Inhibitory Effect of Valine on Trichophyton Mentagrophytes in Normal and Dermatophytic Patients under in Vivo and in Vitro Conditions

1 Aliasghar Gharachorlou, 2 Shahram Gharachorlou
1 Department of Mycology, Tabriz branch, Islamic Azad University, Tabriz-Iran.
2 Department of Pathology, Tabriz branch, Islamic Azad University, Tabriz-Iran.

Aliasghar Gharachorlou, Shahram Gharachorlou, Inhibitory effect of valine on trichophyton mentagrophytes in normal and dermatophytic patients under in vivo and in vitro conditions

ABSTRACT

In this study the effect of valine amino acid on trichophyton mentagrophytes in normal and dermatophytic patients under in vivo and in vitro conditions were studied. From safe and suffering from dermatophytic people's blood sample was obtained and amounts of valine amino acid in this people serums by HPLC method was measured and so mentioned fungi was cultured in cultures media with different concentrations of valine. Each of samples repeated in 3 times and after 2 weeks the diameter of colonies was measured, the results of research statistically were analyzed by using the SAS software and comparison of mean by using the ANOVA was done. Result has shown that the diameter of colony in different concentration of valine decreased in experimental fungi than control group. This appears that the valine amino acid cause decrease in the trichophyton mentagrophytes growth. Thus, probably the mentioned amino acid has inhibitory effects on experimental fungi growth.

Key words: dermatophytosis, valine, trichophyton mentagrophytes, inhibitory effect.

Introduction

Dermatophytosis is one of the dermal mycosis that results from the group of fungus actions in the keratinized tissue (such as hair, nail, and skin keratinized tissue) that called dermatophytes. Dermatophytes is a group of keratinophilic fungus that known from many years ago. Nowadays 41 species of dermatophytes were identified that totally divided into three geniuses (with notice to the asexual phase) with names microsporium, trichophyton, epidermophyton. Dermatophytosis is not contiguous disease and probably specific agents in sufferance to this disease are effective. physical and chemical agents can be effective in reveals of dermatophytosis pathogenesis in human which some people are sensitive and some other are resistance and might be dermatophytes also shown difference susceptible against of this agent. Of physical effective agents can be refer to temperature, moisture and PH that have difference effects on several dermatophytes. Several chemical factors such as hormones, fatty acids and amino acids in skin can be effective in dermatophytes growth. It is clear that human is continuously contact with dermatophytes, therefore, fortunately low amount show disease signs. For example, leg ringworm is one of the most prevalent types of tinea. However, appearance of empirical infection in volunteer peoples has been shown a high percentage of a natural resistance in against of sufferance from infection. Recent studies has shown that composition and rate of amino acids in perspiration of patients with ringworm disease were different with natural cases and were imaged that this is one of the effective agent in appearance of chronic infections [1]. The study that were done

Corresponding Author

Aliasghar Gharachorlou, Department of Mycology, Tabriz branch, Islamic Azad University, Tabriz-Iran.
E-mail: dr.ali.gh1531@gmail.com
Phone +98 914 116 7379
on microsporum gypseum and trichophyton mentagrophytes in India has shown that cysteine hydrochloride amino acids and aspartic acid have inhibitory effect and minimal inhibitory concentration of cysteine hydrochloride for microsporum gypseum is 0.5 gr/dl and for trichophyton mentagrophytes is 0.4 gr/dl were reported. Also, Acid aspartic with 1gr/dl concentration decreased the growth of microsporum gypseum to 100 percent and growth of trichophyton mentagrophytes to 48 percent [2]. Also in one study with adding androgen hormones to dermatophyts culture media, the diameter of colonies were decreased and among hormones, androstenedione has most inhibitory effect and epidermophyton flucosum and trichopyton rubrum has high susceptibility [3,4]. In other study showed that from 24 experimented dermatophy species, only trichophyton mentagrophyts had ability to growth in presence of cysteine 4% molar concentration [5].

In one study with measurement of androgen hormones in patients serum with dermatophytosis result from epidermophyton flucosum and trichophyton rubrum, were considered significant decrease in patients serum testosterone levels with epidermophyton flucosum agent in compared with healthy individual [6]. In the other study observed that fatty acids cause decreases the dermatophytes growth and unsaturated fatty acids with low amounts of carbon, acted more efficiently [7]. In this study the effect of valine with different concentrations on trichophyton mentagrophytes.

Materials and methods

In-vivo pathway

In this study during the years of 88-89 on 340 male which their lesions with regarding the dermatophyte infection were positive in direct experiment were done. Of 20-40 years old peoples we selected 76 individuals that have tinea capitis disease, from these all peoples by catching ferver refer to laboratory to blood sampling. After blood sampling, the samples to separating the serum were centrifuged (2500 round per minute for 10 minutes). Then samples were maintained in refrigerator at -60°C. The cutaneous sample of these peoples were cultured in mycobiotic agar while the fungus strain by macroscopic and microscopic studies were designated as only on peoples serum that suffers from dermatophytosis due to trichophyton mentagrophytes agent to determination of valine amino acid, HPLC experiment were exert.

In-vitro pathway

Materials used in this study includes A: trichophyton mentagrophytes counterfoils. Trichophyton mentagrophytes provided from fungus collections and industrial and infectious bacterial dependent on Iran scientific and industrial researches organization. B: mycobiotic agar culture media produced by germany merck factory. C: valine amino acid produced by germany merck factory. D: tween 80 E: saboroud glucose broth culture media produced by germany merck factory.

Equipment were used in this study includes heater equipped to magnetic mixer, autoclave, disposable 8 centimeter plate, flame light connected to gas, 10 centimeter glassy tube, erlenmeyer flask. This study is tentative types of studies. First, the saboroud glucose broth culture media was provided. Thereby 30 gram of ready powder scaled and added to 1 litter distilled water. Erlenmeyer contain culture media and distilled water was occupied on the magnetic heater and during the boiling mixed. Environment was shaded into 10 centimeter head screwd tubes and was autoclaved.0.5% of tween 80 were shaded into other sterile and head screw tubes. By spike beak fieldoplatin some of dermatophyte colony were achieved and were resolved in tween 80. Contents of each saboroud glucose broth tubes were empties on one of the dermatophytes resolved in tween 80. The samples after closing the curved (the curved should not be quite sealed) for 21 days were kept in laboratory temperature and after 21 days, tubes were centrifuged and upper portion were discarded and from their sediments used to culturing in solid media. 36 gram of mycobiotic agar powder were scaled and added into 2 litter erlenmeyer that 1 litter of this was distilled water. After occupation of magnet into Erlenmeyer were located on magnetic heater while during the boiling assimilated quietly (from this media were provided in more amounts). Into ten of 250cc erlenmeyer that each of them contains 200cc culture media by turn were provided 5 different concentrations of valine (1, 0.75, 0.5, 0.25, and 0.1 percent). As concentration of 1%, 2gram, for concentration of 75%, 1.5 kilogram, for concentration of 0.5%, 1gram, for concentration of 0.25%, 0.5 gram and for concentration of 0.1%, 2 gram of valine was scaled and added. In control erlenmeyer no added any amino acid. Erlenmeyer after autoclaving in temperature at 121 °C and pressure of 15 atmospheres, were divided into 8 centimeter plates and on plates the name of amino acid and their concentration were wrote. Each of trichophyton mentagrophytes counterfoils were cultured in plates contains amino acid and also in plates without amino acid. Cultured plates were located into incubator at temperature of 25° and after 14 days the diameter of
grown colonies were measured. Fungus culturing were done near the gas flame and under sterile conditions. All this stages were repeated in 3 times and growth average of dermatophyt in each concentration of valine was determined and all results by using of SAS statistical software were analyzed [8]. The size of colonies has been exhibited by average. Comparison of fungus colonies size in presence of under studied amino acids, were done by using the ANOVA that amount of p<0.05, were exhibited the significant differences [9].

Results

According to results, the colonies diameter in different concentrations of valine amino acid has significant decreased than control group (p<0.05). Observing the results are indicates that colonies diameter in different concentration of valine amino acids in compared with control group were significantly decreased (p<0.05). In histamine, all concentrations rather than each other have significant differences and minimum average is related to concentration of 1%. Also, minimum colony diameter of trichophyton mentagrophytes is related to concentration of 1% than to maximum average (concentration of 0.1%) is lower than about 2.6 fold.

Discussion:

Achieved results have shown that people distribution in associated with valine amounts in serum was shown that about 50% of diseased peoples. With considering to current study this appears that increase in valine level in serum probably causes hypersensitivity in people against dermatophytosis and were stimulated the dermatophytes growth. Also in Sarasgani and Firozrai study revealed that none of them were inhibited growth of dermatophytes with exception the L-lusin that were elicited to growth inhibition of microsporum gypseum [8].

Table 1: Comparison of colonies diameter in different concentration of phenylalanine in normal and treated groups.

<table>
<thead>
<tr>
<th>Treat level</th>
<th>Mean±Sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>36.20±1.48</td>
</tr>
<tr>
<td>valine</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>21.89±0.50</td>
</tr>
<tr>
<td>0.25</td>
<td>25.92±1.15</td>
</tr>
<tr>
<td>0.5</td>
<td>26.83±1.15</td>
</tr>
<tr>
<td>0.75</td>
<td>25.50±1.15</td>
</tr>
<tr>
<td>1</td>
<td>28.00±2.00</td>
</tr>
</tbody>
</table>

Table 2: phenylalanine amounts in normal and patients to dermatophytosis sera.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>123-310±21.36</td>
<td>84±7.45</td>
</tr>
<tr>
<td>P value</td>
<td>0.0051</td>
<td></td>
</tr>
</tbody>
</table>

mildly effect on microsporum gypseum [8]. In one other study that was done by Garachorlou et al., revealed that asparign and methionine amino acids causes decrease in the trichophyton rubrum and trichophyton verrucosum growth [10]. Acidic amino acids also either was shown inhibitory effect on two dermatophytes that the acid aspartic inhibitory effects on microsporum gypseum growth were determined in pandy study [2]. In current study the inhibitory effect of valine on trichophyton mentagrophytes were assessed and shown that concentration of 0.1% valine causes maximum decrease in trichophyton mentagrophytes growth. The colony diameter in different concentrations of valine in experimental fungi than control group was decreased. This appears that valine causes growth decreasing in trichophyton mentagrophytes. Therefore mentioned amino acid probably has inhibitory effect on growth of trichophyton mentagrophytes.

References

