

Gut microbiota in forty cases of Egyptian relapsing remitting multiple sclerosis

Sherein G. Elgendy^{1*}, Rawan Abd-Elhameed¹, Enas Daef¹, Shereen M. Mohammed¹, Hebatallah M. Hassan¹, Mohamed A. El-Mokhtar¹, Ahmed Nasreldein², Eman M. Khedr²

¹Department of Medical Microbiology and Immunology, School of Medicine, Assiut University, Assiut, Egypt

²Department of Neurology and Psychiatry, School of Medicine, Assiut University, Assiut, Egypt

Received: May 2021, Accepted: September 2021

ABSTRACT

Background and Objectives: Gut microbiota is assumed to play an essential role in the pathogenesis of multiple sclerosis (MS). This study aimed to investigate the abundance of some gut microbiota among Egyptian patients with relapsing remitting multiple sclerosis (RR-MS).

Materials and Methods: Forty cases of RR-MS diagnosed according to McDonald diagnostic criteria (2017) were recruited consecutively from the Department of Neurology, Assiut University Hospitals. The results were compared with 22 healthy age and sex matched control subjects. DNA was extracted from stool and measures made of concentration and copy number of bacterial organisms by real-time PCR using group specific primers for 16S rRNA targeting predominant genera of gut microbiota previously hypothesized to participate in MS pathogenesis.

Results: The mean age was 31.4 ± 8.8 yrs; 75% of the patients were women. The mean and SD of EDSS score was 3.43 ± 1.35 . Seven cases had cervical cord plaques (17%). There were significantly increased copy numbers of *Desulfovibrio*, *Actinobacteria*, *Firmicutes*, and Lactic acid bacteria in patients compared with the control group. In contrast there was a significantly lower level of *Clostridium cluster IV* in the patients. Patients who had EDSS < 3.5 had a significantly higher copy number of *Actinobacteria*, *Bacteroidetes*, and *Bifidobacterium*, compared with patients who had EDSS > 3.5. There was a significant negative correlation between duration of illness and copy number of *Firmicutes*, *Akkermansia*, and Lactic acid bacteria ($P = 0.01, 0.04, \text{ and } 0.004$ respectively).

Conclusion: The changes in gut microbiota are associated with exacerbation of MS disease. Disruption of the intestinal microbiota results in the depletion or enrichment of certain bacteria that may affect the immune balance leading to predisposition to MS.

Keywords: Gut microbiota; Relapsing remitting multiple sclerosis; Egypt

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease characterized by the destruction of the spinal cord and nerve cells in the brain due to an attack of immune cells, causing a wide range of harmful symptoms related to inflammation in the central nervous

system (CNS) (1).

It has several clinical variants, among which the most frequent type is relapsing remitting MS (RR-MS). MS develops in people who are genetically susceptible and likely requires environmental triggers such as low levels of vitamin D, Epstein-Barr virus infection, smoking and dietary habits (2, 3).

*Corresponding author: Sherein G. Elgendy, Ph.D, Department of Medical Microbiology and Immunology, School of Medicine, Assiut University, Assiut, Egypt. Tel: +2-01021887728 Fax: +088-2080278 Email: ShereinElgendy@yahoo.com



Gut microbiota are also thought to be involved in the pathogenesis of MS (4). The main end-products of intestinal microbial fermentation, short-chain fatty acids (SCFAs), Lipid 654, polysaccharide A (PSA), and aryl hydrocarbon receptor (AHR) agonists influence systemic immunity, CNS-resident microglia and CNS-resident astrocytes (5). A compromised immune system due to dysbiosis can initiate MS or exacerbate its relapses (6).

Recently, gut microbiota has been shown to play a fundamental role in various CNS diseases (7). For example, increased levels of *Akkermansia*, *Ruminococcaceae*, *Lactobacillus*, and *Bifidobacterium* and reduced levels of *Lachnospiraceae* occur in fecal samples of Parkinson's disease (8). Another study illustrated that prominent dysbiosis in the gut microbiota of Autism spectrum disorder (ASD) in children compared with healthy control children (9).

Several studies have investigated differences between gut microbiomes in MS patients and healthy individuals. Chen et al. 2016 found increased levels of *Pseudomonas*, *Mycoplana*, *Haemophilus* and *Blautia* and reduced levels of *Parabacteroides*, *Adlercreutzia*, and *Prevotella* microbes in MS (10). A Japanese study reported moderate levels of dysbiosis and significant changes in the abundance of 21 microbial species in RR-MS (11).

A reduced level of *Prevotella* in patients with RR-MS is linked to the expansion of Th17 cells and disease activity (11, 12). Other study found a reduction in the genus *Clostridium* in patients with RR-MS compared with healthy subjects (11). *Clostridia* are responsible for the production of regulatory T cells in peripheral compartments and the increase of the anti-inflammatory cytokine IL-10 (13).

Several studies reported that *Certain Firmicutes* genera were elevated in RRMS individuals and plays a beneficial role in metabolism of bile acids and can produce metabolites with anti-inflammatory activities (10, 14).

Up to our knowledge there has been no study of the relationship between gut microbiota and MS in Egypt. The aim of the present study was therefore to compare the abundance of some gut microbiota in RR-MS patients and a healthy matched control group.

MATERIALS AND METHODS

Ethics statement. This study was approved by the

Local Ethics Committee in Faculty of Medicine, Assiut University and conducted in accordance with the provisions of the Declaration of Helsinki. Informed written consent was obtained from all participants before enrolment.

Study design. This was a prospective study conducted during the period from February 2018 to August 2020 and included 40 newly diagnosed MS patients and 22 age- and sex-matched healthy volunteers as controls. The patients were diagnosed as RR-MS according to the 2017 diagnostic criteria (15). Exclusion criteria included demyelinating diseases other than MS, MS subtypes other than RR-MS, patients with prior GIT surgeries, patients taking antibiotics in the previous 6 months or probiotic supplements, or having a known history of disease which could affect gut microbiota such as rheumatoid arthritis, type-1-diabetes and irritable bowel disease (IBD). During study setup, sample size calculation and power analysis could not be performed because effect size estimates for the gut microbiome were lacking. A detailed medical, neurological history and examination was in all patients including demographic and clinical data, expanded disability status scale (EDSS) score, MS activity status, type and duration of treatment. Each patient had Magnetic resonance imaging (MRI) of the brain as well as visual evoked potentials (VEP), Lumbar puncture was performed in all patients to detect oligoclonal bands in CSF and laboratory investigations to exclude MS mimics such as collagen disease.

VEPs were recorded by using a black and white checker-board pattern on a screen, with a checker size of 36 cm² and a pattern reversing frequency of 1.9 Hz. The subjects were sitting at a distance of 1 m from the screen. The stimuli were presented monocularly. An active recording electrode was attached to the scalp on the midline at the occipital region (Oz according to the 10-20 system), the reference electrode was placed on the midline frontal point (Fz) and the ground electrode on the forearm. Ag/AgCl surface electrodes were used and their impedance was maintained below 5 k Ohm. Two hundred responses were averaged in each run and two runs were performed for each eye. For each subject, the latency and amplitude ("peak to peak") of the P100 component were determined for each eye, as well as relative P100 latency (interocular latency difference). VEP abnormalities were quantified and interpreted as described by Leocani et al. (16).

Stool sample processing and extraction. Stool samples collected from patients before initiation of therapy and stored immediately after collection in screw cap sterile containers at -70°C upon arrival to the Medical Research Center laboratory, Assiut University Hospitals. Total bacterial DNA was extracted from fecal samples using the QIAamp® DNA Stool mini kit (Qiagen GmbH, Germany. Cat. No.12830-50) according to the manufacturer's protocol. DNA concentration and purity were determined using a spectrophotometer (GeneQuart 1300, Germany).

Quantitative real-time PCR (qRT-PCR). Bacterial abundance was quantified by SYBR Green qRT-PCR using 7500 Fast Real-Time PCR System (Applied Biosystems, USA) using group specific primers for 16S rRNA targeting strains according to Pirker et al. (2013) who detect predominant genera of gut microbiota previously hypothesized to participate in MS pathogenesis (17). Primers used in this study are listed in Table 1 (17-19).

The PCR reaction mixture and serial DNA dilution were prepared according to Pirker et al. (2013). Amplification reactions were carried out in a total volume of 20 µL and the standard reaction mixture consisted of 10 µL SYBR green PCR master mix (SensiFAST

SYBR® Cat.No Bio-92020), 0.8 µL of each of the specific primers and 2 µL of template DNA at a final concentration of 20 ng/µL. For *Akkermansia*, the reaction mixture consisted of 12.5 µL SYBR green, 1 µL of each primer and 2.5 µL of template DNA for a total volume of reaction of 25 µl (20). For the negative control, 2 µl of sterile distilled H₂O was added to the reaction solution instead of the template DNA solution (21). The PCR conditions were as follows: 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, primer annealing at 50-70°C gradient for 20 s, and primer extension at 72°C for 45s, with final extension at 72°C for 5 min. The fluorescent products were detected at the last step of each cycle. A melting curve analysis was made after amplification to distinguish the targeted PCR product from the non-targeted PCR product. Analysis of data was carried out by absolute quantification method as previously described (22).

Absolute quantification with a standard. In absolute quantification, real-time PCR reaction is performed using both the experimental samples and known amounts of specific PCR-product, which have the same primer binding sites as that of the specific DNA target and is subsequently diluted to create a

Table 1. Primers targeting 16S rDNA coding regions of bacteria (17-19)

Target organism	Oligonucleotide sequence (5' - 3')	Size (bp)	Ta (°C)
<i>Lactic Acid Bacteria</i>	F: AGC AGT SGG GAA TCT TCC A R: ATT YCA CCG CTA CAC ATG	352-700	60
<i>Akkermansia muciniphila</i>	F: CAGCACGTGAAGGTGGGGAC R: CCTTGCGGTTGGCTTCAGAT	1505	60
<i>Atopobium</i> spp.	F: ACCGCTTTCAGCAGGGA R: ACGCCCAATGAATCCGGAT	120	61
<i>Desulfovibrio</i> spp.	F: GGTACCTTCAAAGGAAGCAC R: GGGATTTCACCCCTGACTTA	191	58
<i>Methanobrevibacter smithii</i>	F: CCGGGTATCTAATCCGGTTC R: CTCCCAGGGTAGAGGTGAAA	123	60
<i>Clostridium cluster IV (Ruminococcaceae)</i>	F: GGTGGCAAAGCCATTCCGGT R: GTTACGGGACGGTCAGAG	182	55
<i>Bifidobacterium</i> spp.	F: TCGCGTC(C/T)GGTGTGAAAG R: CCACATCCAGC(A/G)TCCAC	243	60
<i>Firmicutes</i>	F: GGAGYATGTGGTTTAATTCGAAGCA R: AGCTGACGACAACCATGCAC	126	62
<i>Bacteroidetes</i>	F: GTTTAATTCGATGATACGCGAG R: TTAASCCGACACCTCACGG	122	55
<i>Actinobacteria</i>	F: TGTAGCGGTGGAATGCGC R: AATTAAGCCACATGCTCCGCT	277	55

suitable range of standard concentrations (23). The calibration curves are based on PCR product (24). Standard curve analysis was done by PCR multiplication using the species specific primers. Amplified product was run on agarose gel electrophoresis to ensure the presence of a specific band, and then it was purified (23). Determine DNA concentration using Microplate Spectrophotometer. Standard curves were created using serial 10-fold dilutions of template for each primer. Repeat amplification by Real-time qPCR and record Ct for each dilution. The bacterial concentration of each sample was calculated by comparing the threshold cycle (Ct) values obtained from the standard curve. So, the standard curve is illustrated as plot of threshold cycles (Ct) values of standard dilutions against the logarithm of the amount of bacterial DNA added (20).

Statistical analysis. All data were analyzed with the aid of the SPSS ver.16. Descriptive statistics, cross-tabs and frequency tables were used to describe some of the basic variables. A Shapiro-Wilk test confirmed the data was distributed normally so that an independent sample t-test was performed to compare continuous variables between groups which are expressed as mean \pm SD data. Categorical variables were compared by Fisher's exact 2-tailed test or by the Chi Square test. Kruskal-Wallis H test was used to determine if there were statistically significant differences in the gut microbiome between groups with high and low EDSS or VEP scores. Pearson correlations were assessed between two continuous variables.

RESULTS

The average age of patients was 31 years and 75% were women. Over half of the patients were retired due to clinical deterioration or due to age, while 15% were still in employment. The EDSS score was 3.43 ± 1.35 (mean and SD ranging between 1 and 7). The disease duration varied between 3 months and 96 months, with a mean of 29.90 ± 25.79 months. Of 40 patients, 35 (87.5%) had a history of optic neuritis confirmed by VEP of one or both eyes. The mean number of attacks was 2.9 ± 1.2 with range between 1 and 6. Details of EDSS and VEP scoring are given in Table 2.

There were significantly increased copy numbers of *Desulfovibrio*, *Actinobacteria*, *Firmcutes*, and

Lactic acid bacteria in patients with RR-MS in comparison with the control group. In contrast there was a significant lower copy number of *Clostridium clusterIV* in RR-MS. There were no differences in other species of gut microbiota (*Bifidobacterium*, *Methanobreviabacter smithii*, *Atopobium*, *Akkermansia* and *Bacteroidetes*) (Table 3 and Fig. 1).

Patients who had EDSS ≤ 3.5 (23 cases) had a significantly higher copy number of *Actinobacteria*, *Bacteroidetes*, and *Bifidobacterium*, compared with patients with an EDSS >3.5 (Table 4 and Fig. 2).

Table 5 shows that patients during relapse had a significantly higher copy numbers of *Clostridium clusterIV*, *Akkermansia*, and Lactic acid bacteria than patients during remission. Other gut microbiota showed no significant differences between patients group during activity and during remission. Patients in relapse had significantly higher copy numbers of *Desulfovibrio*, *Actinobacteria*, *Firmcutes*, *Methanobreviabact* and Lactic acid bacteria in comparison with the control group, whereas there was no significant difference between copy numbers in patients in remission and controls.

There were significant negative correlations between duration of illness and copy numbers of three species; *Firmcutes*, *Akkermansia*, and Lactic acid bacteria ($r = -0.42$; $P = 0.01$, $r = -0.33$; $P = 0.04$, $r = -0.64$; $P = 0.004$, respectively) (Fig. 3).

There was no association between the VEP scoring (0-4) and copy number of any microbiota. There was a significant correlation between EDSS scoring (1-7 score) and copy number of *Bifidobacterium* ($P = 0.041$) but not with any other of the gut microbiota. There was no significant association between the presence or absence of spinal cord plaque and copy number of any of the gut microbiota.

DISCUSSION

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) with various clinical manifestations (25).

A combination of genetic and environmental factors contribute to the development of the disease, and advances in analysis of microbial communities has suggested that alterations in gut microbiota are one of the important environmental factors that predispose to the disease (26). The majority of MS patients

Table 2. Demographic and clinical data of the study population

	RR-MS patients (n=40)	Control group (n=22)
Age (years)	31.4 ± 8.8	30.4 ± 6.8
Range	16 - 48	17-45
Sex (female/male)	30/10	17/5
Duration of disease (months)	29.90 ± 25.79	NA
Range	3 - 96	
Number of Attacks mean ± SD	2.9 ± 1.2	NA
Range	1-6	
Disease severity	3.43 ± 1.35	NA
EDSS < 3.5	23 (57.5%)	
EDSS > 3.5	17 (42.5%)	
Patients during relapse	21 (52.2%)	NA
Patients during remission	19 (47.8%)	
Visual evoked potentials (VEPs)		
latency Right Mean ± SD ms	116.42 ± 52.9	NA
Left Mean ± SD ms	115.0 ± 23.2	
VEP score		
0-2	21 (52.2%)	
3-4	19 (47.8%)	
Number and percent of patients with spinal plaque	7 (17.5%)	NA

NA; not applicable

Table 3. Differences in copy number of studied gut microbiota between MS patients and control group

Gut Microbiota	Control (n=22) Mean ± SD	Patients (n=40) Mean ± SD	P-value
<i>Desulfovibrio</i>	11.28 ± 4.44	14.50 ± 4.15	0.02*
<i>Actinobacteria</i>	5.56 ± 0.32	7.38 ± 1.77	0.0001*
<i>Clostridium clusterIV</i>	7.34 ± 1.9	6.96 ± 1.83	0.03*
<i>Firmcutes</i>	8.20 ± 2.00	9.62 ± 1.11	0.027*
<i>Akkermansia</i>	6.188 ± 1.05	6.38 ± 1.38	0.215
<i>Bacteroidetes</i>	7.57 ± 1.28	7.40 ± 1.67	0.75
<i>Bifidubacterium</i>	8.78 ± 1.29	9.79 ± 2.07	0.59
<i>Lactic acid bacteria</i>	6.17 ± 4.40	12.58 ± 3.76	0.0001*
<i>Methanobreviabacter smithii</i>	8.15 ± 1.51	9.05 ± 1.09	0.39
<i>Atopobium</i> group	11.5 ± 2.2	10.26 ± 1.01	0.09

present with defined attacks followed by varying degrees of recovery (a relapsing-remitting MS disease course) (27). The strengths of our study are that it is the first study to be carried out on Egyptian patients, and that we correlated between disease severity and gut microbiota.

The results showed that the gut microbiome of our 40 patients differed from that in healthy controls. Certain microbes were elevated and others were de-

pressed, pointing to a possible role of the intestinal microbial community in the exacerbation of MS disease. The difference in abundance of specific microbial communities in our study compared with that seen by others could be attributed to differences in measurement techniques, primers used, genetic factors, diet habits, and geographical location.

We found higher levels of Lactic acid bacteria, *Firmicutes*, *Actinobacteria*, *Desulfovibrio* and *Bifi-*

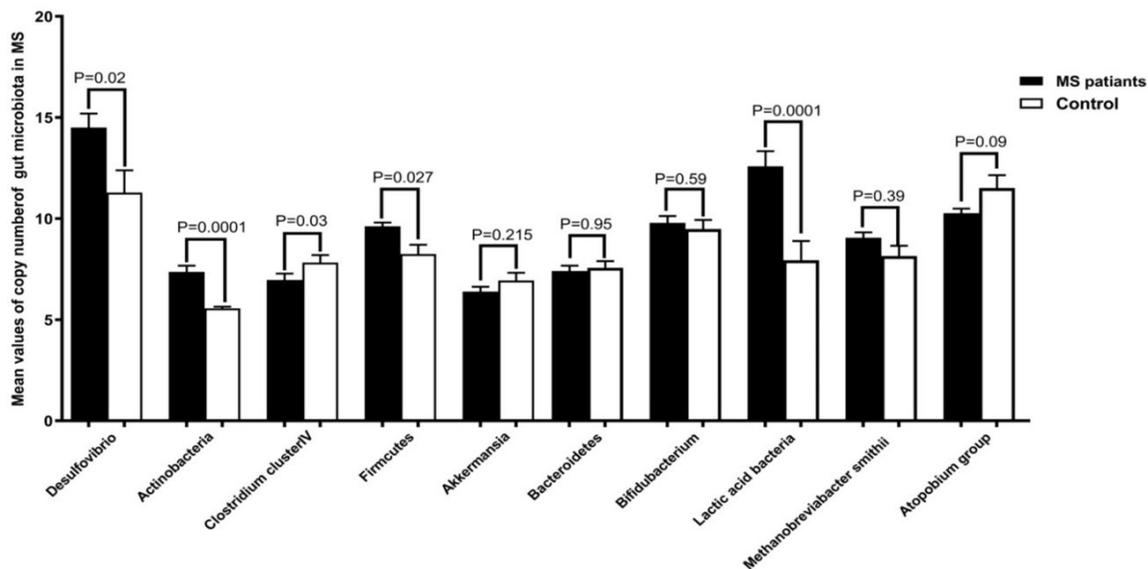


Fig. 1. Copy number of studied gut microbiota between MS patients and control group. There were significantly increased copy numbers of *Desulfovibrio*, *Actinobacteria*, *Firmcutetes*, and Lactic acid bacteria in patients with RR-MS in comparison with the control group. In contrast there was a significant lower copy number of *Clostridium clusterIV* in RR-MS than in control group.

Table 4. Copy number of gut microbiota of patients with EDSS < 3.5, patients with EDSS > 3.5 and control group

Gut microbiota	Control group (n=22)	Copy numbers		P-value controls versus patients with < 3.5	P-value controls versus patients with EDSS> 3.5	P-value EDSS < 3.5 versus EDSS> 3.5
		Patients had EDSS < 3.5 (n=23) Mean ± Std. Deviation	Patients had EDSS > 3.5 (n=17) Mean ± Std. Deviation			
<i>Desulfovibrio</i>	11.28 ± 4.44	15.25 ± 3.7	13.45 ± 4.6	0.007*	0.107	0.22
<i>Actinobacteria</i>	5.56 ± 0.32	8.19 ± 1.4	6.32 ± 1.7	0.0001*	0.016*	0.002*
<i>Clostridium clusterIV</i>	8.023 ± 1.76	7.48 ± 2.1	6.33 ± 1.2	0.805	0.044	0.058
<i>Firmcutetes</i>	8.20 ± 2.06	9.78 ± 1.1	9.40 ± 1.2	0.006*	0.57	0.31
<i>Akkermansia</i>	7.006 ± 1.76	6.29 ± 1.4	6.54 ± 1.4	0.294	0.07*	0.63
<i>Bacteroidetes</i>	7.34 ± 1.30	8.02 ± 1.5	6.64 ± 1.5	0.34	0.503	0.009*
<i>Bifidobacterium</i>	9.55 ± 2.4	10.47 ± 1.9	8.89 ± 2.1	0.097	0.29	0.02*
<i>Lactic acid bacteria</i>	8.29 ± 4.7	13.32 ± 2.5	10.24 ± 6.12	0.0001*	0.40	0.12
<i>Methanobrevibacter smithii</i>	8.45 ± 1.49	8.8 ± 1.1	9.3 ± 1.1	0.31	0.70	0.32
<i>Atopobium group</i>	11.5 ± 2.2	10.34 ± 1.01	10.09 ± 0.97	0.11	0.70	0.62

dubbacterium in the total patient group. Meanwhile, patients with EDSS score > 3.5 had lower *Actinobacteria*, *Bifidobacterium*, and *Bacteroidetes*, than patients with lower scores, pointing to a possible role in the severity of the disease.

There were also differences between patients in relapse (active state) and remission, with the former having higher levels of *Clostridium clusterIV*, *Akkermansia* and Lactic acid bacteria. In contrast Chen et al. 2016 found that there was a trend towards lower

species richness in patients with active disease compared to healthy controls (10). The microbiota of patients in remission exhibited species richness similar to the healthy controls. The difference between our study and that of Chen et al. may be related to dietary habits.

Lactic acid-producing bacteria have an anti-inflammatory role. It includes *Lactobacillus*, and *Streptococcus* genera. Administration of lactic acid-producing bacteria reduces inflammation in EAE through

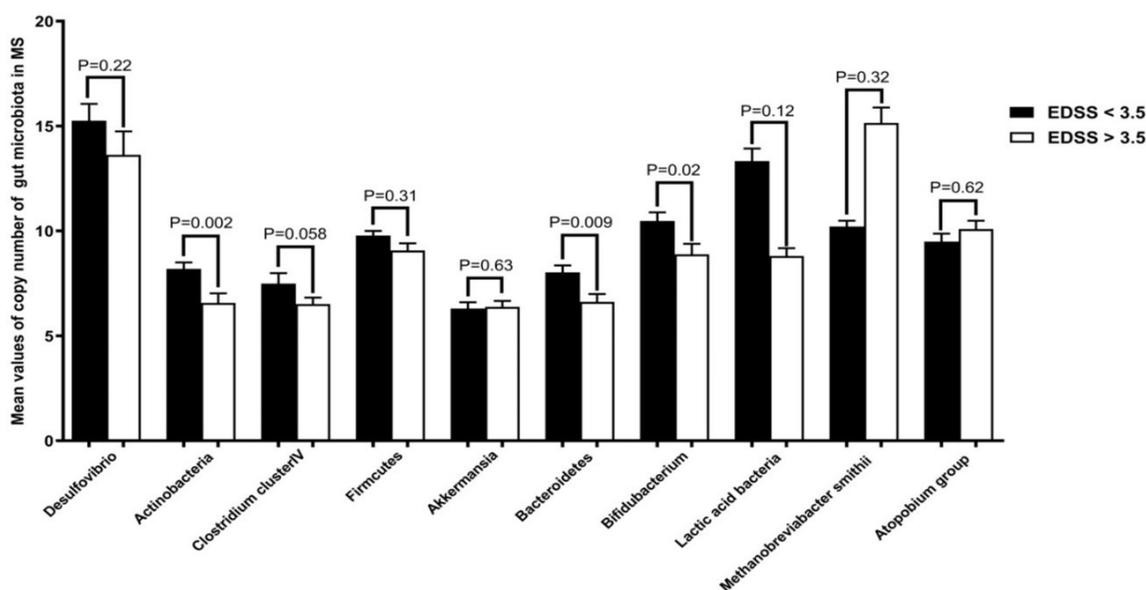


Fig. 2. Copy number of gut microbiota of MS patients and control group according to (EDSS ≤ 3.5 versus EDSS >3.5). Patients who had EDSS ≤ 3.5 (23 cases) had a significantly higher copy number of *Actinobacteria*, *Bacteroidetes*, and *Bifidobacterium*, compared with patients with an EDSS >3.5

Table 5. Copy number of gut microbiome of 22 RR-MS patients (during relapse versus during remission)

Gut microbiome	Control group (n=22)	Patients during relapse Mean ± SD	Patients during remission Mean ± SD	P-value relapse versus remission	P-value control versus relapsing group	P-value control versus remission group
<i>Desulfovibrio</i>	11.28 ± 4.44	15.15 ± 4.6	12.98 ± 3.70	.163	0.017*	0.28
<i>Actinobacteria</i>	5.56 ± 0.32	7.66 ± 1.7	6.30 ± 1.73	.060	0.0001*	0.21
<i>Clostridium cluster IV</i>	8.023 ± 1.76	7.52 ± 2.0	6.25 ± 1.09	.039*	0.771	0.071
<i>Firmicutes</i>	8.20 ± 2.06	9.8 ± 1.0	9.08 ± 1.25	.090	0.004*	0.16
<i>Akkermansia</i>	7.006 ± 1.76	6.97 ± 1.23	5.38 ± .98	.002*	0.32	0.013*
<i>Bacteroidetes</i>	7.34 ± 1.30	7.54 ± 1.51	6.71 ± 1.70	.166	0.557	0.06
<i>Bifidobacterium</i>	9.55 ± 2.4	9.79 ± 2.27	8.99 ± 1.62	.262	0.092	0.722
Lactic acid bacteria	8.29 ± 4.7	13.84 ± 1.65	9.02 ± 4.96	.042*	0.0001*	0.217
<i>Methanobrevibacter smithii</i>	8.45 ± 1.49	9.46 ± 1.03	8.49 ± .97	.110	0.047*	0.61
<i>Atopobium</i> group	11.5 ± 2.2	10.40 ± 1.04	9.52 ± .74	.302	0.136	0.06

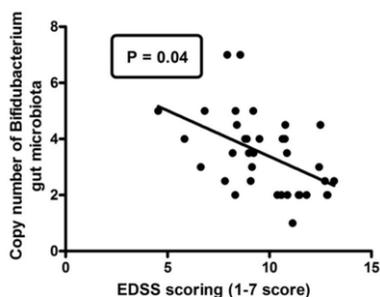


Fig. 3. Correlation between EDSS score and copy number of *Bifidobacterium*. There was a significant correlation between EDSS scoring (1-7 score) and copy number of *Bifidobacterium* (P = 0.04)

induction of T-regulatory cells that down-regulate immune responses by production of IL10 (28). Similarly, analysis of 20 Japanese RR-MS patients demonstrated increased levels of *Actinobacteria*, *Bifidobacterium*, and *Streptococcus* (11). Increased level of Lactic acid bacteria is matching with other studies explaining increased streptococcus species in RRMS in high disease activity (11, 29).

In line with our results, Cosorich et al. (2017) reported that MS patients who had high disease activity, showed higher levels of *Firmicutes* and lower *Prevotella* (*Bacteroidetes*) compared to healthy controls. Interestingly, these changes in bacterial community and disease activity in MS patients were

associated with an increased TH17 cells. TH17 are known for their ability to initiate pro-inflammatory responses and their ability to increase autoimmunity in the brain through the release of IL17 (30, 31). *Firmicutes* also result in slight endotoxemia, which contributes to chronic and systemic inflammation. Also increased level of *Bifidobacterium* coincides with Miyake et al. (2015) and Tremlett et al. (2016) (11, 32).

Prevotella play an important role in the metabolism of phyto- and xeno-estrogens (33, 34). These bacteria can produce metabolites from estrogens that initiate anti-inflammatory responses through maintenance of homeostasis in the intestinal mucosal surface. Therefore, it is possible that the reduction in this species may play a role in the increased inflammation associated with MS (35). Low estrogenic conditions in women such as in menopause and postpartum are specifically associated with increased MS activity (10). Similarly, several reports have shown lower levels of *Prevotella* in other inflammatory conditions such as diabetes mellitus type 1 and autism (36).

Bacteroidetes members have beneficial effects and can modulate immune activity, homeostasis, and neuro-inflammation. They are involved in metabolism of indigestible fibers into short-chain fatty acids (SCFAs), including butyrate which is known to influence immune responses (37). SCFAs promotes T-regulatory cell differentiation and ameliorates CNS inflammation (13). Interestingly, LPS of *Bacteroides* induces lower proinflammatory cytokine levels compared to LPS derived of other Gram-negative bacteria such as that of *Escherichia coli* (38).

Firmicutes and *Bacteroidetes* phylum members in the gut are also involved in generating butyrate and other SCFAs, and many have been to be reduced in MS. *Firmicutes* also has anti-inflammatory effects and its presence is considered a 'sensor and marker of health' (39). (Chen et al. 2016) reported that Certain *Firmicutes* genera (*Blautia* and *Dorea*) were elevated in RR-MS individuals (10). *Firmicutes* family plays a beneficial role in metabolism of bile acids and can produce metabolites with anti-inflammatory activities (14).

Clostridium have been shown to exert anti-inflammatory effects through induction of FoxP3+ T regulatory cells which play a role in suppression of inflammation (40). Treatment of MS patients was associated with restored *Clostridiales* levels (32). Similarly, *Clostridium cluster IV* produces short-chain

fatty acids, which can initiate anti-inflammatory effects (41). Nevertheless, we did not observe any modulation in the levels of *Clostridium* group. Also, contrary to previous reports, we found no changes in levels of *Akkermansia* and *Methanobrevibacter smithii* (4, 12). The observed discrepancies could be due to many factors, including geographical location and life style, as previously mentioned, or differences in patient populations. Microbial changes in MS also included an increased abundance of *Desulfovibrio*. A similar observation has been reported in early pediatric multiple sclerosis (42).

CONCLUSION

Our results suggest that changes in gut microbiota are associated with exacerbation of MS disease. Disruption of the intestinal microbiota may result in the alteration of certain bacteria and their metabolites that may affect the immune balance leading to predisposition to proinflammatory diseases such as MS. The present study will pave the way to the development of better and more effective future therapies such as Probiotics/prebiotic by modulating the composition of gut microbiota restoring the physiological bacterial flora and preventing gut dysbiosis may provide protection from the neurodegenerative disorders like MS. Larger studies that include larger numbers of patients at different stages of the disease together with functional studies are needed to evaluate the role of each microorganism in the modulation of disease severity.

ACKNOWLEDGEMENTS

We acknowledge the Medical Research Center for providing the necessary laboratory equipment for carrying out the experiments. This research was funded by the Grant Office, School of Medicine, Assiut University.

REFERENCES

1. Esmaeil Amini M, Shomali N, Bakhshi A, Rezaei S, Hemmatzadeh M, Hosseinzadeh R, et al. Gut microbiome and multiple sclerosis: new insights and perspec-

- tive. *Int Immunopharmacol* 2020; 88: 107024.
2. Mirza A, Forbes JD, Zhu F, Bernstein CN, Van Domseelaar G, Graham M, et al. The multiple sclerosis gut microbiota: a systematic review. *Mult Scler Relat Disord* 2020; 37: 101427.
 3. Goodin DS. The epidemiology of multiple sclerosis: insights to a causal cascade. *Handb Clin Neurol* 2016; 138: 173-206.
 4. Calvo-Barreiro L, Eixarch H, Montalban X, Espejo C. Combined therapies to treat complex diseases: the role of the gut microbiota in multiple sclerosis. *Autoimmun Rev* 2018; 17: 165-174.
 5. Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med* 2016; 22: 586-597.
 6. Budhram A, Parvathy S, Kremenchutzky M, Silverman M. Breaking down the gut microbiome composition in multiple sclerosis. *Mult Scler* 2017; 23: 628-636.
 7. Ma Q, Xing C, Long W, Wang HY, Liu Q, Wang RF. Impact of microbiota on central nervous system and neurological diseases: the gut-brain axis. *J Neuroinflammation* 2019; 16: 53.
 8. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, et al. Parkinson's disease and parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord* 2017; 32: 739-749.
 9. Pulikkan J, Maji A, Dhakan DB, Saxena R, Mohan B, Anto MM, et al. Gut microbial dysbiosis in Indian children with autism spectrum disorders. *Microb Ecol* 2018; 76: 1102-1114.
 10. Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Paz Soldan MM, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* 2016; 6: 28484.
 11. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, et al. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVA and IV clusters. *PLoS One* 2015; 10(9): e0137429.
 12. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. *Nat Commun* 2016; 7: 12015.
 13. Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity* 2015; 43: 817-829.
 14. Labbe A, Ganopoulosky JG, Martoni CJ, Prakash S, Jones ML. Bacterial bile metabolising gene abundance in Crohn's, ulcerative colitis and type 2 diabetes metagenomes. *PLoS One* 2014; 9(12): e115175.
 15. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018; 17: 162-173.
 16. Leocani L, Rovaris M, Boneschi FM, Medaglini S, Rossi P, Martinelli V, et al. Multimodal evoked potentials to assess the evolution of multiple sclerosis: a longitudinal study. *J Neurol Neurosurg Psychiatry* 2006; 77: 1030-1035.
 17. Pirker A, Stockenhuber A, Remely M, Harrant A, Hippe B, Kamhuber C, et al. Effects of antibiotic therapy on the gastrointestinal microbiota and the influence of *Lactobacillus casei*. *Food Agric Immunol* 2013; 24: 315-330.
 18. Png CW, Linden SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of mucin by other bacteria. *Am J Gastroenterol* 2010; 105: 2420-2428.
 19. Dridi B, Henry M, El Khechine A, Raoult D, Drancourt M. High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. *PLoS One* 2009; 4(9): e7063.
 20. Collado MC, Derrien M, Isolauri E, de Vos WM, Salmiinen S. Intestinal integrity and *Akkermansia muciniphila*, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol* 2007; 73: 7767-7770.
 21. Gałecka M, Szachta P, Bartnicka A, Łykowska-Szuber L, Eder P, Schwiertz A. *Faecalibacterium prausnitzii* and Crohn's disease—is there any connection? *Pol J Microbiol* 2013; 62: 91-95.
 22. Remely M, Dworzak S, Hippe B, Zwielerhner J, Aumüller E, Brath H, et al. Abundance and diversity of microbiota in type 2 diabetes and obesity. *J Diabetes Metab* 2013; 4: 253.
 23. Rose-Meyer RB, Mellick AS, Garnham BG, Harrison GJ, Massa HM, Griffiths LR. The measurement of adenosine and estrogen receptor expression in rat brains following ovariectomy using quantitative PCR analysis. *Brain Res Brain Res Protoc* 2003; 11: 9-18.
 24. Rutledge RG, Côté C. Mathematics of quantitative kinetic PCR and the application of standard curves. *Nucleic Acids Res* 2003; 31: e93.
 25. Ghaderian S, Shomali N, Behraves S, Danbaran GR, Hemmatzadeh M, Aslani S, et al. The emerging role of lncRNAs in multiple sclerosis. *J Neuroimmunol* 2020; 347: 577347.
 26. Waubant E, Lucas R, Mowry E, Graves J, Olsson T, Alfredsson L, et al. Environmental and genetic risk factors for MS: an integrated review. *Ann Clin Transl Neurol* 2019; 6: 1905-1922.
 27. Goldenberg MM. Multiple sclerosis review. *P T* 2012;

- 37: 175-184.
28. Lavasani S, Dzhambazov B, Nouri M, Fak F, Buske S, Molin G, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* 2010; 5(2): e9009.
 29. Cantarel BL, Waubant E, Chehoud C, Kuczynski J, Desantis TZ, Warrington J, et al. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. *J Investig Med* 2015; 63: 729-734.
 30. Cosorich I, Dalla-Costa G, Sorini C, Ferrarese R, Messina MJ, Dolpady J, et al. High frequency of intestinal TH17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. *Sci Adv* 2017; 3(7): e1700492.
 31. Wang Y, Yin Y, Chen X, Zhao Y, Wu Y, Li Y, et al. Induction of intestinal Th17 cells by flagellins from segmented filamentous bacteria. *Front Immunol* 2019; 10: 2750.
 32. Tremlett H, Fadrosch DW, Faruqi AA, Hart J, Roalstad S, Graves J, et al. Gut microbiota composition and relapse risk in pediatric MS: a pilot study. *J Neurol Sci* 2016; 363: 153-157.
 33. Korkina L, Kostyuk V, De Luca C, Pastore S. Plant phenylpropanoids as emerging anti-inflammatory agents. *Mini Rev Med Chem* 2011; 11: 823-835.
 34. Schogor AL, Huws SA, Santos GT, Scollan ND, Hauck BD, Winters AL, et al. Ruminant *Prevotella* spp. may play an important role in the conversion of plant lignans into human health beneficial antioxidants. *PLoS One* 2014; 9(4): e87949.
 35. Moussa L, Bezirard V, Salvador-Cartier C, Bacquie V, Lencina C, Leveque M, et al. A low dose of fermented soy germ alleviates gut barrier injury, hyperalgesia and faecal protease activity in a rat model of inflammatory bowel disease. *PLoS One* 2012; 7(11): e49547.
 36. Leiva-Gea I, Sanchez-Alcoholado L, Martin-Tejedor B, Castellano-Castillo D, Moreno-Indias I, Urda-Cardona A, et al. Gut microbiota differs in composition and functionality between children with Type 1 diabetes and MODY2 and healthy control subjects: a case-control study. *Diabetes Care* 2018; 41: 2385-2395.
 37. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; 54: 2325-2340.
 38. Wisniewski PJ, Dowden RA, Campbell SC. Role of dietary lipids in modulating inflammation through the gut microbiota. *Nutrients* 2019; 11: 117.
 39. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naïve microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; 15: 382-392.
 40. Chu F, Shi M, Lang Y, Shen D, Jin T, Zhu J, et al. Gut microbiota in multiple sclerosis and experimental autoimmune encephalomyelitis: current applications and future perspectives. *Mediators Inflamm* 2018; 2018: 8168717.
 41. Jeraldo P, Hernandez A, Nielsen HB, Chen X, White BA, Goldenfeld N, et al. Capturing one of the human gut microbiome's most wanted: reconstructing the genome of a novel butyrate-producing, clostridial scavenger from metagenomic sequence data. *Front Microbiol* 2016; 7: 783.
 42. Tremlett H, Fadrosch DW, Faruqi AA, Zhu F, Hart J, Roalstad S, et al. Gut microbiota in early pediatric multiple sclerosis: a case-control study. *Eur J Neurol* 2016; 23: 1308-1321.