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Higher Serum Ceruloplasmin Levels May Indicate the Role of Oxidative Stress in Onychomycosis

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ABSTRACT

Background: Onychomycosis is the most common nail infection in the world. The most common causative agents are dermatophytes; however, the disease may appear due to non-dermatophytic molds and yeasts. Ceruloplasmin also takes part in defense mechanisms against oxidative stress. Ceruloplasmin is considered to be associated with approximately 80% of oxidative events in the plasma. The aim of the present study was to evaluate ceruloplasmin level in onychomycosis and to review the association with the parameters of the disease.

Materials and Methods: This is a prospective cross-sectional study and included 112 healthy volunteers and 102 patients followed up with the diagnosis of onychomycosis in the dermatology clinic of Ankara City Hospital between October 2021 and March 2022.

Results: The patients and control group were similar in terms of age and gender. The mean serum ceruloplasmin level in patients (866.6 U/L) was significantly higher than the control group (800.6 U/L) ($p=0.025$). A positive correlation was detected between the duration of the disease and ceruloplasmin levels. Serum ceruloplasmin levels were higher in patients with longer disease duration ($p=0.002$, $r=0.31$). The disease severity of the patients enrolled in the study was scored according to the onychomycosis severity index. Similarly, ceruloplasmin levels were correlated with disease severity ($p<0.001$, $r=0.43$).

Conclusion: Consequently, we believe that higher ceruloplasmin levels in the patients may indicate oxidative stress on tinea unguium. Ceruloplasmin may be a potential marker of disease severity and activity in onychomycosis.

Keywords: Onychomycosis, Ceruloplasmin, Oxidative stress

Introduction

Onychomycosis is the most frequent nail infection in the world and causes thickening and staining of the influenced nail plate. Although it may be detected in any age, the prevalence increases along with aging [1]. Organisms that cause onychomycosis can be categorized as dermatophytes, non-dermatophyte molds and yeasts [2]. Dermatophytes are accepted as the prevaling infectious organisms in onychomycosis; however, non-dermatophyte molds are also reported with increased incidence, especially in hot climates. The uncomplicated dermatophyte infection of the nail is called tinea unguium. The majority (60% to 70%) of dermatophytic nail infections are caused by Epidermophyton floccosum, Trichophyton rubrum and Trichophyton mentagrophytes [1]. The factors that predispose onychomycosis involve advanced age, diabetes mellitus, HIV infection, Down syndrome, psoriasis, peripheral vascular disorders, and traumatic nail disorders [2]. Oxidative stress is an injury in which redox imbalance occurs as a result of

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an increase in destructive free radicals, a decrease in antioxidants and antioxidant defense pathways [3]. Different biomarkers have been detected in oxidative stress to date. Ceruloplasmin is a serum ferroxidase that contains more than 95% of the copper found in plasma. Ceruloplasmin belongs to the group of positive acute phase proteins and acts in defense mechanisms against oxidative stress. The ability of ceruloplasmin to trap transition metal ions appears with preventing the formation of superoxide anion. Ceruloplasmin performs many catalytic functions similar to glutathione peroxidase activity and acts as an oxidase against a wide range of organic substrates. Today, ceruloplasmin is considered to be associated with approximately 80% of oxidative events in the plasma [4].

In our study we aimed to evaluate ceruloplasmin level in onychomycosis and appraise it as an oxidative stress marker in onychomycosis and to review the association with the parameters of the disease.

Materials and Methods

Study design and patients

This is a prospective cross-sectional study and included patients followed up with the diagnosis of onychomycosis in the dermatology clinic of Ankara City Hospital between October 2021 and March 2022, and healthy subjects without any concomitant disease. The diagnosis was confirmed by direct microscopic examination for all patients through a 20% potassium hydroxide preparation. The patients with concomitant systemic or cutaneous diseases, those who received any systemic treatment (e.g., anti-seizure drugs, oral contraceptives) in the last 1 month, and smokers were excluded from the study. The age, gender, disease duration, location and onychomycosis severity scores of the patients were recorded.

Our study was carried out in conformity with the Declaration of Helsinki and approval of the Ethical Committee of Ankara City Hospital was obtained (number: 2027, date: 06.10.2021). Written informed consents were obtained from the patients and volunteers.

Sampling and Measurements

Venous blood samples collected from the patient and control groups after at least 8 hours of fasting were centrifuged at 1,500 rpm for 10 minutes, and the separated serum was stored in a deep freezer at -80 degrees Celsius.

Ceruloplasmin ferroxidase activity levels were measured by the method described by Neselioglu et al. [5]. This is an automated and colorimetric method lying on the enzymatic oxidation of ferrous iron ions (Fe^{2+}) to ferric ions (Fe^{3+}).

Statistical Analysis

The SPSS 23.0 package program was used for data analysis. Categorical variables were shown as numbers and percentages, and numerical variables were expressed as mean (standard deviation) or median [(IQR) interquartile range]. The Kolmogorow-Smirnow test was used to assess whether numeric variables comply with normal distribution. Numerical variables with normal distribution were compared with Student's t-test, and numerical variables without normal distribution were compared with Mann-Whitney U test. The chi-square test was used to compare categoric variables. The correlation between numeric variables was examined through Pearson's or Spearman Analysis. Any p value <0.05 was accepted as statistically significant.

Results

The study included 102 patients followed-up due to onychomycosis and 112 healthy volunteers.

The patients and control group participants were similar in terms of age (p=0.45) and gender (p=0.18) (Table 1).

The onychomycosis subtype detected in all cases was distal lateral subungual onychomycosis. The location of the onychomycosis was foot in 95.1% of the patients whereas 4.9% of the patients had both hand and feet involvement.

Comparison of serum ceruloplasmin levels of the patient and control groups: The mean serum ceruloplasmin level was 866.6±231.9 U/L in the patient group, and 800.6±189.4 U/L in the control group. Ceruloplasmin level was considerably higher in the patient group than in the control group (p=0.025) (Figure 1).

The association between serum ceruloplasmin levels and demographic and clinical characteristics of patients: Serum ceruloplasmin levels were significantly higher in females. (930.1 vs 758.1, p<0.001) There was not any correlation detected between the age and ceruloplasmin levels (p=0.088, r=0.12). A positive correlation was detected between the duration of the disease and ceruloplasmin levels. The median duration of the disease was calculated as 18 (IQR: 9-36) months. Serum ceruloplasmin levels were higher in patients with longer illness duration (p=0.002, r=0.31) (Figure 2).

The disease severity of the patients enrolled in the study was scored according to onychomycosis severity index (OSI) created by Carney et al. [6] The OSI score is available by multiplying area of

Table 1. The distribution of demographic data

	Patient N (%)	Control N (%)	p-value
Gender			0.18
Female	39 (38.2)	53 (47.3)	
Male	63 (61.8)	59 (52.7)	
Age (years), mean (SD)	48.6 ±14.6	47.3 ±12.8	0.45

SD: Standard deviation

involvement (range 1-5) by the score for the closeness of disease to the matrix (range 1-5). Ten points are affixed for the presence of a longitudinal streak or dermatophytoma or for greater than 2 mm of subungual hyperkeratosis. Mild onychomycosis accounts for a score of 1-5, moderate 6-15, severe 16-35. The OSI scores of the patients in this study were between 3 and 33. Median OSI was calculated at 13 (IQR: 7-21). Similarly, ceruloplasmin levels were correlated with disease severity ($p<0.001$, $r=0.43$) (Figure 3). The mean ceruloplasmin levels in patients with onychomycosis on both hands and feet were 1254.8±320.3 U/L and 846.6±209.9 U/L in patients with onychomycosis on the feet alone. Such difference between the groups was statistically significant ($p<0.001$) (Figure 4).

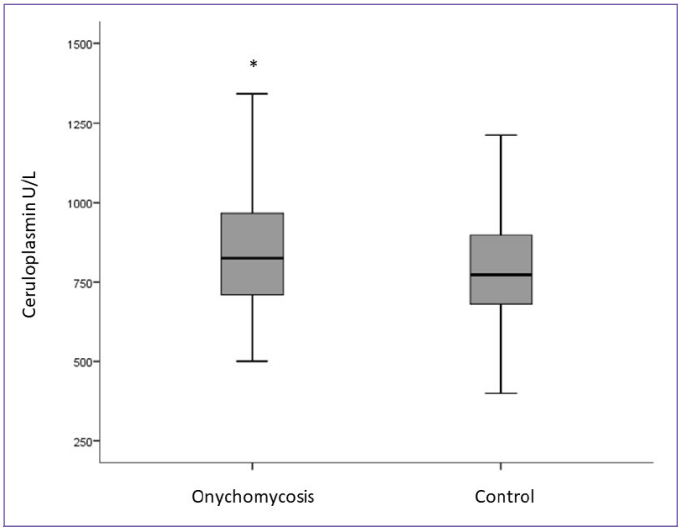

Figure 1. Ceruloplasmin levels in the patient and control groups

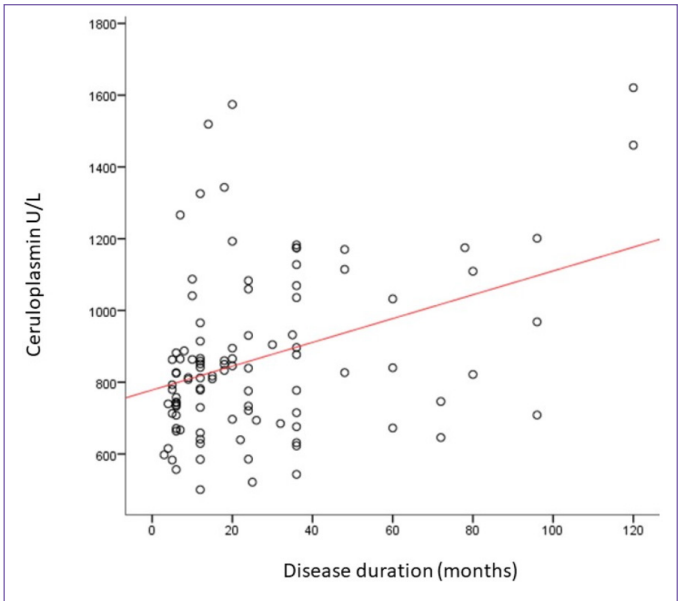

Figure 2. Ceruloplasmin levels and disease duration of the patients

Discussion

Onychomycosis consists of 50% of all nail diseases. Although the prevalence varies according to regions, Gupta et al. [7] analyzed the studies conducted in 2016 and detected the global prevalence of onychomycosis as 5.5%. Onychomycosis may appear at any age; however, the incidence increases with age. Although dermatophytes are the most common microorganisms in onychomycosis, non-dermatophytic molds such as Scopulariopsis brevicaulis, Acremonium spp., Aspergillus spp. may also be detected. Mixed dermatophytic-non dermatophytic infection may also be detected. The presence of non-dermatophytic molds in onychomycosis has often been associated with treatment failure and recurrence due to missed diagnosis. Onychomycosis caused by yeast is most

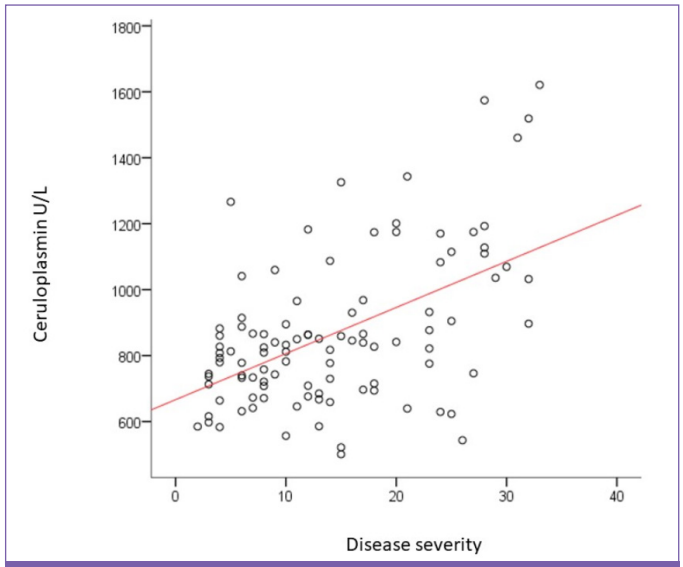

Figure 3. Ceruloplasmin levels and disease severity of the patients

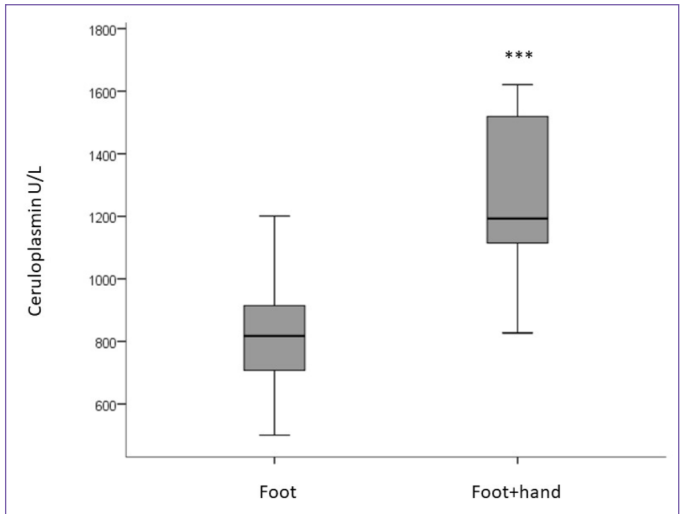

Figure 4. Ceruloplasmin levels and localization of the disease

commonly caused by Candida spp. on fingernails of people who are intensely in contact with water [1]. The presence of oxidative stress has previously been studied in many dermatological diseases such as telogen effluvium [8], seborrheic dermatitis [9], vitiligo [10], skin cancer [11], lichen planus [12], atopic dermatitis [12], and pemphigus vulgaris [13]. Different biochemical markers have been reviewed in aforesaid studies [8,9,10,11,12,13]. We used ceruloplasmin in order to demonstrate the role of oxidative stress in tinea unguium. Half lives of free radicals are as short as several minutes; therefore, in vivo quantity measurement is very difficult. Various markers have been used to assess oxidative stress, however none of them are considered as ideal biomolecules [14].

Since ceruloplasmin belongs to a positive acute phase protein group, plasma concentration increases by 50% following exposure to an injury. Such response developed after a stimulus that initiates the acute phase reaction appears within 24 to 28 hours. Ceruloplasmin is also involved in defense mechanisms against oxidative stress. Ceruloplasmin prevents the formation of superoxide anion due to the ability to bind transition metal ions and acts as a protective antioxidant in free radical reactions. It catalyzes the oxidation reaction of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}). Ceruloplasmin is the principal copper oxidase in the plasma. It mediates the transport of most of the serum copper. It stabilizes the activity of superoxide dismutase enzyme which is the key enzyme of the antioxidant barrier of the body [4,15].

The only study on the role of oxidative stress in tinea unguium is the study conducted by Metin et al. [16]. The thiol/disulfide homeostasis was investigated in 52 patients with onychomycosis and 50 healthy individuals in the aforesaid study. It was observed that the thiol/disulfide balance in the patient group shifted in favor of disulfide indicating the oxidative stress. It was suggested in this study that oxidative stress may have a role in the pathogenesis of onychomycosis [16]. Novikova and Zlotnikova [17] investigated ceruloplasmin levels in patients with chronic recurrent and severe form of herpes infection. They found that ceruloplasmin levels were higher during disease exacerbation and remission periods when confronted with the control group. The ceruloplasmin level in the remission period was found higher than the level in the exacerbation period in that study, and this was attributed to the endogenous antioxidant effect of ceruloplasmin [17]. Kocyigit et al. [18] examined serum trace elements and their associated enzyme values in cutaneous leishmaniasis and found that ceruloplasmin level were more elevated in the patient group than in the control group. Cwynar et al. [4] measured malondialdehyde and ceruloplasmin levels in the blood in order to evaluate the oxidative stress in patients with alopecia areata and found that ceruloplasmin levels were greater in patients with alopecia areata when compared to the control group, and they attributed this to the oxidant/anti-oxidant system imbalance in alopecia areata. Kirmit et al. [15] reported in their study to appreciate the oxidative stress status in psoriasis that ferroxidase levels were higher in the patient group than the control group, and this increase may have occurred as a compensatory response to oxidative stress.

It is known that medications can affect the levels of ceruloplasmin [17]. For that reason we avoided the participants who took any medications from our study. Also high estrogen and progesterone levels can cause high ceruloplasmin levels which suggests that ceruloplasmin levels are influenced by gender [17]. However, our patient and control groups were statistically similar in regards to gender which showed a parallel distribution of males and females in both groups. Ceruloplasmin levels were found significantly higher in the patients than in the control group in our study; and they were correlated with both disease severity and disease duration. The ceruloplasmin level was detected higher in patients with hand and foot involvement where the disease is relatively severer than in those with foot involvement only.

Study Limitations

The principal limitation of our study was lack of other biomarkers of the oxidative stress. Single center design of the study and the small sample size of the patients were the other limitations of our study.

Conclusion

Consequently, we believe that higher ceruloplasmin levels in the patients may indicate the oxidative stress on onychomycosis. However, further studies with larger patient series are needed. An investigation of the effects of systemic antifungals used in the treatment of tinea unguium on ceruloplasmin levels may be the subject of a further study. Furthermore, we believe that ceruloplasmin in onychomycosis may be a potential marker of disease severity and activity due to a strong statistical correlation.

Ethics

Ethics Committee Approval: Our study was carried out in conformity with the Declaration of Helsinki and approval of the Ethical Committee of University of Helath Sciences Turkey, Ankara City Hospital was obtained (number: 2027, date: 06.10.2021).

Informed Consent: Written informed consents were obtained from the patients and volunteers.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: F.Erd., Design: F.Erd., Data Collection or Processing: F.Erd., F.E., E.F.O., Ö.E., Analysis or Interpretation: Y.H., F.Erd., Ö.E., Literature Search: F.Erd., A.Y.İ., Writing: F.Erd.

Conflict of Interest: No conflict of interest was declared by the authors.

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