

# Relationship of amino acid concentrations in blood with occurrence of dermatophytosis

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## Abstract

**Objective** To compare the free amino acid concentrations of the blood in subjects suspected to tinea corporis with healthy individual.

**Methods** Blood samples from 40 adult (18-40 year) patients of mycologically-proven tinea corporis and 44 age- and sex-matched healthy controls were taken and assayed using high performance liquid chromatography for free amino acid levels.

**Results** 90% of patients showed higher free amino acids levels than controls. Methionine did not show significant difference in concentration between infected and healthy individuals. However, concentrations of glutamine, asparagine, phenylalanine, and tyrosine were significantly high in infected individuals with dermatophytes ( $p < 0.005$ ).

**Conclusion** Our finding provide information about the possible role of free amino acids in pathogenicity of dermatophytes.

## Key words

Dermatophytes, high performance liquid chromatography, tinea corporis.

## Introduction

Dermatophytosis, also known as ringworm or tinea, is caused by a group of fungi that infect keratinized tissues in human and animals. These fungi, known as dermatophytes, are classified based on their habitat. Fungi growing on humans are called anthropophilic and those that infect animals are named as zoophilic.<sup>1</sup> Human infection occurs through different ways such as contacting with contaminated soil, hair, or animal scales, and infected individuals.<sup>2</sup> After adhering to keratinized tissues, dermatophytes release enzymes e.g. keratinase, metalloprotease, lipase, and serine protease, which break and damage keratinized tissues. Moreover, severity of inflammatory reactions induced by

dermatophytes adds to the injury caused by fungi themselves.<sup>1,3</sup> There are some common clinical manifestations of dermatophytic infections, including tinea corporis, tinea imbricata, tinea cruris, tinea barbae, and tinea manuum, which are believed to affect about 25% of population in the world.<sup>2</sup> Superficial dermatophytoses are one of the most frequent infections in the last decades, that are going to increase day by day.<sup>4</sup>

Tinea corporis, ringworm of the body, includes any type of dermatophyte infection that does not appear in scalp, beard, hand, feet, or nails. It usually indicates one or more dry, scaly patches. Overweight people, children, wrestlers, and under stress individuals are more susceptible to this dermatophyte infection. The lesions caused by tinea corporis show elevated, scaly edge and erythematous with sharp margin. A wide spectrum of dermatophytes cause tinea

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corporis.<sup>1,5,6</sup>

Dermatophytes penetrate stratum corneum through emergence of germ tubes from the arthroconidia.<sup>6</sup> During infection process, dermatophytes degrade proteins into large peptides, which are subsequently digested into amino acids and short peptides.<sup>7</sup> These amino acids may provide an energy source for dermatophytes. Investigations have indicated that some amino acids, including glutamine, arginine, ornithine, citrulline, tryptophan, valine, and leucine, could be efficient nitrogen source for dermatophytes.<sup>8-10</sup> Some amino acids have also antifungal effects on dermatophytes. On this effect of amino acids, Pandey *et al.*<sup>11</sup> reported that L-cysteine hydrochloride and DL-aspartic acid have absolute toxicity on some species. They showed that antifungal activities of amino acids are stronger in comparison to four antimycotic drugs viz., griseofulvin, 5-bromosalicyl-4-chloranilidene, acidum undecylenicum and nystatin. It has been indicated that the antifungal activity of cysteine is high at 0.9 mm.<sup>12</sup>

There are limited reports about the antifungal activity of amino acids on dermatophytes. In addition, it has not been well understood that what is the relationship of the amino acid concentrations of the blood of infected individual with occurrence of dermatophyte infections. Considering high frequency of dermatophyte infections around the world and possible vital role of amino acids with it, we performed this study. In this study we aimed to determine the relationship of some amino acids, including methionine, asparagine, glutamine, phenylalanine, and tyrosine, with the dermatophyte infections.

## Methods

The skin samples were collected from patients suspected to tinea corporis at Sina Hospital,

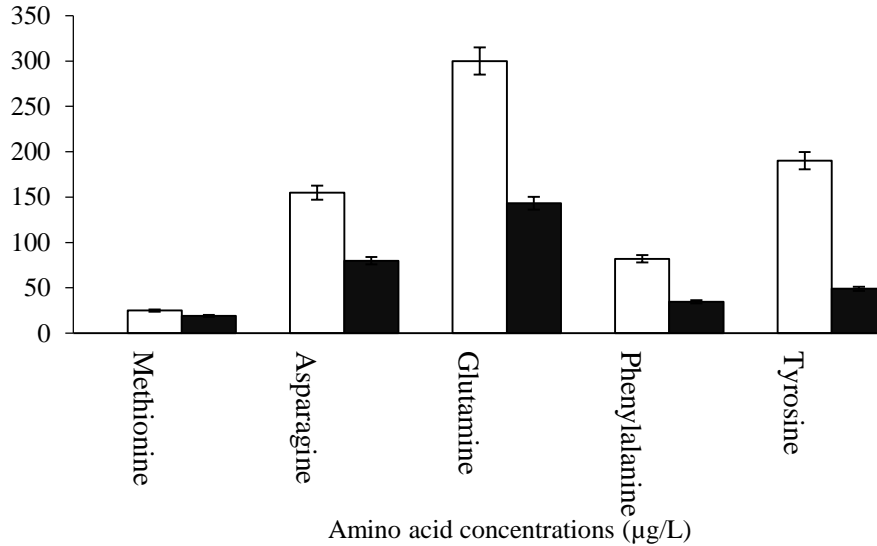
Tabriz, Iran. In order to verify infection by dermatophytes, the skin samples were observed under 40X microscope using potassium hydroxide (KOH) microscopy method. The smear-positive samples were cultured on the mycobiologic agar medium in order to final verification of the infection. Culture media comprised of 10.0g of pancreatic digest of soybean meal, 10.0g of dextrose, 0.4g of cycloheximide, 0.05g of chloramphenicol, and 15.5g of agar, all amount per liter.

The blood samples were taken from 40 male patients in 18 to 40 year age group having mycologically proven dermatophytosis. For comparison, blood samples were taken from 40 age- and sex-matched healthy individuals as controls. Samples from both patients and controls, were assayed for concentrations of methionine, asparagine, glutamine, phenylalanine, and tyrosine. 5 ml of the blood samples were kept at 37°C for 15-20 minutes for formation of the blood clot. After that, samples were centrifuged and serum separated and kept under -70°C.

High performance liquid chromatography (HPLC) was used to measure amino acid concentrations of the prepared samples. All data was analyzed with SAS software, version 6.0.

## Results

The results of analysis indicated that concentrations of amino acids were significantly different between patients and healthy individuals ( $p < 0.05$ ), except methionine that there was not significant difference, as shown in **Figure 1**. The difference of changes of amino acid concentrations was 4.48, 18.20, 47.77, 8.52, and 13.74  $\mu\text{g/L}$  for methionine, asparagine, glutamine, phenylalanine, and tyrosine, respectively. The normal distribution of data showed that 90% of infected individuals' amino acid concentrations were high compared to the



**Figure 1** Comparison of the amino acid concentrations between subjects infected with dermatophytes and healthy individuals. Total of 40 blood samples of suspected subjects were assayed for the amino acid concentrations of the serum using HPLC method. White bars indicate patients and black bars indicate healthy individuals

normal concentration of pertinent amino acids in healthy individuals.

## Discussion

Tinea corporis is one of common superficial fungal infections caused by many types of dermatophytes, such as *Trichophyton rubrum*, *T. tonsurans*, *T. verrucosum*, *T. equinum*, and *Microsporum nanum*.<sup>13</sup> This dermatophytosis is more common in hot climates, children, and wrestlers.<sup>5,14</sup> Fari and Gräser<sup>15</sup> reported that tinea corporis caused by *T. tonsurans* was an epidemic among wrestler children, aged 7-17 years. For dermatophytes, including those causing tinea corporis, amino acids are regarded as a source for growth.<sup>16</sup> Under *in vivo* condition, dermatophytes secrete several types of endopeptidase and exopeptidase in the presence of keratin as a sole nitrogen source.<sup>17</sup> These enzymes digest proteins into peptides and free amino acids to be used as nutrients by fungi.<sup>18</sup> One of these proteins is filaggrin that acts in assembling of keratin bundles. Filaggrin degradation releases free amino acids into

stratum corneum. High levels of free amino acids correlate with high water-holding capacity of stratum corneum.<sup>19</sup> We observed that 90% of studied amino acids (asparagine, glutamine, phenylalanine, and tyrosine) were in higher level in infected people compared to healthy individuals (**Figure 1**).

Methionine is a sulfur containing, essential amino acid in human body. *T. mentagrophytes* is one of the species of dermatophytes that requires methionine for its growth, so that many researchers have used this specific nutrition for determining *Trichophyton* species.<sup>16</sup> Investigations indicated that methionine has no effect on *Epidermophyton floccosum* and its effect on *M. gypsum* is medium.<sup>20,21</sup> Our results indicated that there was not significant difference in the methionine concentration of the blood between individual infected with tinea corporis and healthy individuals (**Figure 1**).

**Figure 1** clearly shows that four amino acids were significantly in high level in patients blood

as compared to healthy ones, among which tryptophan is an essential amino acid and asparagine, glutamine, and tyrosine are non-essential amino acids. There are reports that indicate these amino acids have inhibitory effect on different types of dermatophytes. Gharachorlou, Hashemi<sup>22</sup> demonstrated that tryptophan in different concentrations causes decrease in the growth of *T. verrucosum* prepared from patients of tinea corporis. It was reported that aspartic acid has a toxic effect on dermatophytes.<sup>23</sup> Such reports have also been published for glutamine and tyrosine.<sup>23-27</sup> Bakhshi *et al.*<sup>28</sup> reported that the concentration of glutamates, asparagine, histidine, glutamine, arginine, citrulline, threonine, phenylalanine, and isoleucine are high in patients suspected to dermatophytes compared to healthy individuals. Takahashi and Tezuka<sup>29</sup> reported that content of free amino acids of stratum corneum is larger in aged skin as compared to young skin. In the another study regarding the content of free amino acids, Katagiri *et al.*<sup>30</sup> reported that a drastic decrease in the environmental humidity reduces the total free amino acid levels in the stratum corneum leading to skin surface dryness. There was, however, no reports about the amino acid content of the blood in infected people. We think that increase in the free amino acids in the blood of infected individuals serves as a source of amino acids at the site of infection.

## Conclusion

Our results indicated that patients infected with tinea corporis, had high level concentrations of asparagine, glutamine, phenylalanine, and tyrosine, while there was no difference between methionine between patients and healthy controls. The finding suggests that this increase in content of free amino acid may be to supply amino acids to the site of infection. However, we need more research to clarify the possible mechanisms underlying free amino acid increase

in the blood of dermatophyte infected individuals. This research provides clues to more investigation on possible role of amino acids in infection process with dermatophytes

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