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RESEARCH ARTICLE

Study of free amino acids in stratum corneum and plasma in patients with dermatophytosis and normal subjects

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Abstract

Background: Dermatophytes are a group of fungi that cause infections in keratinized human and animal tissues. Physical and chemical agents can be effective in reveals of dermatophyte pathogenesis in human which some people are sensitive and some other are resistance to it. Amino acid changes may be a risk factor for infection with dermatophytes in mammals.

Methods: Amino acids in plasma and stratum corneum analyzed by HPLC method and the identification of dermatophytosis was based on direct examination and culture.
The results of research statistically were analyzed by software and comparison of mean by using the t- test.

Results: Achieved results between case and control in sole area have shown that cases were significantly increased in: **Aspartate - Tyrosine – Tryptophane - Phenylalanine** and were significantly decreased in: **Citrulline– Ornithine** similarly, in male and female.
The results have shown that people with dermatophytosis in two site near skin lesion and sole area distribution in associated were significantly increased in: **Glutamates - Asparagine - Histidine - Glutamine - Arginine - Citrulline - Threonine - Methionine - Leucine – Ornithine** and were significantly decreased only in: **Glycine .**
In plasma**: Aspartate - Glutamates - Asparagine - Arginine - Threonine - Alanine - Tyrosine - Valine - Phenylalanine - Isoleucine – Leucine** have statistical differences with significant p value ($p \leq 0.05$) between two groups ,case and control similarly, in male and female.

INTRODUCTION

Dermatophytes are a group of closely related fungi that have keratinase and can therefore cause infections in keratinized human and animal tissues (skin, hair and nails), leading to a disease known as dermatophytosis. The etiologic agents of dermatophytosis (ringworm) are classified in three anamorphic (asexual or imperfect) genera, Epidermophyton, Microsporum, and Trichophyton. 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. Several chemical factors such as amino acids can be effective in dermatophytes growth. Amino acid changes may be a risk factor for infection with dermatophytes in mammals (1-5).

Some investigations reveal that inhibitory effects of some amino acids to growth of some dermatophytes for example L-cysteine hydrochloride, L-cysteine, L-aspartic acid, Lglutamic acid and DL-tryptophan and L-tyrosine have the most inhibitory effects on the studied dermatophytes, while arginine L-lysine and L-methionine have moderate effects and the rest of amino acids have less inhibitory, or even stimulatory, effects on the growth of the dermatophytes. *M. canis* and *T. schoenleinii* data indicates that sulfur-containing amino acids and acetic amino acids have greater inhibitory effect against these dermatophytes(6-8).
The other study done by Garachorlou et al (2001-2012), revealed that aspargin and methionine causes decrease in *T.rubrum* and *T. verrucosum* growth and inhibitory effect of valine on *T.mentagrophytes* .Histidine has inhibitory effect on Trichophyton mentagrophytes growth and the inhibitory effect of tryptophan on Trichophyton verrucosum and growth decreasing in *E. floccosum* and study about four amino acids valine , tryptophan , methionine and asparagine in serum was shown increase in valine , tryptophan , methionine and asparagines level in serum probably causes hypersensitivity in people against dermatophytosis (9-12).

Pandy showed acidic amino acids (acid aspartic) had inhibitory effect on *M.gypseum* and *T. mentagrophytes* growth. L-Cysteine hydrochloride exhibited absolute toxicity against both the test pathogens while DL-aspartic acid was found active against *M. gypseum*(13).

Base on this finding we decided to determine the skin (stratum corneum) free amino acids concentration in patients with dermatophytosis and comparative them with normal subjects for first time in world.

Materials and methods:

Dermatophyte processing

From 370 patients with suspected dermatophytosis, 60 patients (females and males, 15-35 years old) were only with skin lesion (no hair or nail) that were diagnosed by direct microscopic examination and culture method. The identification of dermatophytes was based on macroscopic and microscopic colony characteristics, subcultures on specific media and tests and PCR.
60 healthy volunteers (females and males, 15-35 years old) without clinical sign of skin disease or amino acids anomaly participated in the study.

Biochemical processing

Stratum corneum samples:
The scales of the 60 patients with dermatophytosis in two site near skin lesion and sole area and 60 healthy volunteers(normal subjects), at sole area were scraped off with a sterile surgical scalpel Blade No.21 and the scales were collected directly onto a tube. The samples were prepared with added 100 µ double-distilled water (DDW) and 100 µ pure methanol for extracted free amino acids and added 200 µ acetonitrile for remove protein and centrifuge in 2000 rpm 5 min at 10ºC and supernatant were stored at –20°C until used . To standardize the concentration number of cells Concentration factor of each sample was found the samples were diluted with normal saline and shaken in a vortex shaker and stratum corneum cells were counted using a hemocytometer(14).

Plasma samples:
Hole blood of 60 patients with dermatophytosis, and 60 healthy volunteers (normal subjects), were collected.
Collection of 2 ml of Venous blood was drawn into EDTA- containing tubes between 7 Am to 9 Am after overnight fasting and centrifuged for 10 min at 2500 rmp in 10 °C and remove the plasma.
Reversed-phase high-performance liquid chromatography (RP-HPLC) is a powerful method for assaying physiological amino acid concentrations in biological fluids. Four pre-column derivatization methods, with o-phthaldialdehyde (OPA), 9-fluoronylmethyl chloroformate (FMOC-Cl), phenyl isothiocyanate (PITC) and 1-

dimethylaminonaphthalene-5-sulphonyl chloride (dansyl-C l)(15-17) were assessed with respect to their applicability in biological research.

Of this reaction were resolved on a high-performance liquid chromatography (HPLC) reversed-phase column (Younglin Acme 9000)
The amino acid-OPA derivatives were separated on reverse phase 5 µm C18 ultrasphere column Aglient (250 × 4.6 mm I.D.) kept at 30°C.
OPA reagent: mixture was prepared by mixing 200 µl of K-borate buffer pH 8.5 and 40 µl of OPA dissolved in methanol (5 mg ml^{-1}).
Solvent A was a mixture of 50 mm sodium acetate adjusted to pH 7 with acetic acid plus 1% (v/v) tetrahydrofuran. Solvent B was pure methanol.
A mixture include of 25 µ l of supernatant (extract from skin or blood samples) and 25 µl internal standard (100 µm/l) and 50 µl methanol vortex 30" and centrifuge 10.000 rpm for 5 min.50 µl of supernatant was derivatized with OPA reagent (50 µl) for 5 min and 10 µl of the mixture were injected and eluted at a flow rate of 1 ml min^{-1} at 30°C in gradient condition wavelength 34o nm emission 450 nm (18).

Statistical analysis:

The samples were grouped by donor group and analyzed using test paired two samples for mean. P values <0.05 were considered significant. Infections and amino acids were compared using an independent variable t-test. Analysis for trends by sex group used the mean, standard deviation and measure of variance.

Quality control:

For quality control and calibration and precision and accuracy we used Levey Jenning and Westgard multirole(19).

Younglin Acme 9000 for all case and control and test for accuracy we test HPLC Agilent 1200 infinity series for 10% of case and control for precision.We use SIGMA AZ161 for standardization and Recipe Lot 722 level 2 for control.
For remove all errors we take a decision to measure qualitative and compare between patients and normal control and we analyze each amino acids independently and we analyzed the results of each amino acid independently.

Results:

Dermatophyte results:

Trichophyton interdigitale was the most common isolate (41.7%) followed by **T. rubrum** (31.7%), **Epidermophyton floccosum** (16.7%), **Microsporum canis** (3.4%), and **M. gypseum** (1.7%). **Other species** (4.8%) in total male and female.

Biochemical results:

About 80% of free amino acids in stratum corneum is: **Serine – Glycine – Alanine –Ornithine -Threonine – Histidine - Valine** and it is same and common in man and woman in normal objects.Achieved results between case and control in sole area have shown that cases were significantly increased in case in amino acids: **Aspartate - Tyrosine – Phenylalanine-- Tryptophane**
and were significantly decreased in case in amino acids: **Citrulline – Ornithine**
Similarly, in two sex male and female result. and have not any significant different in other amino acids in stratum corneum.(table 1 & diagram 1)

Achieved results have shown that people with dermatophytosis in two site near skin lesion and sole area distribution in associated were significantly increased in near skin lesion in amino acids: **Glutamates - Asparagine - Histidine - Glutamine - Arginine - Citrulline - Threonine - Methionine - Leucine – Ornithine** and were significantly decreased in near skin lesion in only amino acid : **Glycine** and have not any significant different in other amino acids in stratum corneum.(table 2 & diagram 2)

Most amino acids in plasma is: **Glutamine** - **Alanine** - **Glycine** - **Valine** - **Leucine** - **Serine** - **Lysine** - **Threonine** and it is same and common in man and woman .
Achieved results have shown that people with dermatophytosis distribution in associated were significantly increased in**: Aspartate** - **Glutamates** - **Asparagine** - **Arginine** - **Threonine** - **Alanine** - **Tyrosine** - **Valine** - **Phenylalanine** - **Isoleucine – Leucine** have statistical differences with significant p value ($p \leq 0.05$)between two groups ,case and control in male and female**.**(table 3 & diagram 3)

Table1:Comparative changes **in** mean concentration of free amino acids**(µm/l i**n **10^9 stratum corneum cells)** cases with dermatophyte and control (normal skin**)** sole area in male **and female**

results	P(T<=t)	Variace Control	Female Control µm/l	Variance Case	Female Caseµm/l	P(T<=t)	Variance control	Male Control µm/l	Variance case	Male Case µm/l	Amino Acids
#	0.04	2.63	7.99	5.90	10.86	0.05	4.08	9.82	6.82	12.10	Aspartate
=	0.06	0.60	2.86	0.54	2.22	0.06	1.84	3.33	0.81	2.78	Glutamates
=	0.06	0.11	1.50	0.35	1.90	0.06	0.25	1.34	0.45	1.67	Asparagine
=	0.43	810.5	111.4	870.1	114.9	0.32	765	105.4	830	109.9	Serine
=	0.20	34.41	15.28	54.49	13.32	0.27	36.29	16.04	64.99	14.56	Histidine
=	0.47	0.67	0.96	0.99	0.99	0.30	0.45	1.02	0.83	1.18	Glutamine
=	0.34	0.91	1.31	0.99	1.48	0.13	0.28	1.12	0.98	1.48	Arginine
#	0.01	0.95	1.49	0.16	0.73	0.01	0.95	2.33	0.47	1.22	Citrulline
=	0.37	279.2	61.16	237.9	58.63	0.06	278.1	82.50	238.9	70.22	Glycine
=	0.31	39.04	20.04	38.22	18.58	0.11	30.23	21.52	35.71	18.14	Threonine
=	0.48	50.28	36.09	60.37	35.79	0.25	62.88	40.45	63.0	36.90	Alanine
#	0.03	3.08	6.60	4.40	9.04	0.05	3.31	9.57	6.57	11.80	Tyrosine
#	0.05	1.30	2.48	2.0	3.12	0.02	1.30	3.24	3.70	4.61	Tryptophane
=	0.06	0.69	1.43	0.60	1.87	0.06	0.86	1.57	0.62	1.92	Methionine
=	0.11	4.10	10.87	6.77	12.97	0.27	3.73	13.48	5.57	14.75	Valine
#	0.03	1.10	4.59	3.60	6.31	0.04	2.33	7.77	4.30	9.20	Phenylalanine
=	0.07	2.1	6.41	3.40	7.98	0.29	3.25	8.62	4.13	9.38	Isoleucine
=	0.22	4.10	7.34	5.72	8.88	0.37	6.48	9.90	6.93	10.41	Leucine
#	0.04	95.84	34.60	99.93	26.19	0.01	134.2	39.18	75.70	26.94	Ornithine
=	0.20	5.41	6.10	6.37	5.23	0.34	2.99	6.44	6.71	5.59	Lysine

= mean no statistical differences and # mean significant differences

Table2:Comparative changes in mean concentration of free amino acids(µm/l in 10^9 stratum corneum cells) cases with dermatophyte (two site near skin lesion and sole area) in male and female

results	P(T<=t)	Variace lesion	Female lesion µm/l	Variance Case	Female Caseµm/l	P(T<=t)	Variance lesion	Male lesion µm/l	Variance case	Male Case µm/l	Amino Acids
=	0.18	21.35	12.2	5.90	10.86	0.16	23.46	14.78	6.82	12.10	Aspartate

#	0.01	60.11	11.10	0.54	2.22	0.01	72.23	12.31	0.81	2.78	Glutamates
#	0.01	8.75	3.56	0.35	1.90	0.01	9.24	4.97	0.45	1.67	Asparagine
=	0.48	440.5	113.2	870.1	114.9	0.48	430.1	110.2	830	109.9	Serine
#	0.01	78.24	25.77	54.49	13.32	0.01	144.1	29.88	64.99	14.56	Histidine
#	0.01	23.25	9.98	0.99	0.99	0.01	34.12	11.23	0.83	1.18	Glutamine
#	0.01	186.2	21.66	0.99	1.48	0.01	185.1	24.12	0.98	1.48	Arginine
#	0.01	34.47	15.44	0.16	0.73	0.01	43.12	17.77	0.47	1.22	Citrulline
#	0.01	387.8	34.77	237.9	58.63	0.01	396.6	37.49	238.9	70.22	Glycine
#	0.02	19.65	22.45	38.22	18.58	0.02	22.77	21.29	35.71	18.14	Threonine
=	0.49	48.97	36.65	60.37	35.79	0.47	76.90	37.88	63.0	36.90	Alanine
=	0.25	6.68	9.87	4.40	9.04	0.23	7.22	12.11	6.57	11.80	Tyrosine
=	0.23	1.49	2.51	2.0	3.12	0.21	1.43	3.22	3.70	4.61	Tryptophane
#	0.01	1.50	3.07	0.60	1.87	0.01	1.12	3.21	0.62	1.92	Methionine
=	0.21	4.88	13.13	6.77	12.97	0.18	6.68	15.01	5.57	14.75	Valine
=	0.10	6.42	5.92	3.60	6.31	0.12	6.40	8.88	4.30	9.20	Phenylalanine
=	0.33	3.66	8.01	3.40	7.98	0.33	4.50	9.01	4.13	9.38	Isoleucine
#	0.05	6.42	10.01	5.72	8.88	0.05	7.44	12.45	6.93	10.41	Leucine
#	0.05	78.81	33.84	99.93	26.19	0.05	88.95	34.77	75.70	26.94	Ornithine
=	0.20	8.48	6.12	6.37	5.23	0.18	8.58	7.11	6.71	5.59	Lysine

= mean no statistical differences and # mean significant differences

Table3:Comparative changes in mean concentration of free amino acids in plasma in cases with dermatophyte and control (normal skin)

results	P(T<=t)	Variace Control	Female Control µm/l	Variance Case	Female Case µm/l	P(T<=t)	Variance control	Male Control µm/l	Variance case	Male Case µm/l	Amino Acids
#	0.01	4.51	3.80	5.95	6.34	0.04	3.48	4.57	5.78	6.18	Aspartate
#	0.01	406.2	55.71	411.3	81.36	0.01	902.8	73.25	1108	99.97	Glutamates
#	0.04	25.67	35.38	120.3	43.71	0.01	54.60	42.34	155.8	48.99	Asparagine
=	0.07	134.9	113.5	1303	124.7	0.08	584.1	117.6	1350	139.9	Serine
=	0.29	226.2	71.34	253.6	60.58	0.13	290.1	74.15	1110	81.97	Histidine
=	0.13	6490	651.2	6778	508.5	0.36	8204	620.8	9995	608.3	Glutamine
#	0.01	182.5	56.13	621.54	76.53	0.02	362.9	67.38	949.2	77.71	Arginine
=	0.23	39.56	29.81	52.77	29.79	0.08	59.84	32.12	82.78	34.79	Citrulline
=	0.42	2944	238.4	6747	223.4	0.26	4421	253.4	6970	241	Glycine
#	0.04	791.2	111.4	3702	159.07	0.01	927. 3	110.3	3578	135.9	Threonine
#	0.01	6585	355.4	6627	427.4	0.01	3264	343.5	9934	461.5	Alanine
#	0.01	172.7	63.19	267.9	79.18	0.01	384.3	68.00	503	86.93	Tyrosine
=	0.15	69.96	51.37	64.20	57.12	0.18	374.9	58.17	313.8	62.68	Tryptophane
=	0.26	54.95	28.48	48.97	29.45	0.06	44.55	32.76	67.70	37.93	Methionine
#	0.03	1428	207.5	1450	245.2	0.01	4309	236.7	5715	285. 5	Valine
#	0.01	10.26	53.17	83.12	67.27	0.01	209.1	63.44	407.2	75.85	Phenylalanine
#	0.01	151.9	62.44	154.3	77.93	0.01	688.9	77.76	661.4	99.79	Isoleucine
#	0.01	132.9	115.7	354.4	139.2	0.01	1168	130.6	1752	156.3	Leucine
=	0.22	147.1	49.25	205.5	53.35	0.07	309.4	78.07	1394	66.66	Ornithine
=	0.33	288.1	112.4	491.4	101.4	0.41	1206	110.9	1287	112.9	Lysine

= mean no statistical differences and # mean significant differences

Diagram1:Comparative in mean concentration of free amino acids(µm/l in 10^9 stratum corneum cells) in control (normal skin) and cases (with dermatophyte) in sole area with significant in male and female

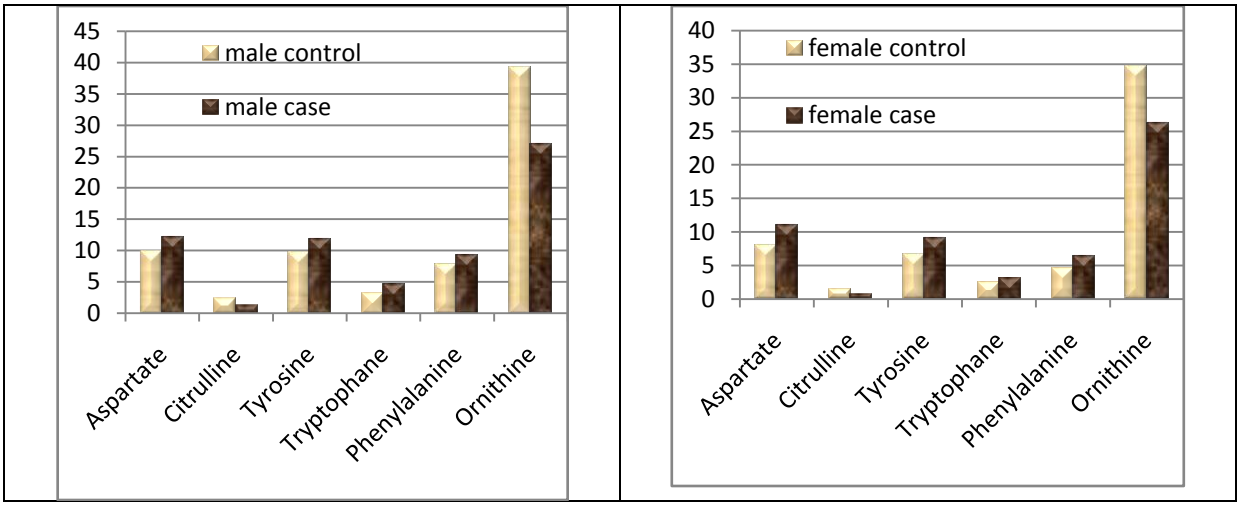


Diagram2:Comparative in mean concentration of free amino acids(µm/l in 10^9 stratum corneum cells in cases with dermatophyte(two site near skin lesion and sole area) with significant in male and female

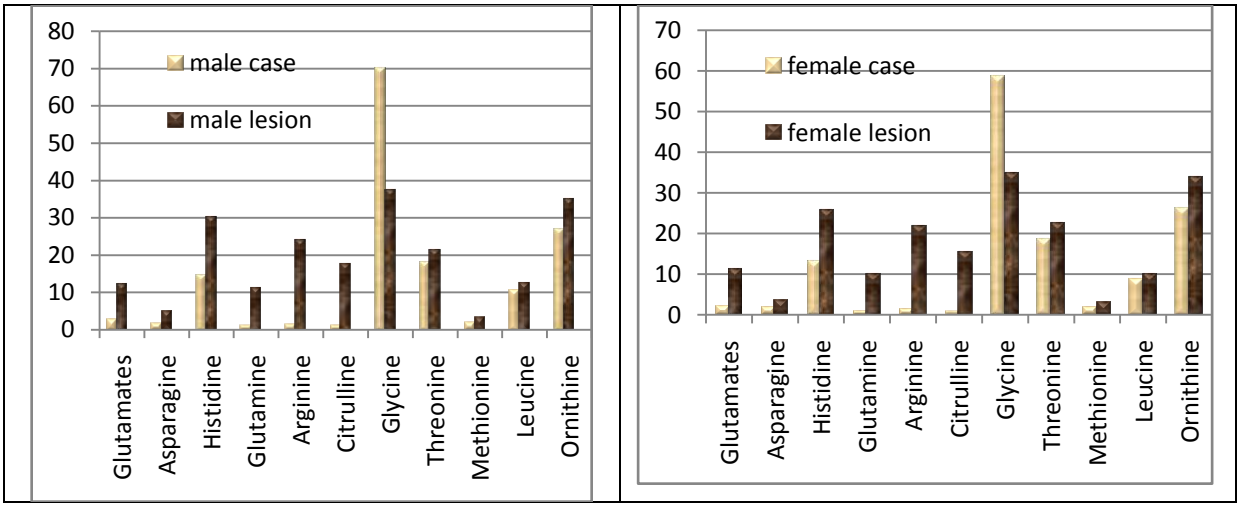
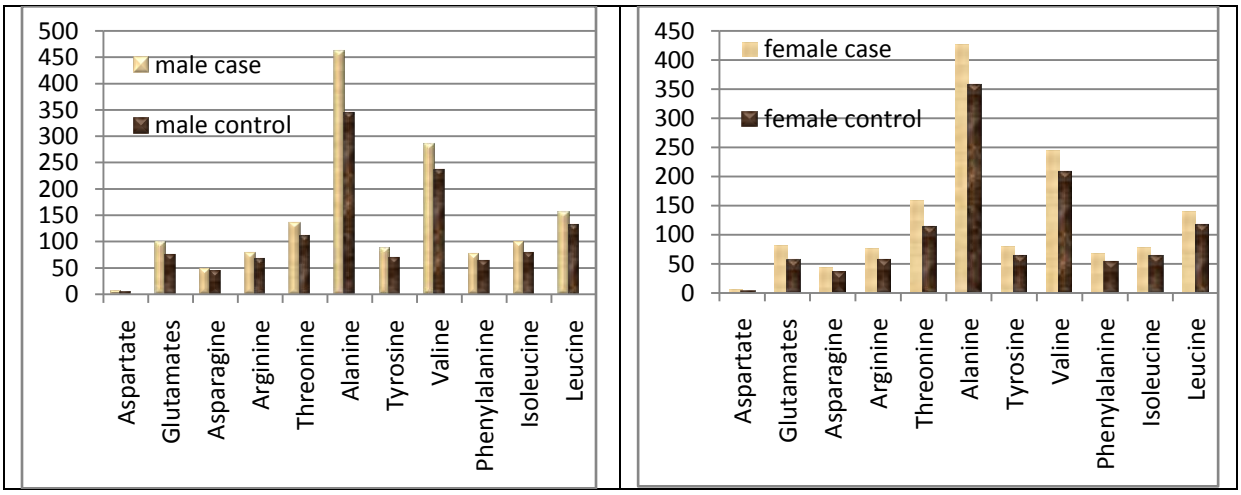


Diagram3:Comparative in mean concentration of free amino acids µm/l in plasma in cases with dermatophyte and control(normal subject) with significant in male and female



Discussion:

The stratum corneum is the first defense layer which stands in the face of pathogenic microorganisms. Identification of the components in stratum corneum can provide plenty of information on its resistance against superficial infections.

One of these compound is filaggrin,that is histidine-rich .In adition to filaggrin's role in the assembly of keratin bundles during terminal differentiation, its hydrolysis is carefully regulated to generate free amino acids that contribute to the water-holding properties of the stratum corneum. The conversion of filaggrin to amino acids is preceded by the dephosphorylation of a large precursor molecule (profilaggrin) that is then susceptible to proteolytic degradation into lower-molecular-weight poly peptides . Proteolysis occurs within the stratum corneum, liberating hygroscopic free amino acids.A blockade of this sequence leads to a build-up of filaggrin and amino acids in the stratum corneum. Without these molecules, the amount of water retained in the stratum corneum is decreased. Because water .Has a profound effect on the plasticity and elasticity of the stratum corneum, its absence is associated with inflexibility, cracking, scaling, and flaking of the skin(20,21).

Profilaggrin and filaggrin. A disturbance in the degradation of profilaggrin, the principal component of keratohyalin granules, may be responsible for a variety of stratum corneum abnormalities(22).

Production of any dermatophyte proteases is repressed by small molecules such as carbohydrates and amino acids.Arthroconidia from infected material are stimulated to germinate by components of the urea cycle and by certain amino acids. **Leucine**, for example, stimulates the germination of *T. mentagrophytes* arthroconidia . Carbohydrates do not stimulate germination of conidia of this species and during log-phase growth most of the proteolytic enzymes of *T. rubrum* are repressible in vitro by small molecules such as amino acids(2,4,5,23,24).

We have any report and investigation about stratum corneum amino acids in patient with dermatophytosis but in this way there are some invitro research reveal coloration between amino acids and dermatophyte.

For examplesome investigation have reported that **L-lusin** were elicited to growth inhibition of *M. gypseum* and **Argenine** also in concentration of 1 and 0.1 gr/dl have inhibitory effects but were not causes complete growth inhibition even in concentration of 1 gr/dl. **Methionin**e also have no effect on *E.floccosum* and were shown mildly effect on *M. gypseum*. The results showed that **L-cysteine hydrochloride**, **L-cysteine, L-aspartic acid, Lglutamic acid** and **DL-tryptophan** and **L-tyrosine** had the most inhibitory effects on the studied dermatophytes, while **Arginine L-lysine** and **L-methionine** had moderate effects and the rest of amino acids had less inhibitory, or even stimulatory effects on the growth of the dermatophytes. *M. canis* and *T. schoenleinii* has a different sensitivity to amino acids. This data indicates that sulfur-containing amino acids and acetic amino acids have greater inhibitory effect against these two dermatophytes(6-8).

In the other study that was done by Garachorlou et al.,reveled that **Aspargin** and **Methionine** amino acids causes decrease in the *T.rubrum* and *T.verrucosum* growth and the inhibitory effect of **valine** on *T,mentagrophytes* were assessed and shown that concentration of 0.1% **valine** causes maximum decrease in *T.mentagrophytes* growth. In one other study by Garachorlou et al.,reveled that **Histidine** has inhibitory effect on *T. Mentagrophytes* Growth and the inhibitory effect of study the inhibitory effect of **Tryptophan** on *T. verrucosum* were assessed and shown that

concentration of 1% **Tryptophan** causes maximum decrease in *T. verrocosum* growth and that **Tryptophan** causes growth decreasing in *E. floccosum*(9-12).
Acidic amino acids also either was shown inhibitory effect on two dermatophytes that the **Aspartate(aspartic acid)** inhibitory effects on *M. gypseum* growth were determined in pandy study . Some of amino acids were assayed at 1% concentration for their toxicity against the mycelial growth of two dermatophytes viz., *M. gypseum* and Trichophyton mentagrophytes. **L-Cysteine** hydrochloride exhibited absolute toxicity against both the test pathogens while **DL-Aspatate(aspartic acid)**was found active against *M.gypseum* only. The minimum inhibitory concentrations of **L-cysteine** hydrochloride was found to be 0.5 and 0.4% against *M. gypseum* and *T. mentagrophytes*, respectively, at which it showed mycostatic nature. However, the amino acid exhibited mycocidal activity at 0.9 and 0.8% against *M. gypseum T. mentagrophytes*, respectively(13).

We analyzed the results of each amino acid independently .Most amino acids in stratum corneum in normal skin is common and same in man and woman: **Serine-Glycine-Alanine-Ornithine** about 80% of free amino acida in stratum corneum.
Other amino acid Included essential: **Histidine – Isoleucine – Leucine – Lysine – Methionine -Threonine – Tyrosine – Valine – Phenylalanine** and non-essenttial**: Arginine – Asparagine –Citrulline -Glutamic acid – Glutamine – Taurine –Carnosine** that have Lowest concentrations in the skin.
And it is natural that smaller amounts of essential amino acids are present in the skin. Because the stratum corneum of the skin and Whatever secreted surface of skin Includes waste that are often non-reuptake.
In current study how that most amino acids in plasma is: **Glutamine - Alanine - Glycine - Valine - Leucine - Serine - Lysine - Threonine** and it is same and common in man and woman.
Our results for normal skin and plasma are also in very good agreement with the most recent experimental results (14,16,25-32).
We have any report research about stratum corneum amino acids in skin in patients with dermatophytes for comparison whit our data.
Patterns of tests results are very different between people with dermatophytosis in two sites near skin lesion and sole area distribution and also between case and control in sole area.
Our research shows that due to the concentration, amino acids can effect stimulation or inhibition of dermatophytes growth in stratum corneum.
In current study this appears that case and control in sole area had significantly increasion in **Aspartate - Tyrosine – Phenylalanine - Tryptophane** and significantly decreasion in **Citrulline– Ornithine** in case in compare to control. Due to concentration have probably causes Prevent the spread of the disease in people against dermatophytosis because this results (due to concentration) is agreement with invitro research (6-13).
People with dermatophytosis in two site near skin lesion and sole area increased in amino acids near lesion compare with case in sole area: **Glutamates - Asparagine - Histidine - Glutamine - Arginine - Citrulline - Threonine - Methionine - Leucine – Ornithine.** probably stimulated the dermatophytes growth.This results is agreement with previous invitro research.Because arthroconidia are stimulated to germinate by components of the urea cycle (**Arginine – Ornithine – Citrulline)** and by certain amino acids **Leucine**, for example, stimulates the germination of Ttrichophyton mentagrophytes arthroconidia.amino acids (**Glutamates - Glutamine - Arginine – Ornithine – Citrulline)** best of nitrogen source and **Asparagine - Histidine** good nitrogen source for fungus (1,2,23,24,33,34).
In current study in plasma show that most amino acids in plasma is: **Glutamine - Alanine - Glycine - Valine - Leucine - Serine - Lysine - Threonine** and it is same and common in man and woman.
Achieved results in plasma have shown that people with dermatophytosis distribution in associated were significantly increased in**: Aspartate - Glutamates - Asparagine - Arginine - Threonine - Alanine - Tyrosine - Valine - Phenylalanine - Isoleucine – Leucine** and have same results in male and female .

This study have led to an understanding of mechanisms, and variation and virulence factor. We need more research for confirm or refute the suggestion that these changes in amino acids are primary or secondary otherwise active or passive, or a way to fight against the further spread within the body or to invade region is limited or stimulation factor depended on concentration

References

1- Rippon J W(1982). Medical mycology; the pathogenic fungi and the pathogenic actinomycetes, Eastbourne, UK; WB Saunders Company.

2- Rippon J(1988). Dermatophytosis and dermatomycosis. Medical mycology, **3**: 169-275.

3- Weitzman I, Summerbell R C (1995). The dermatophytes. Clinical microbiology reviews, **8**(2): 240-259.

4- Vermout S, Tabart J et al(2008). Pathogenesis of dermatophytosis. Mycopathologia, **166**(5-6): 267-275.

5- Achterman R R, White T C(2012). Dermatophyte virulence factors: identifying and analyzing genes that may contribute to chronic or acute skin infections. International journal of microbiology Volume 2012 , Article ID 358305, 8 pages.

6- Sarasgani M, Firoozraee M(2006). The Effect of Amino Acids on the Growth of Epidermophyton Floccosum and Microsporum Gypseum. Razi Journal of Medical Sciences, **13**(51): 131-138.

7- Sarasgani M, Firoozrai M, Hashemi S J(2008). The effect of amino acids on the growth of Microsporum canis and Trichophyton schoenleinii. Tehran University Medical Journal, **6**6(3): 158-164.

8- Sarasgani M, Firoozrai M(2010). The Effect of Amino Acids on the Growth of Trichophyton rubrum and Trichophyton verrucosum. Razi Journal of Medical Sciences, **17**(77): 32-39.

9- Gharachorlou A, Gharachorlou S(2011). Inhibitory Effect of Valine on Trichophyton Mentagrophytes in Normal and Dermatophytic Patients under in Vivo and in Vitro Conditions. Advances in Environmental Biology, **5**(6): p 1198.

10- Gharachorlou A, Issabeagloo E(2011). Survey of Methionine Effects on Epidermophyton Floccosum Growth in Normal and Dermatophytic Patients under in Vivo and in Vitro Conditions. Advances in Environmental Biology, **5**(6): p 1303.

11- Gharachorlou A, Hashemi s J et al(2011). Survey of methionine and aspargine amino acids effects on trichophyton rubrum and trichophyton verrucosum growth in safe peoples and suffering peoples to dermatophytosis under in-vivo and in-vitro conditions. international journal of academic research, **3**(1):50-153.

12- Gharachorlou A, Ghorbani A et al(2012). Survey of tryptophan effects on trichophyton verrucosum growth in normal and dermatophytic patients under in vivo and in vitro conditions. Annals of Biological Research, **5**(7):1984-1987.

13- Pandey D, Chandra H et al(1983). Antimycotic activity of some amino acids against dermatophytes. Arzneimittel-Forschung, **34**(5): 554-556.

14- Takahashi M, Tezuka T(2004). The content of free amino acids in the stratum corneum is increased in senile xerosis. Arch Dermatol Res, **295**(10): 448-452.

15- Fürst P, Pollack L et al(1990). Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. Journal of Chromatography A, **499**: 557-569.

16- Liu H, Worthen H(1992). Measurement of free amino acid levels in ultrafiltrates of blood plasma by high-performance liquid chromatography with automatic pre-column derivatization. Journal of Chromatography B: Biomedical Sciences and Applications, **579**(2): 215-224.

17- Mark H, Harding C R(2013). Amino acid composition, including key derivatives of eccrine sweat: potential biomarkers of certain atopic skin conditions. International journal of cosmetic science, 35(2):163-8.

18- Di Martino C, Delfine S et al(2003). Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. New phytologist, **158**(3): 455-463.

19- Westgard J O, Barry P L et al(1981). A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem, **27**(3): 493-501.

20- Scott I R, Harding C R et al(1982). Histidine-rich protein of the keratohyalin granules: Source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum. Biochimica et Biophysica Acta (BBA)-General Subjects, **719**(1): 110-117.

21- Jackson S M, Williams M L et al(1993). Pathobiology of the stratum corneum. Western journal of medicine, **158**(3): 279.

22- Mendez-Tovar L J(2010). Pathogenesis of dermatophytosis and tinea versicolor. Clinics in dermatology, 28(2):185-189.

23- Kunert J(2000). Physiology of keratinophilic fungi. Revista Iberoamericana de Micologia, 77-85.

24- Peres N T d A, Maranhão F C A et al(2010). Dermatophytes: host-pathogen interaction and antifungal resistance. Anais brasileiros de dermatologia, **85**(5): 657-667.

25- Pratzel H, Fries P(1977). Modification of relative amount of free amino acids in the stratum corneum of human epidermis by special factors of the environment. I .The influence of UV-irradiation (author's transl). Arch Dermatol Res, **259**(2): 157-160.

26- Koyama J, Horii I et al(1984). Free amino acids of stratum corneum as a biochemical marker to evaluate dry skin. J Soc Cosmet Chem, **35(4):** 183-195.

27- Horii I, Nakayama Y et al(1989). Stratum corneum hydration and amino acid content in xerotic skin. British Journal of Dermatology, **121**(5): 587-592.

28- Denda M, Hori J et al(1992). Stratum corneum sphingolipids and free amino acids in experimentally-induced scaly skin. Arch Dermatol Res, **284**(6): 363-367.

29- Liu H(2000). Measurement of blood plasma amino acids in ultrafiltrates by high-performance liquid chromatography with automatic precolumn o-phthaldialdehyde derivatization. Method Mol Biol, 159**:** 123-140

30- Sylvestre J P, Bouissou C C, et al(2010). Extraction and quantification of amino acids in human stratum corneum in vivo. Br J Dermatol, **163**(3): 458-465.

31- Visscher M O, Robinson M et al(2011). Amputee skin condition: occlusion, stratum corneum hydration and free amino acid levels. Archives of dermatological research, **303**(2): 117-124.

32- Rawlings A V, Voegeli R(2013). Stratum corneum proteases and dry skin conditions. Cell and tissue research, **351**(2): 217-235.

33- Kunert J(1981). Organic sulphur sources for the growth of the dermatophyte Microsporum gypseum. Folia microbiologica, **26**(3): 201-206.

34- Kunert J(1985). Metabolism of sulfur-containing amino acids in the dermatophyte Microsporum gypseum I. Neutral amino acids. Journal of basic microbiology, **25**(1): 29-37.