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The Comparative Study of Skin Amino Acids In Dogs With Ring Worm And Healthy Dogs

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ABSTRACT

Background | Stratum corneum consists of keratinocyte cells, the first defense layer that stands against pathogenic microorganisms. Identifying the components of stratum corneum provide information on its resistance against superficial infections. It contains higher amount of amino acidswhich most of them can serve as source of nitrogen. | Objective: | The objective is to investigate and compare the quantity of amino acids in dogs with dermatophytosis and healthy dogs. | Material&Method: | 30 dogs suspectedof dermatophytosis and 30 healthy dogs were used. Skin scraping samples from bothwere taken. Amino acids were analyzed by High-performance liquid chromatography (HPLC). | Results: | Significant differences were found for the rate of aspartic acid, serine and asparagine. | Conclusion: | Aspartic acid seems to play inhibitory role whileasparagine and serinehave stimulatory role in colonization of dermatophytes. These properties of aspartic acid can be used in manufacture of more effective antifungal agents especially for chronic type. |

KEYWORDS : Stratum corneum, Dermatophytosis, Amino acids, Dogs

INTRODUCTION

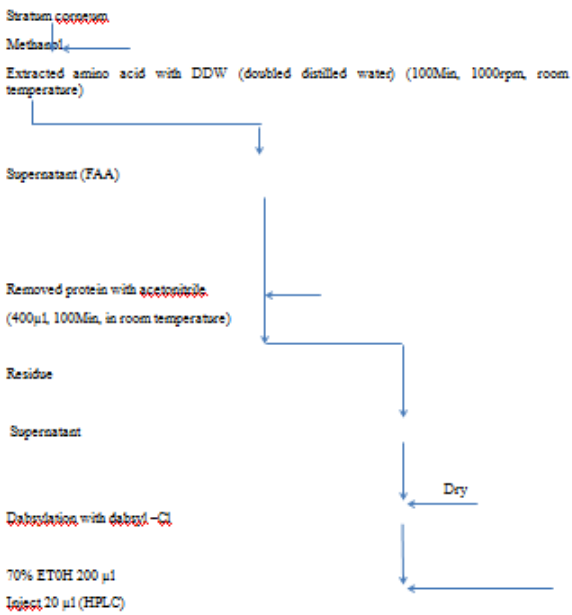
The skin surface is made up of the stratumcorneum layer, sweat, sebaceous glands and hair follicles. The stratum corneum layer consists of keratinocyte cells which act as a sponge in preserving the skin. They are made up of proteins, carbohydrates, lipids, acids, salts and their derivatives. This layer is the first defense layer in the face of pathogenic micro-organisms. Identifying the components of stratum corneum can provide plenty of information on resistance or lack of resistancein the face of superficial infections (2). Amino acids, compared to other substances, contain the most amount of stratum corneum and have a significant role in regulating the skin buffer system and skin moist, thereforeplay a vital role in maintaining the structure of stratum corneum (2, 5, and 13). Different concentrations of amino acids have different effects on the growth of dermatophytes and, like any other substances,they are needed in a certain concentration for the growth of the organism and in higher concentration tend to decrease growth. Depending on the type of the amino acid and the organism, the required dosage is different. The increase and decrease in amino acids of the skin can pave the way for colonization or resistance (18). In a study by Chandra and Pandy (1984) conducted on*Microsporumgypseum* and *Trichophyton mentagrophytes* in vitro, cysteinehydrochloride and aspartic acids were shown to have an inhibitory effect on these two dermatophytes (15).

A study by Nguyenet al (1981) on the effect of cysteine on the growth ofdermatophytesshowed that from 24 dermatophytes species in this study only*Trichophytonmentagrophytes var. quinqianum* can grow in a concentration of cysteine 4% molar (14). The study by Sarasgani and Firoozrai (2006) on the effect of amino acidson the growth of *Microsporumgypseum* and *Epidermophytonfloccosum* conducted invitro, cysteinehydrochloride,cysteine, aspartic acids and trichophyton at a concentration of 1% were shown to be the most effective amino acids in decreasing the growth of thesedermatophytes (17).

Given that stratum corneum amino acids can be used by dermatophytes as a source of nitrogen (9), the amount of amino acids in the skin of dogs diagnosed with dermatophytosis and healthy dogs have been compared in this study and since dermatophytosis is known as a zoonotic disease, dogs have been chosen as house pets that in many cases act as carriers (6). Determining the amount of amino acids in the skin of diagnosed dogs can provide more useful information on the infection and its getting chronic especially in view of the fact that the disease, in its chronic form, requires a longer therapy as long as 6-12 months and in many cases, is recurring.

METHODOLOGY

This is a cross sectional study conducted on 30 male dogs, younger than two years who have been diagnosed with dermatophytosis and referred to veterinary clinic. The same number of healthy dogs makes up the control group. Sampling of skin defects suspected of dermatophytosis has been done on dogs in the trial group with sterile scalpel. Each sample was recorded under the patients' names and was sent to the lab for direct examination and culture. A questionnaire has been designed to record patients' data including age, sex, and history of disease, infected area, other diagnosed diseases and nutrition status. In the laboratory for every sample, a smear with 20% of Potassium hydrochloride prepared. Upon observationof septatedmycelium, as well as round and barrel like arthroconidia,dermatophytosis was immediately confirmed on the slide. However,to determine the type of dermatophyte, the defected skin was inoculated inmycobioticagar (Difco). Given that dermatophytes grow relatively slowly, the cultures usually turns positive in the room temperature (25Ċ) between 10 days and three weeks and then the dermatophytetype is determined. In the next step, from normal areaon the skin of the dogs with dermatophytosis and also near the affectedarea, skin scrapes are taken and are kept in plates. The same is conducted on the control dogs. Later the specimens are prepared for extracting amino acids through HPCL as shown below (20):



The data were then analyzed with Student's t-test.

RESULTS

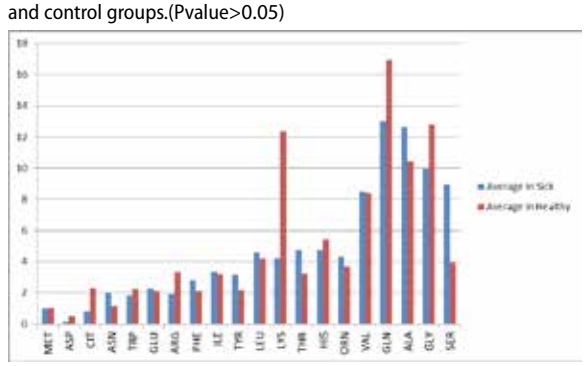
In this study, from these 30 dogs with dermatophytosis, *Microsporumcanis*grew in all cultured media.

The HPLC method was applied in this study on sick and healthy dogs and the results shown as follows:

Table 1: The mean score in samples of patients and healthy dogs along with their mean standard deviation

Amino Acids	Mean in Patients	Mean in Controls	Standard Deviations in Patients	Standard Deviations in Controls
(ASP) Aspartic acid	0.1	5.0	1.0	4.0
(CIT) Citrulline	0.8	2.3	0.5	1.7
(MET) Methionine	0.9	1.1	0.3	0.1
(TRP) Tryptophan	1.8	2.2	0.6	8.0
(ARG) Arginine	1.9	3.3	1.8	4.2
(ASN) Asparagine	1.2	7.1	2.1	0.8
(GLU) Glutamic acid	3.2	2.1	1.3	0.6
(PHE) Phenylalanine	8.2	2.1	0.7	9.0
(TYR) Tyrosine	2.2	2.2	9.0	0.9
(ILE) Isoleucine	4.2	3.2	8.0	0.9
(LYS) Lysine	4.2	4.12	8.3	9.8
(ORN) Ornithine	3.1	5.2	3.7	4.3
(LEU) Leucine	6.1	1.6	2.4	6.4
(HIS) Histidine	1.8	3.9	5.4	4.7
(THR) Threonine	1.2	1.1	3.2	8.4
(VAL) Valine	6.3	3.6	8.4	5.8
(SER) Serine	6.1	8.9	9.3	8.9
(GLY) Glycine	8.6	9.4	12.8	9.9
(ALA) Alanine	9.5	3.5	10.4	12.6
(GLN) Glutamine	5.7	8.5	16.9	12.6

There was a significant difference (P value<0.05)betweenthe case and control groups;so that the rate of aspartic acid in patients was less than in healthy dogs and the rates of serine and asparagine were morethan healthy dogs. As concerns the amino acids of phenylalanine, leucine, ornithine, glutamic acid, glutamine, histidine, arginine, citrulline, ornithine, tyrosine, alanine, tryptophan, methionine, valine, isoleucine, lysine, there is no significant difference between the case and control groups.(Pvalue>0.05)



1-Bar diagram of the comparison of mean scores in samples of patients and healthy dogs

DISCUSSION:

Stratum corneum is known to be the most superficial skin layer in which many microorganisms including dermatophytesare colonized to cause disease. Identifying the components of the stratum corneum can increase our information on resistance or lack of resistance against superficial infections. Compared to other substances, amino acids have the highest rate in stratum corneum. Their main origin (70%-100%) is matrix proteins called filaggrin whichare rich in histidine and as a result of hydrolyzation release free amino acids and a few of them are derived from sweat glands (4). Dermatophytes are able to use amino acids as a sourceof nitrogen and this process happens in mycelium through enzyme L-amino acid oxidase. These enzymes transform amino acids into keto acid and ammoniac (16, 9). Oxygen consumption during the de-ammonization leads to the destruction of stratum corneum and mycelium penetration (1).

Besides being used as a source of nitrogen, amino acids are also used in metabolical pathways. The best source of nitrogen for them include glutamic acid, glutamine and urea cycle amino acids namely arginine, citrulline and ornithine. Asparagine, proline, serine, alanine, glycine, histidine and tyrosine are also rather good source of nitrogen for them which are used at different rates by various species. Tryptophan, valine, isoleucine and phenylalanine are rather poor nitrogen sources (16, 9 and 8).

Aspartic acid, methionine and cysteine are not good source of nitrogen for dermatophytes and have inhibitoryeffects on their growth (3).

Increased pH could be a susceptible factor for all kind of cutaneous infections. Skin dog pH is in the range of (5.5- 7.2) (7). This pH in spinal cord region is more and is in the range of (6.4-9.1) (11).Dermatophytosis is more often see in young dogs than in old dogs because their immune system has not been completed enough (10). The most agents of dermatophytosis in dogs are *Microsporumcanis* and *Microsporumgypseum* (12).

In this study, there was a significant difference between case and control groups with regard to the rate of aspartic acid, serine and asparagine. (P-value<0.05) The rates of aspartic acid in the patient group were lower than in control dogs, and serine and asparagine were higher than in patients group.

In a study by Pandy and Chandra (1984) on two dermatophytosis of *Microsporumgypseum* and *Trichophyton mentagrophytes* in-vitro,aspartic acid was found to have an inhibitory effect on the growth of dermatophytes; that is in a concentration of 1 gram per deciliter; it decreased the growth of *Microsporumgypseum* and *Trichophyton mentagrophytes*down by 100% and 18%respectively (15). In the 2006Sarasganiand Firoozrai study on the effects of amino acids on the

growth of *Microsporumgypseum* and *Epidermophytonfloccosum*in-vitro, aspartic acid in a concentration of 1% was proved to have the biggest effect on the growth of these two dermatophytes (17). In a study by Sarasgani, Firoozrai and Hashemi (2008) on the effects of amino acid on the growth of*Microsporumcanis*and *Trichophytonschoenleinii*in-vitro, aspartic acid in aconcentration of more than 1% were shown to have the biggest inhibitory effect on the growth of these two dermatophytes (18).Another study by Sarasgani (2010) on the effect of amino acids on the growth of *Trichophytonrubrum* and *Trichophytonverrucosum*which was done in culture media showed that aspartic acid in a concentration of 1% has the highest inhibitory effect on the growth of these two dermatophytes (19).Thus the inhibitory effect of aspartic acids on the growth of dermatophytes in the present study which was conducted on layers of stratum corneum of case group can be confirmed. The lower rate of aspartic acids in case group compared to control dogs can be the reason behind the better growth and colonization of dermatophytesin the absence of these amino acids. In this study a significant difference was seen between the rate of serine among dogs of the case and control groups so that its rate in case group was higher than in control dogs(P-value<0.05). Serine is one of the best sources of nitrogenfor the growth of dermatophytes (8). In the Sarasgani and Firoozrai study (2006), serine at a concentration of 1 gram per deciliter in amedia lead to the decrease in the growth of the two dermatophytes*Microsporumgypseum*and *Epidermophytonfloccosum* (17). In the Sarasgani, Firoozrai and Hashemistudy on two dermatophytes*Microsporumcanis* and *Trichophytonschoenleinii*, serine at a concentration of 0.1% can increase the growth of the two dermatophytes in media (18). In another study by them (2010), serine at aconcentration of 1% led to a decreasein the growth of the two dermatophytes*Trichophytonverrucosum* and *Trichophytonrubrum* (19). It seems that serine can have both inhibitory and simulating effectin-vitro on the growth of different dermatophytes. In the present study, since the rate of serine in infected dogs is higher than in control dogs (P-value<0.05), it can be said that serine has a stimulatory role in the growth and colonization of dermatophytes.Asparagine is a good source of nitrogen for dermatophytes growths (9). In this study, the rate of asparagine in infected dogs is higher than in control dogs (Pvalue<0.05), therefore itcan be said that asparagine has a simulating role in the growth and colonization of dermatophytes. In this study, no significant difference was found between the trial and control groups in the rate of amino acids of phenylalanine, leucine, threonine, tyrosine, alanine, ornithine, tryptophan, methionine, valine, isoleucine, and lysine. (P-value>0.05)

Given that there is a significant difference between the two groups in terms of rate of aspartic acid which is less in patient dogs compared to the healthy ones,and the fact that this amino acids plays an inhibitoryrole in the growth of dermatophytes, it can be said that lower amount of the aspartic acidcan turn the disease chronic and cause its recurrence. Concerning serine and asparagine amino acids where a significant difference has been noted between the case and control groups more in the diagnosed dogs than in healthy ones, and since these two amino acids are good source of nitrogen, any increase in their quantity can make the disease chronic necessitating a longer therapy (9).

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