

Investigation of Mannose-Binding Lectin Level and Deficiency in Patients With Dermatophytosis

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Background: Dermatophytosis is a cutaneous fungal infection with a worldwide occurrence. In dermatophyte infections, the release of keratinocyte cytokines, in the presence of dermatophyte antigens, causes an acute phase response; subsequently, the acute-phase proteins are produced by hepatocytes. Mannose-binding lectin (MBL), an acute-phase protein, also acts as a kind of pattern recognition receptor. MBL deficiency plays a role in susceptible viral, bacterial, fungal and parasitic infections.

Objectives: Some research has been conducted on the role of acute-phase proteins in dermatophyte infections. This study has been designed to determine the serum MBL levels in patients with dermatophytosis.

Materials and Methods: This cross-sectional study, included 96 healthy individuals and 105 patients with dermatophytosis, in access sampling procedure. Microscopic examinations were conducted and cultivated to detect dermatophytes, and in the cases that the identification of different dermatophyte species was necessary, complementary examinations were conducted. Additionally, the enzyme-linked immunosorbent assay (ELISA) was used to determine the serum MBL levels of healthy individuals and patients. Various tests (Chi-square, Fisher exact, Mann - Whitney, Kruskal Wallis, Kendal tau correlation coefficient and ROC curve analysis) were used to examine the relationships between variables, when the $P < 0.05$ were considered as significant level.

Results: The mean serum MBL level of healthy individuals and patients, was $1.53 \pm 1.87 \mu\text{g/mL}$ and $1.97 \pm 2.03 \mu\text{g/mL}$ ($P = 0.039$), respectively. Using ROC curve analysis, the MBL level was established as a significant predictor of dermatophytosis ($P = 0.042$). MBL deficiency (serum level $< 1 \mu\text{g/mL}$) was more common in healthy group (56.2%) than the patients with dermatophytosis (41.0%).

Conclusions: The findings showed that the increased concentrations of serum MBL in patients with dermatophytosis play a role in this fungal infection. The high frequency of MBL deficiency in healthy individuals was compared with patients indicated that MBL deficiency is not a predisposing factor of this type of infection.

Keywords: Mannose-binding lectin; Dermatophytosis; Mannose-binding lectin Deficiency

1. Background

Dermatophytosis is a common fungal skin infection found worldwide. The hair, skin and nails are infected by a homogenous group of keratinophilic fungi called dermatophytes (1, 2). Dermatophytes are classified as *Trichophyton*, *Microsporum*, or *Epidermophyton* (2, 3) based on their cultural anamorphic stages (sexual or imperfect). They are further divided into 3 groups, antropophilic, zoophilic, or geophilic, according to their ecology (1, 2). A quantitative analysis of a purified cell wall of a dermatophyte showed that the cell wall is basically composed of glucose containing polysaccharide and N-acetyl glucosamine polymers, which are probably mannan, chitin and protein. A small amount of galactosamine and lipid

exist in the cell wall (4-7).

Dermatophytes are colonized on the keratinized tissue of stratum corneum (2, 8). Immune responses against dermatophytic factors begin with the reaction of the stratum corneum against the fungus (8). Keratinocytes detect pathogens by using different recognition receptors, such as mannose receptors, which produce cytokines and chemokines (9). In response to zoophilic dermatophyte infections, keratinocytes can produce various kinds of cytokines, such as proinflammatory cytokines (Interleukin 1β (IL- 1β), IL-6, IL-6sR, IL-17), chemokines (eotaxin and eotaxin-2, IL-8, MCP-1) and immunomodulatory cytokines (IL-7, IL-10, IL-15, IL-16), while infections with antropophilic dermatophytes induce a limited number of cytokines (eotaxin-2, IL-8, IL-16) (9).

Implication for health policy/practice/research/medical education:

Mannose-Binding Lectin (MBL) deficiency appears to play a role in recurrent skin infections and skin inflammatory diseases, indicating that its mechanisms are related to the innate immunity pathway and the removal of apoptotic and immune complexes. To clarify this relationship, we conducted clear and comprehensive studies on MBL serum levels of patients with dermatophytosis compared with a control group.

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Cytokines that are produced during the inflammatory process, stimulate the production of acute phase proteins, including tumor necrosis factor α (TNF α), IL-1 β , IL-6, interferon- γ (INF γ), transforming growth factor β (TGF- β) and likely IL-8 (10). Based on some previous researches, IL-8 released from keratinocytes in presence of dermatophytic antigens (thrichophytin) that induce the acute phase responses during dermatophytic infection (8). MBL is a subfamily of proteins known as collectins (calcium-dependent collagenous lectins); the members of this family containing collagenous regions and lectin domains (11-15). Collectin gene is located on chromosome 10 (16). There are two MBL genes: MBL-1, which is a pseudogene, and MBL-2, which is encoding the MBL-2 protein (16). MBL synthesizes in the liver and acts as an acute phase protein (17-18). It also acts as a kind of pattern recognition receptor (17).

About 5% - 7% of the world population has been affected with MBL deficiency (12, 18, 19) but a 30% frequency of this deficiency has been reported in Caucasian communities (18). Three-point mutations on the gene of MBL-2 in codons 52, 54, 57 of the first exon of the MBL gene have been shown with D, B and C, these mutations cause amino acid substitutions and also impair the MBL function (17, 18, 20, 21). In addition to the structural gene mutation, a number of polymorphisms in the promoter region of the MBL gene have been described. These are locuses of H/L, X/Y and P/Q (in 5' untranslated region) and are located in positions -550, -221 and +4 of the MBL gene (12, 22-24). Seven haplotypes are reported for MBL-2, four of which are associated with lower MBL serum concentrations including HYPD, LXPA, LYPB and LYQC (18, 25). The other haplotypes, which produce normal levels of MBL, including HYPA, LYPA and LYQA (18). The MBL concentration is characterized by haplotypes inherited from parents (20, 26). MBL deficiency plays a role in susceptible viral, bacterial, fungal and parasitic infections (13, 22, 26-28). Recently, many researchers have been investigated the MBL deficiency by studying individuals with recurrent infections and autoimmune disorders, but so far, comprehensive studies have not been conducted on skin diseases (21).

2. Objectives

MBL deficiency appears to play a role in recurrent skin infections and skin inflammatory diseases, indicating that its mechanisms are related to the innate immunity pathway and the removal of apoptotic and immune complexes (18). To clarify this relationship, we conducted clear and comprehensive studies on MBL serum levels of patients with dermatophytosis compared with a control group.

3. Materials and Methods

This was a cross-sectional study using availability and non-probability sampling methods. The mycology labo-

ratory of Razi Hospital, Tehran, Iran was selected as the sampling location (2010 - 2011) after microscopic tests (hydroxide potassium 20% (KOH 20%)) the infected people who had experienced symptoms of dermatophytosis were confirmed. With their consent, the sampling was repeated. Thus, 105 patients with dermatophytosis were selected. All of the skin, hair and nail samples were kept in sterile plates and also 5 mL venous blood samples in sterile testing tubes without preservative and transferred to the mycology laboratory of the medical faculty of Tehran University and underwent the following procedures: cultivation on mycobiotic agar (QUELAB, Canada), Sabouraud dextrose agar (QUELAB, Canada), teased mount preparation, slide culture, complementary examinations (cultivation on sterile rice grain media, creation of pigment on corn meal agar with 1% dextrose (BIOMARK, India), urea hydrolysis (QUELAB, Canada), nutritional testing (Trichophyton agar 2,3,4) (BIOMARK, India) and in vitro hair perforation test), serum isolation (serum samples were maintained in two separate microtubes at 20°C) and an ELISA test (Sanquin, The Netherlands).

The sera of ninety six healthy individuals (control group), who had no dermatophyte infection or other kind of infections that may increase the MBL serum level, were collected, then the samples were assessed using ELISA test. Data analysis was performed using SPSS 16.0 software. Chi-square, Fisher exact, Mann - Whitney, Kruskal Wallis, Kendal tau correlation coefficient and ROC curve analysis were used to identify the relationship between variables and the $P < 0.05$ were considered as significant.

4. Results

The results showed that out of 105 patients with dermatophytosis (positive microscopic test), 96 had positive cultures (91.4%), 5 had negative cultures (4.8%) and 4 had infected cultures (3.8%). The most common isolated dermatophyte in this study was *Trichophyton verrucosum*, detected in 31 cases (32.3%); other common types were *Epidermophyton floccosum* 29 cases (30.2%), *T. mentagrophytes* in 15 cases (15.6%), *T. Rubrum* in 8 cases (8.3%), *T. tonsurans* and *Microsporum canis*, each in 6 cases (6.2%) and *T. violaceum* in only one case (1.0%). In this study, the tinea infection were more frequent among men (65.7%) than women (34.3%), 20% of patients had an underlying disease (cardiovascular disease, diabetes, psoriasis and thalassemia) and 80% had no underlying disease.

The mean MBL serum level of 96 healthy individuals was 1.53 ± 1.87 $\mu\text{g/mL}$, while the mean MBL serum level of 105 patients with dermatophytosis was 1.97 ± 2.03 $\mu\text{g/mL}$. There were significant differences between the mean MBL serum level of healthy and patient groups ($P = 0.039$), using the Mann-Whitney test. There were not any specific differences between MBL serum levels of both genders, using chi-square tests ($P = 0.621$), significant differences were not observed, using Mann-Whitney tests, between the MBL serum levels of patients with dermatophytosis

at different age groups ($P = 0.697$). Chi-square test were used to investigate the MBL serum level in relation to the dermatophytosis species, no specific differences were observed ($P = 0.662$). By using ROC curve analysis, the MBL serum level became a meaningful indicator of dermatophytosis ($P = 0.042$).

In the present study, in order to determine the MBL deficiency, the cut-off points, 1 $\mu\text{g}/\text{mL}$, 0.5 $\mu\text{g}/\text{mL}$ and 0.3 $\mu\text{g}/\text{mL}$, were measured. Using the Fisher exact test, significant differences were observed when the concentration was $< 1 \mu\text{g}/\text{mL}$ ($P = 0.03$) (Table 1). With a cut-off point of $< 1 \mu\text{g}/\text{mL}$, 56.2% of healthy individuals and 41.0% of patients had MBL deficiency. No significant differences were observed when the Fisher exact test in relation with either genders or underlying diseases were used ($P = 0.756$). In order to examine MBL deficiency associated with the age of patients with dermatophyte infections the Mann-Whitney test was used and no significant differences were observed ($P = 0.579$). Finally, using the Chi-square test, there were no significant differences among various dermatophyte species ($P = 0.683$).

Table 1. Determining MBL Deficiency Serum Level in Healthy Participants and Patients

MBL Deficiency, $\mu\text{g}/\text{mL}$ ^a	Healthy, No. (%)	Patient, No. (%)	P Value
1			
MBL < 1	54 (56.2)	43 (41)	0.03
MBL ≥ 1	42 (43.8)	62 (59)	0.03
0.5			
MBL < 0.5	35 (36.5)	28 (26.7)	0.135
MBL ≥ 0.5	77 (73.3)	61 (63.5)	0.135
0.3			
MBL < 0.3	34 (35.4)	24 (22.9)	0.05
MBL ≥ 0.3	62 (64.6)	81 (77.1)	0.05

^a Examining the MBL deficiency level between healthy participants and patients by the use of Fisher exact test and serum level cut-offs of 1 $\mu\text{g}/\text{mL}$, 0.5 $\mu\text{g}/\text{mL}$ and 0.3 $\mu\text{g}/\text{mL}$ revealed significant differences in serum concentrations $< 1 \mu\text{g}/\text{mL}$ ($P = 0.03$). Individuals with a serum level of $< 1 \mu\text{g}/\text{mL}$ are considered to have MBL deficiency.

5. Discussion

MBL is a key component of the innate humoral immune system and also a component of the complement system (16, 25). MBL enables the phagocytic cells to detect polysaccharides (N-acetyl-D-glucosamine, mannose, N-acetyl mannosamine, fructose and glucose), which are presented on microbial surfaces (bacteria, fungi, viruses and parasites) with a combination of sugar and on certain parts of host cells. These cause opsonophagocytosis either directly or by activating the lectin pathway of the complement system (17, 20, 25, 29). There are a number of microorganisms that appear to be appropriate sub-

strates for binding MBL, including the lectin pathway involved in complementary activation, such as a fungal cell wall, that is relatively rich in mannan (14). Although the direct binding of MBL to dermatophytic species is not proven, it is suggested by the N-acetyl glucosamine and mannan in the cell wall of dermatophytes (6, 7).

In this study, 96 healthy individuals (individuals without any infections, including dermatophyte and other types of infections) and 105 patients with dermatophytosis were evaluated using ELISA in order to study the clinical value of the MBL serum level. Considering the mean MBL serum level in the control group, individuals with a serum level of $> 1.53 \mu\text{g}/\text{mL}$ were considered as patients; we observed an increased MBL serum levels in 49 patients with dermatophytosis. There were significant differences between the mean MBL serum levels of the patients and healthy individuals ($P = 0.039$). The increased MBL serum level in patients suggested that MBL binding to dermatophytes and the role of MBL as an acute-phase protein against dermatophytes. To prove that MBL binds to dermatophyte species would require further research.

There was no difference in MBL serum levels with regards to age, gender or underlying diseases in 105 patients with dermatophytosis, which was in accordance with the results of Lambourne (30). There was also no difference in the MBL serum level of patients infected with different dermatophyte species ($P = 0.662$). Keratinocytes can produce a range of cytokines, such as proinflammatory cytokines, chemokines, etc. in response to zoophilic dermatophyte infections. When infections with anthropophilic species induce a limited number of cytokines (9), we would expect more significant increases in the MBL serum concentrations of infected patients with zoophilic species rather than infected patients with anthropophilic species. However, inequalities in the numbers of these two species may affect the findings of this study. Using ROC curve analysis, the MBL serum level was determined to be a meaningful indicator of dermatophytosis ($P = 0.042$).

MBL deficiencies are common in human populations. Recently, many researchers have been studied the MBL deficiencies, which enhance the immunity against bacterial, fungal, parasitic and viral infections (13). There is no defined cut-off point for MBL deficiency (30). In several studies, different cut-off points for deficiency have been considered, but in the present study, we examined the cut-off points of 1 $\mu\text{g}/\text{mL}$, 0.5 $\mu\text{g}/\text{mL}$ and 0.3 $\mu\text{g}/\text{mL}$ in order to determine the MBL serum deficiency level in patients and healthy individuals. Using the Fisher exact test, a meaningful difference in $< 1 \mu\text{g}/\text{mL}$ was observed ($P = 0.03$) (Table 1). Considering a cut-off point of $< 1 \mu\text{g}/\text{mL}$, 56.2% of healthy individuals and 41.0% of patients had MBL deficiencies. The significantly higher percentage of MBL deficiency in the healthy group suggested an inverse relationship between MBL deficiency and dermatophytosis. Further and more detailed studies are needed to confirm this relationship.

The findings of this study showed that the patients with dermatophytosis have higher MBL serum levels compared with healthy individuals. Also, the acute phase protein plays a role in dermatophyte infections. It is well known that mutations in MBL genes are associated with reduced serum levels, which is a risk factor of infection. In this study, the high frequency of MBL deficiency in the healthy participants compared with the patients indicates an inverse relationship between MBL deficiency and dermatophytosis.

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Authors' Contribution

None declared.

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