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Oral Manifestation and Presence of *Candida albicans* in Multiple Sclerosis Disease with Different Treatment Modalities

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ABSTRACT

Aims Multiple sclerosis is a chronic demyelinating disease, which is considered an autoimmune disease impacting the central nervous system. Patients with MS may have certain orofacial manifestations and oral microbiological changes as a result of different treatment modalities. This study aimed to investigate the changes of *Candida albicans* in normal oral flora during different modalities of treatment and compare them with healthy individuals.

Materials & Methods 120 Patients were volunteered and divided into four groups of 30 people: 1) patients with MS taking Natalizumab or Tysabri, 2) patients with MS taking Betaferon, 3) patients who were recently diagnosed with MS and were in a variety of progressive stages, 4) healthy individuals (control). The swap samples were taken from the participants, particularly from the oral cavity. PCR real-time program investigated the presence of Candida albicans. All patients were examined under standardized conditions by a single examiner, and the oral cavity was examined in an artificial light using a mouth mirror.

Findings Regarding the presence of Candida albicans, there was a significant difference between the naive MS patient group and healthy individuals (p=0.036). However, the difference between the Tysabri group and the healthy group was not significant (p>0.05), while in the Betaferon group, the presence of *Candida albicans* significantly increased (p=0.0001).

Conclusion Oral *Candida albicans* increase in naive MS patients compared to healthy individuals. This increase indicates that oral *Candida albicans* may involve in MS development. On the other hand, orofacial manifestations decrease in all MS treatment groups.

Keywords *Candida albicans*; Natalizumab (Tysabri); Betaferon; Oral Manifestations

CITATION LINKS

[1] The immune response in multiple sclerosis [2] Study of ABO system and multiple sclerosis disease in Iraq [3] Comparing the quality of life among patients with relapsing remitting multiple sclerosis in Iraq using different disease modifying therapies [4] Oral and facial manifestations of patients with multiple sclerosis [5] Trigeminal neuralgia secondary to multiple sclerosis: From the clinical picture to the treatment options [6] Oral colonization by candida species in patients with multiple sclerosis [7] Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria [8] Goldenberg multiple sclerosis review [9] Infection risks among patients with multiple sclerosis treated with fingolimod, natalizumab, rituximab, and injectable therapies [10] Multiple sclerosis: Pathogenesis and treatment [11] Interferon- mechanisms of action in multiple sclerosis [12] The possible role of oral microbiome in autoimmunity [13] Bacterial variation in the oral microbiota in multiple sclerosis patients [14] Effectiveness of D,L-2-hydroxyisocaproic acid (HICA) and alpha-mangostin against endodontopathogenic microorganisms in a multispecies bacterial- fungal biofilm in an ex vivo tooth model [15] Oral cavity and candida albicans: Colonisation to the development of infection [16] Isolation and identification Candida spp from urine and antifungal susceptibility test [17] Candidal prevalence in diabetics and its species identification [18] Oral colonization by yeasts in HIV-positive patients in Brazil [19] Oral manifestations, microbial study and salivary iga study in asthmatic patients receiving prednisolone [20] Fungal infection in a patient with multiple sclerosis [21] Association between multiple sclerosis and Candida species: Evidence from a case- control study [22] Fungal toxins and multiple sclerosis: A compelling connection [23] Experimental autoimmune encephalomyelitis development is aggravated by candida albicans infection [24] Multiple sclerosis treatment and infectious issues: Update 2013 [25] Risk of invasive fungal infections among patients treated with disease modifying treatments for multiple sclerosis: A comprehensive review [26] Dysphagia in multiple sclerosis [27] Oropharyngeal dysphagia in multiple sclerosis [28] Central pain in multiple sclerosis- Prevalence and clinical characteristics

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Introduction

Multiple Sclerosis (MS) is a disorder in the autoimmune system and is considered a complex chronic multifactorial disease that harms the spinal cord and the brain. It may affect every part of the body and cause widespread symptoms. Furthermore, the symptoms of MS usually occur in inflammation and demyelination zone, which may cause sensory, motor, and visual disorders and can last for days and weeks [1-3]. Orofacial manifestations include dysphagia (any difficulty in swallowing function), dysarthria (abnormal speech production), and Trigeminal Neuralgia (TN) [4]. Trigeminal neuralgia secondary to multiple sclerosis is similar to idiopathic and classical TN, which is most commonly characterized by frequent unilateral, sudden, brief, stabbing, or electrical shock-like pain with a consistent spread on one or more branches of the trigeminal nerve. The attacks last from less than a second to 2 minutes and are usually triggered by stimulating the mucous or cutaneous trigeminal territories, which are known as trigger zones [5].

The causes of MS are not identified clearly until now. However, it is recognized that MS affects the immune system, which plays a very important role in its progression. These predisposing factors are Epstein–Barr Virus (EBV), genetic, environmental factors, teenage obesity, vitamin D deficiency, and smoking [1]. In histopathological examination, damage was detected in plasma cells, mononuclear phagocytes, dendritic cell infiltration B-lymphocytes, and T-lymphocytes. The T helper 1 (Th1) and Th17 pathways are involved in MS demyelination and pathogenesis [6]. There is no single test that can positively diagnose MS; i.e., diagnostic criteria that combine clinical symptoms, laboratory evidence, and MRI imaging may evolve with time. The diagnosis is confirmed for every case, according to the 2017 MC Donald criteria [7]. The different types of MS are as follows: 1) Relapsing-Remitting MS (RRMS), which is a common type and represents about 85% of MS cases, 2) Secondary Progressive MS (SPMS), and 3) Primary Progressive MS (PPMS), which represents approximately 15% of MS cases [8].

The challenge in treating MS is that patients cannot fully recover. However, treatment can help control disease by minimizing immune-mediated inflammation [9]. Various mechanisms are used in treatment, such as Natalizumab, Fingolimod, Alemtuzumab, Interferon beta-1a, Interferon beta-1b, and glatiramer acetate [10, 11].

The association between the host and the microorganism inside the human body is very important in the pathogenesis of each disease, like multiple sclerosis, Parkinson's, and Alzheimer's [12], as well as diabetes, cardiovascular and respiratory [13]. Additionally, evidence of fungal antigens from different Candida species was found in the cerebrospinal fluid and blood of MS patients. A small number of studies work on the correlation between oral candida and MS. A study in 2020 by da Cunha *et al*. [6] discussed the relationship between oral Candida and MS. They found that the colonization of oral *Candida albicans* is higher in MS patients compared to healthy individuals.

The study aimed to assess the oral *Candida albicans* in naive MS patients and MS patients with different treatment modalities and compare them with the healthy group.

Materials and Methods

This study is a prospective cohort study performed at Baghdad Teaching Hospital. Samples were collected from January 20, 2022, to July 17, 2022. Real-Time PCR (RT-PCR) was used to detect the presence or absence of *Candida albicans*. RT-PCR was used as it is one of the most sensitive and accurate methods. It was sufficiently sensitive to detect as little as 0.001 parasites per reaction. The qPCR experiment was performed using a Sa-Cycler96 instrument (SACACE Company; Italy).

Groups and treatment

120 patients voluntarily participated in the study and were divided into four groups: the first group consisted of 30 patients with multiple sclerosis taking Natalizumab (Tysabri).

The second group consisted of 30 patients with multiple sclerosis taking Betaferon. The third group consisted of 30 patients who were recently diagnosed with MS and were in a variety of progressive stages (naïve MS group), and the fourth group consisted of 30 healthy volunteers (control group). The inclusion criteria in the study were age between 18 and 55 years and diagnosis of multiple sclerosis based on MC Donald 2017 criteria. The exclusion criteria were severe gingivitis and severe periodontitis (good oral hygiene), pregnancy, HIV, receiving chemotherapy and/or radiotherapy, diabetes, asthma, and any other endocrine disease. Figure 1 shows the flow chart of the experiment (the study design)

Samples and DNA extraction

Participants were asked not drink, eat, brush their teeth, or use mouthwash for at least 30 minutes before swab collection. Firstly, the samples were taken from the oral cavity using a sterile swab, which was rotated and rubbed vigorously over the mucosa. The pressure was put on the swabs to pick up deeply seated microorganisms. The swab was taken from the inner surface of the cheeks, the inner surface of the upper and lower lips, the hard palate, and the dorsum of the tongue. The swab was rotated for 15-20 seconds for sample collection. The swab was kept in an Eppendorf tube with 200µL in DNA and RNA shield until DNA extraction. After that, DNA was extracted using a special kit (iNtRON Biotechnology Company; South Korea) and according to the extraction protocol.

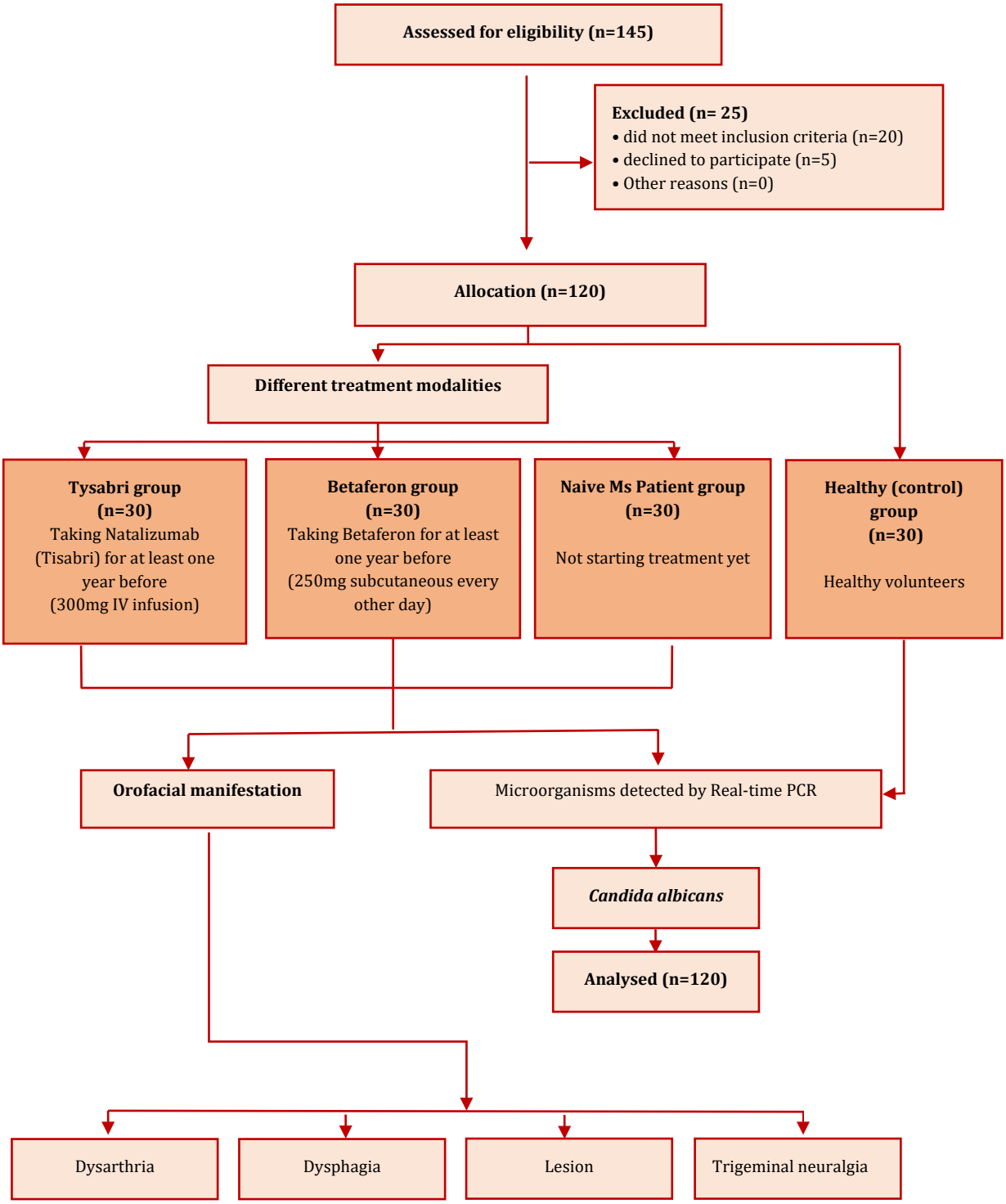

Figure 1) Flow chart of study design

Real-time PCR

The real-time-PCR proceeded as follows: after preparing the SYBR FAST qPCR master mix, the required volume of each component was calculated to form a 20µL final volume (Table 1). The target region within the genome of the bacteria was targeted by specific forward and reverse primers at 55°C. The nucleotide sequences of the primers are shown in Table 2 [14].

Table 1) Each component of the required volume to form a 20µL final volume

Component	20µL (Final volume)
KAPA SYBR FAST qPCR master mix (2X) universal	10Ml
Forward primer	0.5µL
Reverse primer	0.5µL
Nuclease-free water	Up to 4µL
Template DNA sample volume	5µL

Table 2) Forward and reverse primer sequences for *Candida albicans*

Primer	Sequence
Forward	GGGTTTGCTTGAAAGACGGTA
Reverse	TTGAAGATATACGTGGTGGACGTTA

Real-time PCR program

The tubes were sealed and placed in different temperatures, which were programmed in the following steps: The first step was enzyme activation at 95°C for 5 minutes. Then, the denaturation step was performed at 95°C for 30sec.

After that, annealing was performed for 30sec at 55°C for *Candida albicans*. Lastly, the extension was run for 30 and 15sec at 72°C and 90°C, respectively. Figure 2 shows the results curve of *candida albicans*.

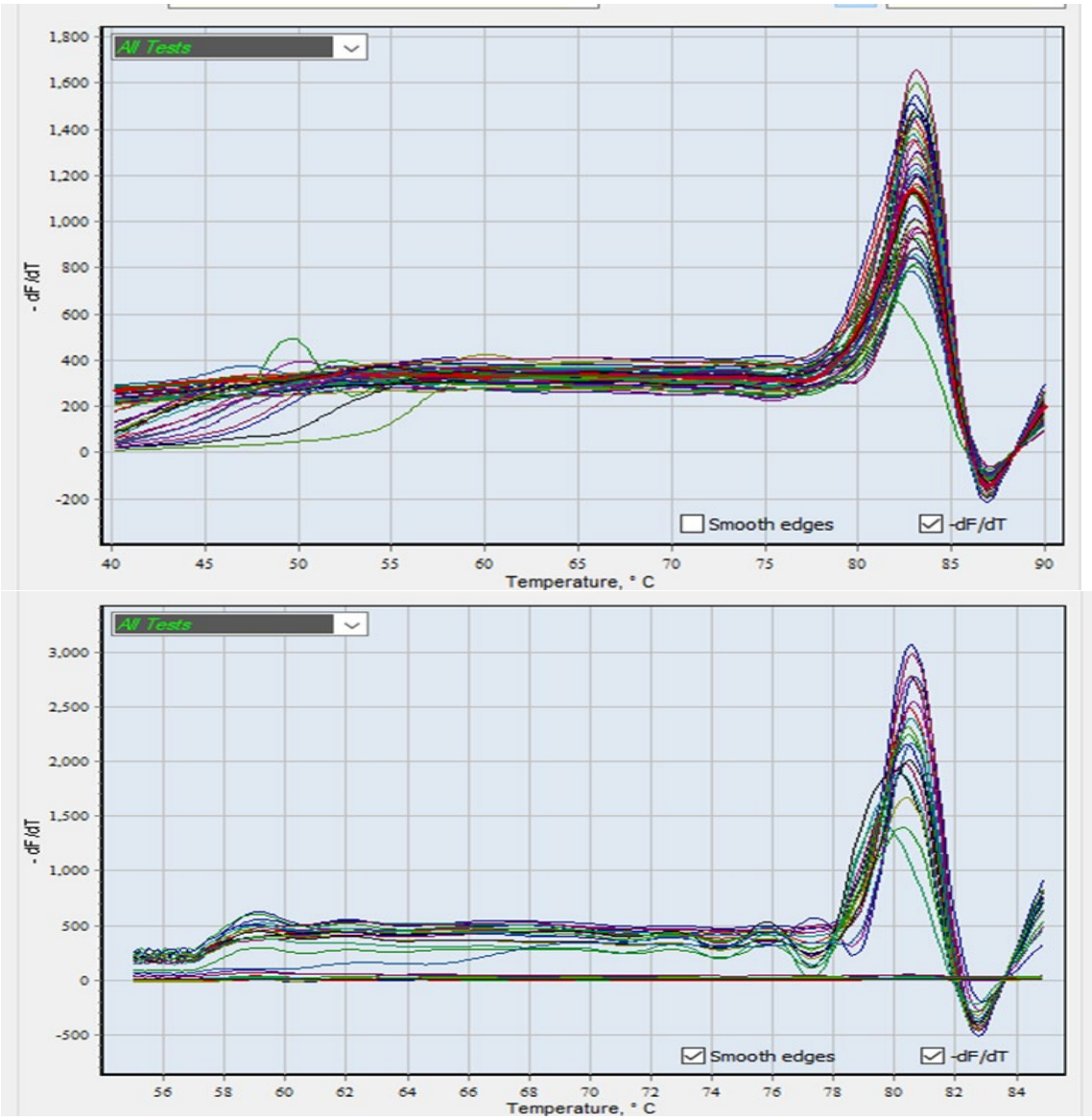

Figure 2) RT-PCR cycling for *Candida albicans* curve

Orofacial manifestation

All patients were examined under standardized conditions by a single examiner, and oral cavity was examined in an artificial light using a mouth mirror. The oral soft tissue examination procedure was performed in sequence according to directions suggested by the World Health Organization (WHO, 2013). The examination procedure started with the lips, the upper and lower sulcus, the retro-molar area, the upper and lower labial mucosa, and the buccal mucosa. Then, the hard and soft palate, the dorsal, margins, and inferior surface of the tongue, and the floor of the mouth were also examined. A questionnaire was completed to evaluate oral facial manifestations in three groups of MS patients (the Tysabri group, the Betaferon group, and naive MS patients). The oral examined manifestations were dysarthria, dysphagia, lesions, and trigeminal neuralgia. The criteria for examining the patients were in the case sheet.

Statistical analysis

Data analysis was done using SPSS 22.0 software. A p-value of less than 0.05 was considered statistically significant and was automatically calculated by the

linear trapezoidal method. The data were analyzed using the Chi-Square test to compare the four groups with the different treatment modalities. Moreover, Fisher's exact test was used to compare both groups. The difference between the mean ages in the four groups was measured by a one-way Analysis of Variance (ANOVA).

Findings

40 of the subjects were male and 80 were female. There was no significant difference among the four groups in demographic characteristics, such as age, age of onset, and gender (p>0.05; Table 3).

There was no significant relationship between gender and Candida status (p=0.597; Table 4).

There was a significant difference in the prevalence of *Candida albicans* among different groups (p=0.0001; Table 5). A significant difference was observed between the Naive MS group (p=0.036) and Betaferon group (p=0.0001) with the Healthy group, as well as between Betaferon group (p=0.001) and Naive MS group (p=0.020) with Tysabri group. However, no significant difference was found between the Betaferon group and the Naive MS group (p=0.056), also between the Tysabri group and the Healthy group (p=0.500; Table 6).

Table 3) Comparison of demographic characteristics in four studied groups (each group = 30 people)

Demographic characteristics	Tysabri group	Betaferon group	Naive MS group	Healthy group	p
Age (years)	31.13±7.14	32.47±7.82	30.90±7.90	30.07±6.20	0.544
Age of onset (years)	25.63±6.64	28.80±7.17	30.10±7.99	-	0.056

Table 4) Candida status in female and male patients

Gender	Candida positive	Candida negative	Total	p
Male	31	9	40	0.597
Female	62	18	80	

Table 5) Comparison of *Candida albicans* prevalence in different groups (each group = 30 people)

Candida status	Tysabri group	Betaferon group	Naive MS group	Healthy group	p
Negative	12 (40.0%)	0 (0%)	4 (13.3%)	11 (36.7%)	0.0001
Positive	18 (60.0%)	30 (100%)	26 (86.7%)	19 (63.3%)	

Orofacial manifestation

Dysphagia was observed in the Tysabri group (6.66%) and Naive MS group (26.6%). Dysarthria was observed in the Tysabri group (20.0%), the Betaferon group (606%), and the Naive MS group (26.0%). Lesions were only seen in the Naive MS group (3.33%), and trigeminal neuralgia was only seen in the Betaferon group (3.33%; Table 7).

Table 6) Pairwise comparison of the studied groups in terms of *Candida albicans* prevalence

Groups	p
Naive MS group & Healthy group	0.036
Betaferon group & Healthy group	0.0001
Betaferon group & Naive MS group	0.056
Tysabri group & Healthy group	0.500
Tysabri group & Naive MS group	0.020
Tysabri group & Betaferon group	0.0001

Table 7) The percentage of orofacial manifestations in different groups (each group = 30 people)

Orofacial manifestation	Tysabri group	Betaferon group	Naive MS group
Dysphagia	6.66	0	26.6
Dysarthria	20.0	6.6	26.0
Lesions	6.0	0	3.33
Trigeminal neuralgia	0	3.33	0

Discussion

The relationship between host and microorganisms plays important role in the regression or progression of several autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease [13]. Although bacteria comprise the majority of microorganisms in the oral cavity, fungi also form a small but important part and cannot be neglected [15]. Candida species are the most common oral cavity-colonizing fungus and are normally prevalent in healthy individuals. This is medically important because they are the most common opportunistic mycosis worldwide [16]. According to da Cunha *et al.* [6], *Candida albicans* is the most frequently isolated species in the oral cavity. It is the most common oral fungal agent, which is highly infective due to its higher pathogenicity level and adhesion characteristics [17].

Candida albicans

The current study found that the prevalence of *Candida albicans* in the naive MS group was significant compared to the healthy group. To the best of our knowledge, this is the first study to investigate a group of MS patients that were not yet receiving any medication (the naive MS group) and compare them with healthy individuals. Therefore, we cannot compare our results with those of another study. Host immunity is considered essential in the colonization of oral Candida. Thus, Candida prevalence in the oral cavity of patients with diabetes mellitus, HIV, asthma, organ transplants, and immunocompromised patients is generally higher compared to healthy patients [1, 15, 17-19].

Moreover, it has been proposed that Candida spp. colonization is linked with the progression of MS due to the presence of fungal antigens and antibodies of different Candida species in the blood and cerebrospinal fluid of MS patients [6, 20]. Furthermore, Benito-Leon *et al.* [21] suggested a positive serologic correlation between *Candida albicans* infection and multiple sclerosis as compared with healthy patients.

In addition, Purzycki & Shain [22] suggested that various fungal toxins can enter the bloodstream and reach the Central Nervous System (CNS) astrocytes [22]. Fraga-Silva *et al*., in a study on Experimental Autoimmune Encephalitis (EAE), suggested that previous infection with *Candida albicans* causes more clinical signs of the disease [23].

The prevalence of *Candida albicans* was highly significant in the Betaferon group compared to the healthy group. When the Betaferon group was compared with the naive MS patient group, the results were nonsignificant (p=0.056). Although the results were statistically nonsignificant, oral *Candida albicans* was slightly increased in this group. The results of the present study are consistent with the results of the previous study by Murua *et al*. [1], which found that oral Candida colonization was higher in MS patients receiving different medications than in healthy individuals (7 out of 10 volunteers receiving Betaferon were positive). Furthermore, it was shown to cause the inhibition of T-cell proliferation and activation [11]. Moreover, the immune system suppression caused by the Betaferon medication may explain why *Candida albicans* increase during use, which is in agreement with another study that suggested that immunomodulation is usually associated with an increased risk of infection [24].

The results were unexpectedly nonsignificant when comparing the presence of *Candida albicans* in the Tysabri and healthy groups. Therefore, a detailed future study on the relationship between Tysabri and *Candida albicans* is required. Interestingly, when the Tysabri group was compared with the naive MS group, the results were significant, and the presence of *Candida albicans* in the Tysabri group was reduced compared to the naive MS group. This result agrees with expert opinions, i.e., generally, the incidence of fungal infections is low among patients treated with monoclonal antibodies [25]. Several clinical trials and observational studies have reported the absence of fungal infections during treatment with Tysabri, indicating the efficacy and safety of Natalizumab [25]. Additionally, Tysabri is a monoclonal antibody that probably binds to specific epitopes. This reduces its side effects due to its specific effect on the immune system [6, 25]. The results were highly significant when comparing the Tysabri and Betaferon groups. These results are in agreement with the findigs of previous studies, suggesting that Tysabri has a lower association with Candida colonization than interferon because Tysabri binds to specific epitopes [6, 25].

Orofacial manifestation

An examination was conducted to assess orofacial manifestations in the three groups of MS patients (the Tysabri group, the Betaferon group, and naive MS patients). The oral manifestations were dysarthria (abnormal speech production), dysphagia (any difficulty in swallowing function), lesions, and trigeminal neuralgia. The prevalence of dysarthria in the naive MS group was 26.0%, in the Betaferon group was 6.6%, and in the Tysabri group was 20.0%. Lassemi *et al*. [4] reported a prevalence of 44.3%, which was similar to a Swedish study. A study in South Africa reported a prevalence of 57%, and a study in the USA reported 23% [4]. These variations may be due to variations in the number and specifications of the samples and the various tools used to examine the patients.

The prevalence of dysphagia was 26.6% in the naive MS group, 0% in the Betaferon group, and 6.6% in the Tysabri group. In previous studies, the results were 21% in the study of Lassemi *et al*. [4], and 29% in the study of De Pauw *et al*., which involved 308 patients [26]. In another study executed by Poorjavad *et al*. [27] in Isfahan, the prevalence of dysphagia in MS patients was approximately 31.7%. However, this variation could be due to the various definitions of dysphagia, the patient criteria selection, and the total number of patients.

Trigeminal neuralgia was 0% in the naive MS group, 3.33% in the Betaferon group, and 0% in the Tysabri group. The prevalence of trigeminal neuralgia in previous studies, such as the study by Osterberg *et al*. in Sweden, was 4.9% [28]. A study of the prevalence of trigeminal neuralgia before the diagnosis of MS was not available. However, in Lassemi *et al*. [4] study, a 5.7 % was recorded. This is the first study to assess lesions and ulceration in the oral cavity. This was chosen because MS patients (naive or receiving medication) suffer from immune system alterations, and one of the signs of immune system suppression is lesions or ulceration in the oral cavity. Lesion prevalence was 3.33% in the naive MS group, 0% in the Betaferon group, and 6% in the Tysabri group. It was found that the naive MS patient group showed more orofacial manifestations than the other two groups. This increase was because the naive MS group received no treatment, and MS treatment helps control the progression of the disease, thus, the oral and facial manifestations.

Conclusion

Oral *Candida albicans* increase in naive MS patients compared to healthy individuals. This increase indicates that oral *Candida albicans* may involve in MS development. On the other hand, orofacial manifestations decrease in all MS treatment groups.

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Ethical Permission: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (Research Ethics Committee) of the College of Dentistry, Baghdad University (protocol code 461722 19/01/2022). Informed consent was obtained from all subjects involved in the study.

Written informed consent has been obtained from the patient(s) to publish this paper.

Conflict of Interests: The authors declare no conflict of interests.

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