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Molecular Detection of Fungal APR1 Gene in Serum of Multiple Sclerosis Patients: A Personalized Medicine Research

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Abstract:
Due to the lack of reliable biomarkers and a thorough understanding of the etiology of multiple sclerosis (MS), the treatment strategy in MS requires a personalized medicine framework that goes beyond the precision medicine idea. A patient-centered approach is necessary for personalized treatment, and the identification of pathophysiological processes should be employed to help classify diseases. Intracellular aspartic proteinase-A enzyme is expressed by the *APR1* gene and is one of the important factors in the development of systemic candidiasis caused by *Candida albicans*. The aim of this study was molecular detection of fungal DNA in serum of MS patients and to evaluate the expression of the *APR1* gene in *C. Albicans* isolates obtained from patients with multiple sclerosis (MS) and controls. The samples were obtained from 100 MS patients with candidiasis and 100 matched controls of healthy individuals during 2018 - 2019. The evaluation of *APR1* gene expression was performed using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. There was a statistically significant difference in *APR1* gene expression of *C. Albicans* strains between MS patients (mean± SD: 0.5008 ± 0.09518) and the control group (mean± SD: 0.7513±0.10505) (P = 0.000). The mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment (P = 0.000). Differences in active fungal infection between patients and controls indicate the importance and possible role of fungi in MS patients. The results suggested that *APR1* gene expression in *C. Albicans* strains isolated from MS patients may be an important factor for invasive *C. Albicans* strains in the progression of MS disease. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system.

INTRODUCTION

To better meet the requirements of each patient, customized medicine often focuses on managing a patient's condition. Informed permission from the patient allows them to participate in the decision-making process, which is crucial to personalized medicine for physicians. According to the European viewpoint, illness taxonomy is influenced by the characterization of pathophysiological processes, with the health of each individual at the center of customized care. Realizing the objectives of customized medicine is made possible by integrating multidisciplinary involvement and research, data processing, infrastructure and resources, and collaborative decision-making (1). Due to the absence of reliable biomarkers and a thorough understanding of MS etiology, personalized medicine, as opposed to precision medicine as discovered in cancer, would be the preferred therapy strategy in

multiple sclerosis (MS). Multiple sclerosis (MS) is one of the inflammatory diseases of the central nervous system (CNS) demyelinating which mainly results in the neurological disability in the young population is increasing (1). The clinical manifestations of MS vary in patients and depend on the location of the injured nerves. The cause of MS is unknown but it is clear that environmental factors and hereditary factors are involved in determining the likelihood of getting MS (2). The broad spectrum of symptoms of MS considerably impacts the quality of life experienced by patients and their families to a greater extent than several other chronic diseases (3). Although etiological factors are not established, some data suggest that non-genetic (environmental) factors, especially infectious agents (e.g. fungal or bacterial infections), may play a role in MS (4). Numerous infectious agents are suspected of triggering MS, and emerging evidence suggest links between established MS and gut microbiota (5). Though most studies focus on bacteria, fungi may also play an important role (6). Many links between fungi and diseases involving idiopathic inflammation have been found recently. Among these, *Candida albicans* stands out as one of the most consistent risk factors (7). Nevertheless, some features of MS epidemiology are not explained and strongly suggest a key role of other infectious agents like *Candida albicans* (8). Antibodies against fungi are a risk factor for psoriasis (9), and fungicides improve symptoms (10). Antibodies against fungal mannoproteins are a risk factor for ankylosing spondylitis (21), systemic lupus erythematosus (11), sarcoidosis (12), and Crohn's disease (13). Few groups have considered the role of fungi in MS. In 1981, Truss reported the resolution of symptoms in five MS cases following antifungal therapy (14). In 2008, Ramos and colleagues reported finding serum antibodies against *Candida* in seven out of eight MS patients, while finding none in 10 healthy controls (17). In 2010, this association was replicated in a larger case-control study (18). Endo proteinases, carboxy peptidases, and amino peptidases are vacuolar proteases that have intracellular proteolytic activities in *Candida albicans*. Intracellular proteinase A is one of the most important enzymes and is produced by the *APR1* gene in *C. Albicans* (19). It is demonstrated that this enzyme has functions in the *Candida* genus, such as nutrition, help with penetration and invasion, infection of host tissues, and the suppression of the immune system. In response to several types of stress, a group of genes is activated in *C. Albicans*, where each type of stress is responded to by a particular set of genes (20). It is suggested that the increase of *APR1* gene expression will impact *C. Albicans* compatibility in the change of environmental conditions. Fungal infections can play a major role in the development of MS, and fungal infections in MS may be due to immune disorders (21). *Candida* species can have an unconstructive effect in the clinical course of MS even though they may not be the main etiology of the disease. *Candida* infection among MS patients has not been adequately studied. However, the possibility that MS is caused by an infective agent has been put forward (22).

MATERIALS AND METHODS

Patients and Controls

One hundred MS patients with fungal infections were included in this study. The samples were obtained from four subtypes of MS disease. Of 56 patients with the relapsing-remitting subtype (RR). A total of 44 patients belonged to the other MS subtypes, including: i) secondary progressive (SP), ii) primary progressive (PP) and iii) progressive-relapsing (PR) subtypes. The control samples and MS patients were similar in age and sex. The expanded disability status scale (EDSS), which ranges from 0 to 10, was defined as measuring impairment in half-point increments between a normal neurologic examination and death from MS. Samples came from Yazd's Shahid Sadoughi Hospital.

Molecular Detection of Fungal DNA in Serum

In this study, 100 cases of patients with MS and 100 samples of healthy individuals were collected. To extract DNA from serum-infected patients and control people, the proposed method was used in kit DNG - PLUS. In this method, we moved a 100 - µL sample of serum to a 1.5 mL tube and added 400 µl Lysis buffer to the sample, and then the sample was shaken for 30 - 20 seconds. After that 350 µl of isopropanol was added to the tube then inverted the tube 10 times for 10 minutes centrifuge at 12000 rpm. Then decanted the Supernatant and 1 mL of wash solution (alcohol 70 %) was added to the sample. After mixing the sample with the wash solution, inverted the tube 10 times and centrifuge at 12000 rpm. After discharge of the supernatant, the sediment in the bottom of the tube dried for 10- 50 min at 65 °C on a heater block device (Nasl Omid Pajoohesh, Iran). After drying the sediment 30-35 µl of Dionysed sterile distilled water was added to the tube and solved the sediment slowly with the finger and vortex. Then set the sample for 5 min at 65 ° C. We stored the extracted DNA for long-term use at -20 ° C. finally, the optimized test was done on all samples of patients and control with positive and negative control, and the results were examined on the electrophoresis gel (1.5 percent gel). The sequence of a dedicated primer pair for 18srRNA is based on the previous literature (22) that is presented in Table 2. PCR Thermo Cycler (Biorad, Germany) was programmed for 35 cycles.

PCR amplification was carried out according to pervious studies (15, 16) in a final volume of 20

Table 1. Clinical Data of MS Patients

Clinical Information	MS Patients
Total individuals with MS	100
Age at onset in years, Mean ±SD	23.4±4.6
Female/male ratio	1/2
Disease duration in years, Mean ±SD	2.5±1.8
EDSS	
≤ 3	82
3.5 - 6	14
> 6	4
different treatment	
used MS & antifungal drugs	39%
did not receive any MS and antifungal drugs	40%
received MS drugs without using any antifungal drugs	21%

Table 2. Sequences of Primers

Primer Name	sequences of primers	Product size PCR
APR1	F: 5'-TCCACCAATCTACAATGCCA-3 R: 5'-ATTTCAGCCAATGAGGATGG-3	300 base Pair
18srRNA	F: 5'-CGGGGAAACTCACCAG-3' R: 5'-AAGGGCATCACAGACC -3'	575 base Pair

µL containing: 10 µL PCR master mix (PCR buffer, MgCl2, dNTP, 0.2 units of Taq polymerase), 1µL reverse primer, 1µL forward primer, 1µL template DNA and 7µL distilled water (Amplicon, Denmark).

Expression of Fungal APR1 Gene in Multiple Sclerosis Patients

A source for gene expression was taken from 31 of the 100 blood samples taken from MS patients that were reported to have fungal infections. Out of 100 main MS patients, 39 instances utilized both MS and antifungal medications at the time of the sample, while 40 cases did not. Without utilizing any antifungal medications, all 21 of the remaining MS patients got MS medications. From hospital blood donors and volunteer students, 100 control samples were collected.

Candida albicans Isolation

The clinical and control isolates of *C. Albicans* obtained from individuals were cultured onto sabouraud dextrose agar (SDA) (Merck, Germany) at 37°C for 24 hours. For isolating the yeast cells, *Candida* colonies were harvested from SDA (Merck, Germany) media and placed into a 1 mL microfuge tube with 0.5 mL distilled water. For equaling the cell number in different samples of *Candida*, the OD of the cells was read at 550 nm. Subsequently, *Candida* cell suspensions were heated at 100°C for 10 minutes and centrifuged at 4500 × g at 4°C for 15 minutes. Finally, the supernatants were used in the reverse transcriptase-polymerase chain reaction (RT-PCR) process.

RT-PCR for APR1 gene

To evaluate the expression of the *APR1* gene, the RT-PCR reaction was performed in three steps: 1) RNA extraction, 2) RT reaction and 3) PCR reaction.

RNA extraction

Total RNA was extracted using RNX plus buffer (Cinagen, Iran). Briefly, about 2×10^6 fungal cells were transferred to 1mL of RNX-plus buffer (Cinagen, Iran) in an RNase-free Microtube, mixed thoroughly, and left at room temperature for 5 minutes. A volume of 200 µL of chloroform (Merck, Germany) was added to the slurry and was mixed gently. The mixture was centrifuged at 13200×g at 4°C for 15 minutes; the supernatant was transferred to a new tube and was precipitated with an equal volume of isopropanol (Merck, Germany) for 15 minutes on ice. The RNA pellet was washed using 75% ethanol (Merck, Germany), briefly dried, and Resuspended in 15 µL of RNase-free water. The purified total RNA was quantified by a Thermo Scientific NanoDrop ONE (Thermo, USA).

RT reaction

A sample of RNA was used for first-strand cDNA synthesis, using 100 pmol oligo-dT (Takapoo zist, Iran), 15 pmol dNTPs (Takapoo zist, Iran), a 20 U RNase inhibitor (Takapoo zist, Iran), and a 200 U M-Mulv reverse transcriptase (Takapoo zist, Iran) in a 0.02 mL final volume. Twenty µL of solution were obtained and maintained at room temperature for 10

minutes. These solutions were incubated at 42°C for 60 minutes in a heat block (Nasl Omid Pajoohesh, Iran) and then at 70°C for 10 minutes.

PCR reaction

Two-pair primers (SinaColon, Iran) including *APR1* as the proteinase-A gene, and 18SrRNA as the housekeeping gene were selected for this study [See table 1] (21). PCR amplification was carried out in a final volume of 20 µL containing: a 10 µL PCR master mix (PCR buffer, MgCl2, dNTP, 0.2 units of Taq polymerase), 1µL reverse primer, 1µL forward primer, 1µL template cDNA and 7µL distilled water (Amplicon, Denmark). The PCR gradient showed that the best annealing temperature was considered 55.5°C for both *APR1* and 18SrRNA genes.

Agarose Gel Electrophoresis

To show the cDNA bands of *APR1* and 18SrRNA genes, the PCR products were run on 1.5% (w/v) agarose gel (Merck, Germany). A 1kb DNA ladder (Bioflux, USA) was used as a marker at this stage.

Statistical analysis

The statistical analysis of the expression of the *APR1* gene in *C. albicans* strains isolated from MS patients and from controls was performed using the independent T-test (SPSS, version 16) method. The differences were considered to be significant at $P < 0.05$. The correlation between *APR1* gene expression with age at onset in years and EDSS was investigated using the Pearson correlation test, and the correlation between the *APR1* gene with MS Patients and sex was detected using the Chi-square (X2) test in this software program.

RESULTS

Prevalence of fungal infections in patients' serum

The data analysis of sex, age, disease duration, EDSS, and different treatments of MS are shown in Table 2. A total of 100 MS patients and 100 healthy controls participated in this study; 68 of the formers were male, and 32 were female. MS was observed in patients ranging from 16 to 40 years (mean age: 23.4 ± 4.6 years). The mean disease duration ranged from 2 to 10 years (mean duration: 2.5±1.8 years). The mean EDSS ranged from 0.5 to 7 (mean value: 2.25 ± 1.5), and in most of the patients, the EDSS was less than 3 (82%). But most patients (40%) did not take any MS and antifungal drugs, indicating an unequal drug distribution system In Iran. Among these patients, 31 cases had serum fungal infection. The PCR gradient showed that the best annealing temperature was considered 55.5°C for 18SrRNA gene. The detection limit of the PCR assay was determined by 40 copy of fungal DNAs using serial dilutions where the number of genomes in each was determined (Fig. 1).

Human DNA, mouse, herpes simplex virus 1, herpes

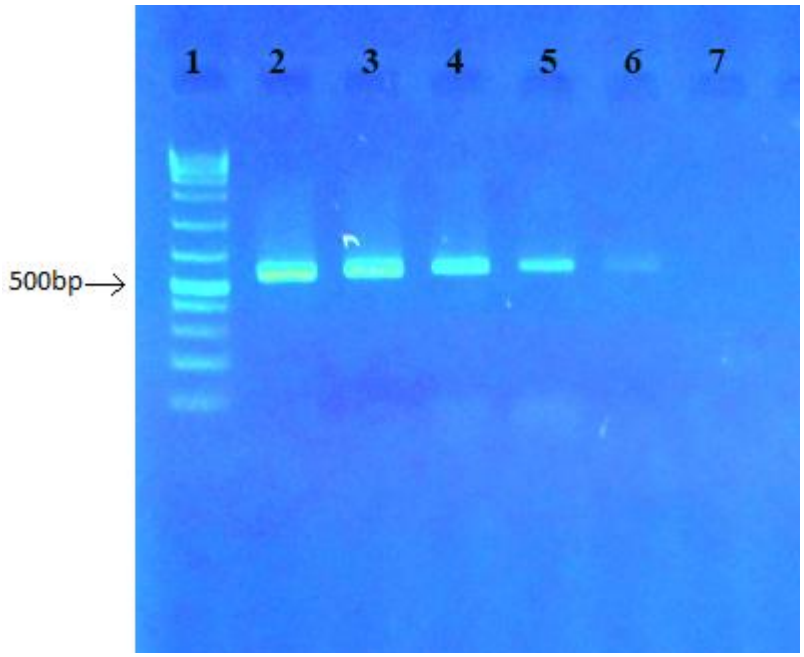

Fig1. Optimization of LOD (Optimized by fungal DNA)
1. Marker size: 100bp DNA Ladder 2. Fungal product size, 575 bp
3. The number of 40000 fungal DNA in a PCR reaction
4. The number of 4000 fungal DNA in a PCR reaction
5. The number of 400 fungal DNA in a PCR reaction
6. The number of 40 fungal DNA in a PCR reaction
7. The number of 4 fungal DNA in a PCR reaction

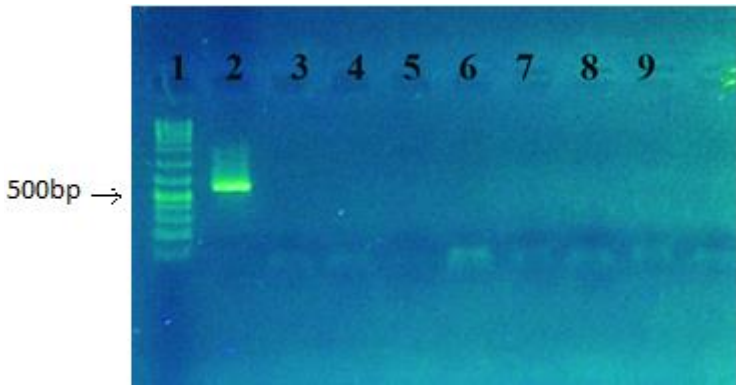

Fig2. Specificity of PCR test for fungal identification.
1. Marker size: 100bp DNA Ladder 2. Positive control sample (Fungal product 575 bp)
3. Mouse DNA 4. Herpes simplex virus1 5. Herpes simplex virus2
6. Hepatitis B Virus DNA 7. Adenovirus DNA 8. Staphylococcus aureus DNA
9. Negative Control

simplex virus 2, hepatitis B virus, adenovirus and Saccharomyces Cerevisiae were used to determine the specificity of the PCR test with positive and negative control samples (Fig. 2).

Fisher test was used to determine the significant relationship between fungal DNA presence in patients and controls. Then, the presence of the 18srRNA gene was analyzed by molecular PCR test and significance level for this difference was determined by Fisher test 0.052 and the results were writing in table 3.

Table 4 shows that 19 individuals had never taken any medicine, 11 had only taken MS medications, and 1 had both MS and antifungal medications. This shows how susceptible MS patients are to fungus infections and how directly antifungal medications affect these patients' therapy.

As shown in Table 5, the correlations between *APR1* gene expression and clinical variables were investigated. There were statistically significant correlations between *APR1* gene expression and age at disease onset as well as EDSS (P = 0.000), whereas no significant correlation was found between the gene expression and MS patients well as sex (P > 0.05). Because of some limitations in doing laboratory examinations, among the clinical features of MS patients, only the EDSS of the MS patients was

Table 3. Fisher's exact test results

Group	Frequency/ percent	Fungal DNA in serum	No fungal DNA in serum	Total
MS Patient	Frequency	31	69	100
	Percent	31%	69%	100%
Control	Frequency	4	96	100
	Percent	4%	96%	100%

Table 4. Distribution of drug use in MS patients with fungal infection in serum

different treatment	Case of patient	
did not receive any drugs	19	61.2%
received MS drugs without using any antifungal drugs	11	435.48%
MS & antifungal drugs	1	3.22%

Table 5. Relationship between APR1 Gene Expression and Age, Sex and EDSS

Clinical Variables	APR1 Gene Expression, Mean± SD	Correlation, P Value
Sex		0.38[2]
Male	0.5227±0.11149	---
Female	0.5200±0.11733	---
Age at onset in years		0.000
EDSS		
≤ 3	0.5223±0.06414	
3.5 - 6	0.4431±0.12960	
> 6	0.1527±0.02903	

Table 6. Mean Ratio of APR1/18SrRNA in MS Patients and Controls

MS patients, No.31	0.5008 ± 0.09518
Controls, No.4	0.7513±0.10505
P value	0.000

investigated after 9 months. Of the treatment of these patients with antifungal drugs and the level of fungal DNA in serum and consequently fungal infection decreased but there was no significant relationship between fungal infection and incidence of MS. But this demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The results showed that the mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment, representing a statistically significant difference between what occurred before and after treatment with antifungal drugs (P=0.000). Our findings demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs.

In this study, *APR1* gene expression was investigated in *C. Albicans* strains isolated from 31 MS patients (Table 6). According to the data, there were no statistically significant differences in the mean ratio of *APR1* to 18SrRNA genes in *C. Albicans* strains isolated from MS patients with candidiasis. The number of people susceptible to fungal infection was significantly higher in the MS group (n=31) than in the control group (n=4), which is a significant difference. That is, people with MS are susceptible to fungal contamination and further destruction, indicating the need for antifungal drugs in these individuals. In contrast, the mean ratio in *C. Albicans* strains isolated from 31 of MS patients (0.5008 ± 0.09518 unit/mg) was significantly less than that of the 4-control group (0.7513± 0.10505 unit/mg) (P = 0.000). The results of *APR1* and 18SrRNA gene expressions showed different expressions among MS patients and controls. As observed, the *APR1* and 18SrRNA in control subjects, it is more expressed than in MS patients. This may be due to immune system involvement in MS patients, the immune system is involved with monocytes, macrophages, and cytokines. High immune activity may reduce the existing fungal infection in healthy individuals.

The comparison of *APR1* gene expression showed that the gene expression increased in *C. Albicans* strains obtained from controls when compared to MS patients. This concept means that fungal contamination cannot cause MS in humans but as a risk factor can lead to the destruction and weakening of MS patients. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system.

DISCUSSION

Varied molecular and epidemiological evidence supports the role of infections in MS. Fungal infection is the most strongly associated infection, though the underlying mechanisms are not firmly established. Several *Candida* species are widely distributed throughout the human population as both commensal organisms and as intermittent pathogens (22). In the clinical arena, blood cultures are the main assay for routine detection of candidiasis (23). However, microbiological confirmation is difficult because blood cultures can be negative in up to 50% of autopsy-proven cases of deep-seated candidiasis or may only become positive late in the infection (23). The aim of this study was molecular detection of fungal DNA in serum of MS patients and to evaluate the expression of the *APR1* gene in *C. Albicans* isolates obtained from patients with multiple sclerosis (MS) and controls. In this study, we investigated the molecular characterization of the fungal DNA in serum patients with MS and healthy individuals in the PCR method. Our idea was to investigate the role of fungal infection in MS disease. So we collected 100 samples of serum with MS and 100 healthy control samples. The results of the Fisher's test showed that 31% of the samples of MS patients had a fungal infection, whereas that of healthy individuals was only 4%. This difference is quite significant and indicates the possible effect of fungal infections on MS. Rasmos et al. In 2008 investigated the presence of anti-*Candida Albicans* antibody and the parasitic, Famata, Glabrata, and Crocus strains in serum of MS patients using Western blot and immunofluorescence and slot-blot analysis. The results of the slot-blot technique indicate the presence of different pathogenic species in patients (except one) as opposed to controls. Following DNA extraction from the full blood of the patients, they studied the Molecular Study of the presence of the fungal DNA using PCR. The fungal DNA was present in the environmental blood of the MS patients against the control. In addition to serum, CSF of patients and controls was evaluated for the presence of specific antigens against different fungal species using the slot blot technique, Results showed antibodies against *Candida* Famata in CSF of patients, unlike controls (24). Another interesting study was conducted by Yoshitomi et al. In 2005. They observed that injecting a fungal-purified beta-glucan antigen into SKG mice genetically susceptible to rheumatoid arthritis caused the disease. Beta-glucan injection into mice causes the activation of the dendritic receptor 1 dendritic TBs and initiates innate immune responses

and eventually rheumatoid arthritis (25).

In addition, Pisa and colleagues reported in 2011, a fungal infection of *Candida* Famata in a patient with MS. The findings of slot blot, PCR, and immunofluorescence all suggest this fungal infection in this patient and the necessity of investigating more types of fungal infections in MS patients to prescribe appropriate medications (26). Other studies have been made about the possible relevance of fungal infections and other nervous system diseases such as Alzheimer›s and Parkinson›s disease. Proteomic analysis of the brain of Alzheimer›s patients indicates the presence of fungal proteins. The presence of the fungal DNA in the brain of patients with Alzheimer›s was investigated using the PCR method and the presence of the fungal DNA in the brain of Alzheimer›s patients was found in comparison with the control. Interestingly, the Aβ peptide has been shown to have antimicrobial activity against Candida albicans. This finding could be a good guide to the physiological function of this peptide. By proving the presence of fungal proteins in the brain of Alzheimer›s patients, it can be said that fungal agents have entered the brain of patients. Of course, the number and type of fungal species vary in different patients. Considering the presence of fungal in the brain, it seems that the peptide is synthesized to perform its antifungal activity. Continuous synthesis of this peptide results in the accumulation of amyloid peptides Therefore, fungal infection induces accumulation of Aβ peptides which in turn accumulates tau protein and neuronal filamentous waste and ultimately neurodegeneration; it also stimulates the secretion of inflammatory cytokines. Some patients with Alzheimer's disease have Cryptococcus infection, however, after the antifungal treatment, their clinical symptoms are greatly reduced. Another interesting point is that there is inflammation and vascular dystrophy in many Alzheimer's patients, which is consistent with the finding that fungal agents cause vascular inflammatory reactions (26). In addition to Alzheimer's disease, the association of fungal infections with Parkinson's disease has also been studied. Patients with Parkinson's also suffer from motor problems, such as problems with facial muscle movements, in addition to motor symptoms. These immobility problems usually occur in patients with salivary dermatitis (Seborrheic dermatitis) and increased sebum secretions, which is due to overactive parenchyma and systemic effects of melanocyte-stimulating hormone (MSH). Treatment of Parkinson's patients with the combination of L-dopa also reduces sebum secretions. According to 2016 research Trusted Source, both MS and PD can affect a person's physical and cognitive functioning. These conditions typically have more severe physical effects than cognitive effects, particularly during the early stages of the diseases. Some of the symptoms of MS and PD are similar (26). The yeast in Malaysia needs certain internal lipids for growth. Yeast Malazia increases androgen secretion by increasing sebum secretion in patients infected with this fungus, triglycerides, and cholesterol are usually high, but the levels of squalene and free fatty acids are significantly lower than in healthy individuals. The free fatty acid is formed from triglycerides through the function of bacterial lipases such as propidium acne. These findings indicate that the imbalance between microbial flora leads to changes in skin lipid composition and provides conditions for the pathogenesis of other infectious agents. The pathogenesis of these factors is mediated by lipase and phosphatase since it initiates inflammatory responses through the release of arachidonic acid from sebum lipids. The general characteristics of all these diseases are similar to MS and similar mechanisms are involved in their occurrence. Fatty acids have direct effects on keratinocytes. The arachidonic acids metabolized in the cyclooxygenase pathway, in turn, are a source of proinflammatory eicosanoids that cause inflammation and damage to the stratum corneum. The globsa is the most pathogenic species of this genus and has more lipase and phosphatase enzymatic activity. This yeast appears to be involved in allergic reactions through other mechanisms as well. Some of these yeast enzymes, such as proteases, glycosidases such as beta-glucuronidase, and leucine arylmidaz, are considered antigens and produce atopic immune responses (27). To the best of our knowledge, there is not enough information about *APR1* gene expression in *C. Albicans* isolated from MS patients, so this study can be considered a novel research study. Previous studies indicated the effect of environmental factors (non-genetic), especially infectious agents, on MS (28). There is a hypothesis about the association between some pathogenic yeasts, such as *C. Albicans C. famata*, *C. parapsilosis, C. glabrata*, and MS (29). In MS patients with candidiasis, demyelinated lesions have been shown in the central nervous system (30). Intracellular proteinase A is one of the most important enzymes in *C. Albicans* that is expressed by the *APR1* gene. This enzyme has an important role in cell survival under stress conditions in *C. Albicans*, and the amount of this enzyme increases in the first few hours of nitrogen starvation when compared to mycelium growth (31). The present study showed that the expression of the *APR1* gene in controls was more than that of MS patients. This difference could be related to both the situation of yeast cells in the human body and the existence of immune disorders. The immune system is involved in infectious diseases, such as candidiasis, and also in MS. In *Candida* infection, monocytes/macrophages synthesize chemokines and cytokines, leading to increased immune responses

(32). There are serious immune or metabolic deficiencies in patients with opportunistic fungal infections and these patients are also under immunosuppressive therapy (33). The present study showed that all MS patients were recently involved in *Candida* infection and some of them did not use any antifungal drugs. Some of these patients were treated with MS drugs and have immune dysfunction. Of the treatment of these patients with antifungal drugs and the level of fungal DNA in serum and consequently fungal infection decreased but there was no significant relationship between fungal infection and incidence of MS. But this demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The results showed that the mean values of EDSS were 1.4074 ± 0.0082 after antifungal treatment and 2.0519 ± 0.1123 before antifungal treatment, representing a statistically significant difference between what occurred before and after treatment with antifungal drugs (P = 0.000). Our findings demonstrated that the progression of MS decreased after the treatment of patients with antifungal drugs. The number of people susceptible to fungal infection was significantly higher in the MS group (n=31) than in the control group (n=4), which is a significant difference. That is, people with MS are susceptible to fungal contamination and further destruction, indicating the need for antifungal drugs in these individuals. In contrast, the mean ratio in *C. Albicans* strains isolated from 31 of MS patients (0.5008 ± 0.09518 unit/mg) was significantly less than that of the 4 control group (0.7513± 0.10505 unit/mg) (P = 0.000). It was detected that there are correlations between *Candida* infection and the increase in MS progression. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system. Regarding the significant role of proteinase-A in the production of new proteins in *C. Albicans*, the *APR1* gene was found to be an important gene in the adaptation of this yeast in different situations. It was also found that there is a correlation between MS patients with fungal infections and the etiology of MS with fungal agents (34). In conclusion, the Fisher test was used to determine the significant relationship between fungal DNA presence in patients and controls showed that the MS patients people had fungal infections in serum more than control people. In addition, the expression of the *APR1* gene in *C. Albicans* was higher in controls than in MS patients using the RT-PCR technique. This study finds that the over-expression of the *APR1* gene can be an important factor in the development of candidiasis caused by *C. Albicans*. In addition, the results here will be useful precursors for achieving the future goal of further research of intracellular proteinases in *Candida* species because of their importance in human pathogenesis. The results of this study propose that *Candida* infection may be associated with the increased progression of MS. Therefore, it is suggested that future studies should make clear how *Candida* species act in the pathogenesis of MS. The comparison of *APR1* gene expression showed that the gene expression increased in *C. Albicans* strains obtained from controls when compared to MS patients. Because fungal infections in the serum causes more activity of the body's immune and defense system and directly affect the activity of the immune system, it further destroys the central nervous system. Given the role of fungal agents in autoimmune diseases such as MS, further studies are suggested. Fungal infection can be investigated using other molecular methods, along with serological and culture methods. It is also recommended that other Target genes be examined as well as the presence of DNA, antigen and fungal antibodies in the cerebrospinal fluid of patients.

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