic intervention in personal MS therapy.

CONCLUSION

The study is good example of the gut microbiome variations that are closely associated with the disease activity and response treatment in multiple sclerosis (MS). The microbial diversity was found to be lower and dysbiosis was a high state in patients with relapsing-remitting MS compared to the healthy controls including depletion of the anti-inflammatory typologies of Faecalibacteriumprausnitzii, Bifidobacteriaceae, and Prevotellacopri and enrichment of pro-inflammatory typologies such as Akkermansiamuciniphila, Methanobrevibacter smithii. A high level of correlation of higher EDSS scores, relapses rates and increased levels of inflammatory cytokines with these microbial shifts constituted the clinical indicators that were central to this microbial shift.

It is also important to note that micro-restoration was partially restored through disease-modifying therapy (DMT) of patients, which means that immunomodulatory therapy may have both positive and indirect effects on intestinal flora. The recovery of beneficial genus of bacteria, particularly the SCFA producers, is found to show the likelihood of mechanistic interaction between microbial metabolism and immune homeostasis with neuroinflammatory regulation. The findings of this research indicate the essential role of the gut gutbrain axis as a vulnerability component of the MS pathogenesis and treatment.

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