Clinical and Experimental Health Sciences

Serum Amino Acid Profile in Chronic Sinusitis

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ABSTRACT

Objective: Serum amino acid profile is known to vary in many diseases. The changes in the serum amino acid profile provide important information about diseases and the effectiveness of treatment. The aim of this study is to investigate whether serum amino acids are effective in the development of CRS.

Methods: A total of 23 healthy volunteers between the ages of 20 and 40 years were allocated to the control group (Group 1) and 27 patients with chronic rhinosinusitis were allocated to the study group (Group 2). The patients whose history, symptom, and examination findings were consistent with chronic sinusitis and who also who had sinusitis findings on a paranasal sinus tomography and were diagnosed with chronic sinusitis for at least one year were included in Group 2. A total of 32 serum-free amino acid levels were measured in both group using the LC-MS/MS system. In the study, the JASEM amino acid kit was used for LC-MS/MS analysis.

Results: The serum taurine level was found to be statistically significantly lower in Group 2 compared to Group 1 (p:0.002). A significant alteration was not observed in the serum levels of remaining 31 amino acids.

Conclusion: According to the data we obtained from the study, decreased serum taurine level may be a factor in the etiopathogenesis of chronic rhinosinusitis and therefore taurine supplementation may be considered as a new therapeutic target in the treatment of chronic rhinosinusitis.

Keywords: Chronic sinusitis; Taurine; Human serum; Amino acids; LC-MS/MS

1. INTRODUCTION

Rhinosinusitis (RS) is the inflammation of nasal mucosa and paranasal sinus mucosa. It is associated with symptoms including nasal obstruction, post-nasal discharge, facial pain and a sensation of pressure, a smelling disorder, cough. and a sensation of fullness in the ear (1-3). RS develops primarily due to viral but also bacterial and fungal infections (4). The disease is defined as chronic rhinosinusitis (CRS) if symptoms and signs of the inflammation last for longer than three months. CRS is a chronic inflammatory disease which may develop due to both infectious and non-infectious factors (5). CRS is a very common disease and represents an important public health problem. In Europe, a high prevalence of up to 19.7% were reported (6). This ratio is between 14-16% in America (7).

CRS is a multi-factorial disease. The causes include anatomic, congenital, genetic, neoplastic, allergic and endocrinologic factors; sinonasal ciliary dysfunction; and smoking (8-9). The exact etiology and mechanism of CRS has not yet been revealed. The pathophysiology of sinusitis should be investigated to determine two important points in detail. One of them is that acute sinusitis is not recovered completely and the second one is that acute sinusitis tuns into chronic inflammatory disease

Many scientific studies have investigated regarding the relationship between diseases and biomolecules like amino acids, metabolites, enzymes and hormones for better understanding the pathophysiology of the diseases. Amino acids are required for the synthesis of many important molecules like protein, hormones, and neurotransmitters (10). Amino acids also regulate the immune response that defends the body against diseases through the activation of T and B lymphocytes, natural killers and macrophages; cellular redox including gene expression and lymphocyte proliferation; and producing antibodies, cytokines and other cytotoxic substances (11). For example, taurine is found in high concentrations in phagocytes and inflammatory lesions (12).

Taurine, a sulfur amino acid, is the most abundant amino acid in the body. Taurine plays a role in cyto-protection

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and the regulation of inflammation through protecting the tissue against oxidative damage. It has been suggested that a deficiency in taurine influences immune cell functions since it is found in high levels in leukocytes (13,14). While taurine plays a role in many physiologic events, it is found in high concentrations in inflammatory cells where oxidative activity is high and in retina, kidney, heart tissues where oxidative products are at high levels (15-16).

In various studies in the literature, the association between different disease groups like oncologic diseases (hepatocellular cancer (17), breast cancer and lung cancer (18),colon cancer (19), renal cell cancer (20), cervical intra-epithelial neoplasia and cervical squamous cell cancer (21)), metabolic disorders including diabetes mellitus and metabolic syndrome (22), renal disorders (23), sepsis (24), nasal polyposis (25), and serum/plasma amino acid profiles was investigated with regard to biochemical parameters.

Based on the data mentioned above, it was suggested that patients with CRS could have an altered serum amino acid profile. For these analyses, the strongest technique, the LC-MS/MS method, was used for detection of the amino acid profile of the patients with CRS and discussed light of the literature.

2. METHODS

2.1. Materials

Ethics committee approval was obtained from the Erzurum Regional Research and Training Hospital (2020/14-157). Twenty-three healthy volunteers between the ages of 20 and 40 (Group 1) and 27 patients with CRS (Group 2) who were admitted to Erzurum Regional Research and Training Hospital in Turkey were included in the study. Healthy volunteers who were matched with the patient group for age, gender, and body mass index were included in Group 1. A detailed medical history was obtained from all participants and a basic rhinologic examination, a nasal endoscopic examination, and a routine ear-nose-throat examination were done. The patients whose history, symptom, and examination findings were consistent with chronic sinusitis and who also who had sinusitis findings on a paranasal sinus tomography and were diagnosed with chronic sinusitis for at least one year were included in Group 2.

Exclusion criteria were as follows: smoking and/or alcohol use, atopic dermatitis, asthma, nasal polyposis, acute sinusitis, chronic drug use, a history of systemic or topical steroid use for any reasons during the last three months, pregnancy, lactation, the presence of an upper respiratory tract infection during the study, and hepatic, renal, hematologic, cardiovascular, metabolic, neurologic, of psychiatric disorders or malignity (25).

In this study, JASEM quantitative amino acid LC-MS/MS kits were used. These kits include amino acid standards, internal standard, analytical column and mobile phase (JASEM Laboratory Systems and Solutions A.S).

2.2. Analytic Chemical Analysis

2.2.1. Measurement of Free Amino Acids

Blood samples were taken into gel tubes from the antecubital vein following one night of fasting. Biochemistry tubes were centrifuged at 3500 rpm for 10 min to separate the serum, and serum samples were stored at – 80 °C until the day of examination and tested after they reached room temperature.

For both patient and control groups, 50 µL of serum samples were transferred into Eppendorf tubes, 50 µL internal standard was added to them, and they were vortexed for 10 sec. 700 µm of amino acid solvent solution (Mobile Phase A: Mobile Phase B, V:V:1:4) was added to each tube. Each sample was vortexed for one min and centrifuged at 3000 rpm and at 4 °C for eight min thereafter. The superior phase was separated and filtered using 0.45 µm filters. Samples were measured in triplicate with the LC-MS/MS system (Agilent 6460 Triple Quadropol, USA). Chromatography and mass spectrometry conditions of the LC-MS/MS method that was used for the separation and definition of amino acids are presented in Tables 1 and 2. Thirty-two amino acids defined as the result of the analysis are listed in table 4. Table 5 gives m/z values for amino acids. MRM chromatograms of the control and patient groups of amino acids obtained from the study are given in figure 2. MRM chromatogram and mass spectrum of the control and patient groups of taurine are also given in figure 3 and figure 4, respectively.

Table 1. *Solvent Composition Schedule during the gradient elution for LC-MS/MS*

Time Flow: 0.7 mL/min Column	Change Solvent Composition A: MPA (Jasem AA Kit) AA Kit) Jasem AA Column	B: MPB (Jasem
1.00 min	78.00 %	22.00 %
4.00 min	70.00 %	30.00 %
5.00 min	70.00 %	30.00 %
5.10 min	22.00 %	78.00 %
9.00 min	22.00 %	78.00 %

Table 2. *Mass conditions*

Parameters	Value (+)	Value(-)
Gas Temp (°C)	150.00	150.00
Gas Flow (L/min)	11.00	11.00
Nebulizer (psi)	40.00	40.00
SheathGasHeater	375.00	375.00
SheathGasFlow	11.00	11.00
Capillary (V)	2000.00	0.00
VCharging	0.00	0.00
Injection Volume (µl)	1.00	
Ion source	AJS ESI	
Ion Mode	Positive	

2.3. Statistical Analysis

An independent samples t test was used for the comparison of the mean differences between groups. Analyses were done using the IBM SPSS 20.0 package program. A p level of<0.05 was accepted as statistically significant.

Table 3. *Demographic data of groups 1 and 2*

Variable	Grup 1 (n=23)	Grup 2 (n=27)	p-value
Gender(Male/Female)	11/12	14/13	p:0,654
Age(years)	33,50±5,30	34,26± 4,72	p:0,780
BMI (kg/m2)	25,1±1,20	24,8±1,16	p:0,802

Table 4. *Serum free amino acid values (nmol/mL) in patients with CRS compared with controls.*

Amino Acids	Group 1 (n:23) Mean±SD	Group 2 (n:27) Mean±SD	p-values
1-Methyl-L-Histidine	5,86±5,74	3,18±1,54	0,172
3-Amino Isobutyric acid	1,93±1,26	1,95±1,27	0,977
3-Methyl-L-Histidine	7,58±1,79	7,27±2,96	0,777
Beta-Alanine	1,28±0,69	1,12±0,51	0,569
DL-5-Hydroxy Lysine	1,48±0,34	1,30±0,48	0,341
Ethanolamine	8,46±4,22	9,10±7,32	0,575
Gamma-aminobutyric acid	0,35±0,13	0,40±0,19	0,569
L-2-Aminobutyric acid	2,97 ±1,89	3,41±2,96	0,699
L-Alanine	596±198	653±391	0,691
L-Anserine	23,48±5,90	25,84±16,25	0,672
L-Arginine	86,26±31,34	81,39±47,78	0,790
L-Asparagine	62,28±17,57	62,75±33,13	0,969
L-Carnosine	10,51±4,46	11,78±6,94	0,632
L-Citrulline	34,31±10,70	27,37±13,18	0,212
L-Cystine	53,55±24,76	50,08±32,03	0,789
L-Glutamine	547,13±131,71	557,79±286,24	0,916
L-Glycin	478,58±200,78	495,83±269,25	0,873
L-Histidine	92,46±20,72	94,65±43,57	0,887
L-Isoleucine	83,67±30,52	79,50±48,17	0,819
L-Leucine	167,60±43,62	174,05±107,04	0,862
L-Lysine	232,72±68,92	250,16±141,81	0,731
L-Methionine	33,21±11,81	30,49±16,28	0,674
L-ornithine	148,23±62,09	148,10±78,20	0,981
L-Phenylalanine	110,14±31,53	124,76±76,50	0,583
L-Proline	330,71±163,51	346,13±225,04	0,863
L-Serine	195,20±55,47	203,60±104,80	0,825
L-Threonine	150,56±57,25	158,35±82,51	0,809
L-Tryptophan	72,04±18,47	65,54±38,29	0,635
L-Tyrosine	76,13±24,15	80,42±52,32	0,816
L-Valine	251,90±57,57	258,66±144,30	0,892
*Taurine	104,15±20,01	59,69±34,28	0,002
Trans-4-hydroxy L-proline	8,08±3,14	6,55±3,06	0,286

**: p<0.05*

Table 5. *m/z values for amino acids*

Amino Acids	m/z
1-Methyl-L-Histidine	170.1→126.2
3-Amino Isobutyric acid	104.1→86.2
3-Methyl-L-Histidine	170.1→124.1
Beta-Alanine	90.1→72.1
DL-5-Hydroxy Lysine	163.1→128.1
Ethanolamine	62.1→44.2
Gamma-aminobutyric acid	104.0→87.1
L-2-Aminobutyric acid	104.2→58.3
L-Alanine	90.2→44.2
L-Anserine	241.1→109.1
L-Arginine	175.2→70.2
L-Asparagine	133.1→74.2
L-Carnosine	227.1→110.1
L-Citrulline	176.4→159.3
L-Cystine	241.0→74.2
L-Glutamine	147.1→84.2
L-Glycin	76.2→30.1
L-Histidine	156.1→110.1
L-Isoleucine	132.2→69.2
L-Leucine	132.2→43.3
L-Lysine	147.1→84.2
L-Methionine	150.1→104.1
L-ornithine	133.2→70.3
L-Phenylalanine	166.1→120.1
L-Proline	116.2→70.2
L-Serine	106.2→60.2
L-Threonine	182.1→165.0
L-Tryptophan	205.1→188.1
L-Tyrosine	182.1→165.0
L-Valine	118.2→72.2
Taurine	126.1 →44.3
Trans-4-hydroxy L-proline	133.2→68.2

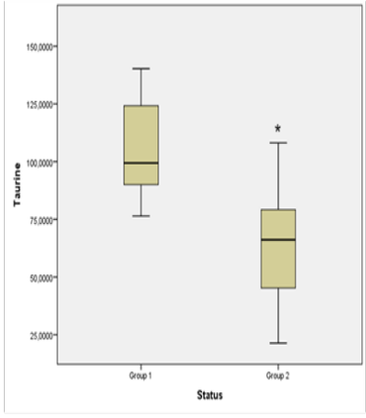

Figure 1. *Box-plot distribution of serum Taurine levels in Group 1(control) and Group 2 (patient).*Significant at p < 0.05 when compared to control*

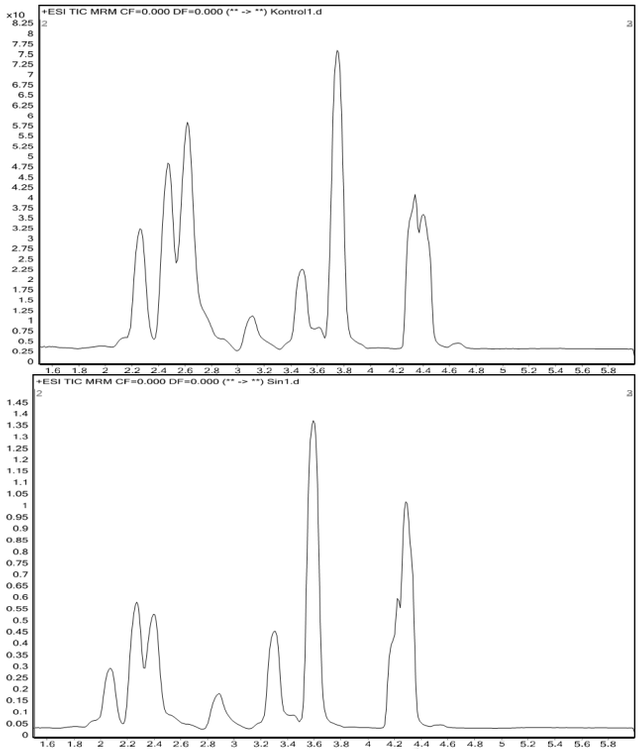

Figure 2. *Typical MRM chromatogram for amino acids obtained from the control group (A) Typical MRM chromatogram for amino acids obtained from the control group (B).*

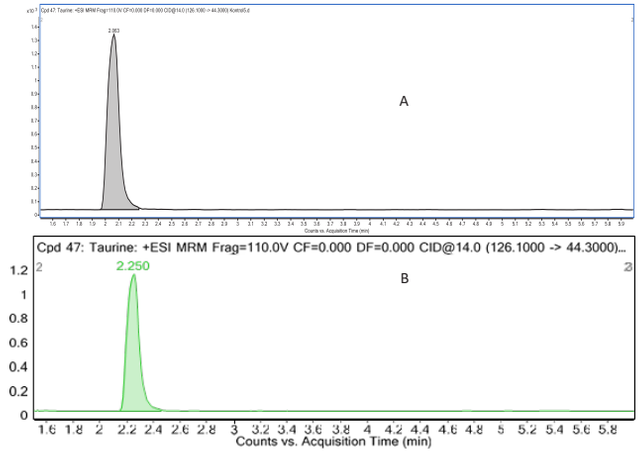

Figure 3. *Typical MRM chromatogram for Tauirine MRM choromatogram: A Control Group, B Patient Group*

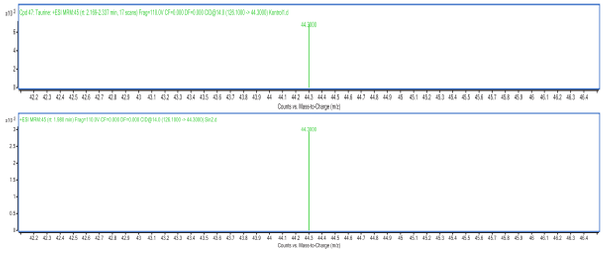

Figure 4. *Product ion mass spectra for Taurine: A Control Group, B Patient Group*

3. RESULTS

The average age of the individuals is 33,50±5,30 in group 1 and 34,26± 4,72 in group 2, and the average age between the two groups is similar (table 3).In Group 1, 11 patients were male and 12 were female while Group 2 included 14 males and 13 females. The female/male ratio was similar between groups and the mean BMI values were similar between groups (table 3). The serum-free taurine level was found to be statistically significantly lower in Group 2 compared to Group 1 (table 4, figure 1). A significant difference was not found between groups with regard to the remaining 31 free amino acid levels (table 4).

4. DISCUSSION

In CRS, long term neutrophil infiltration in paranasal sinuses and ROS are proposed to lead to the infection becoming chronic (26). Neutrophil granulocytes release proteolytic enzymes, and the protease amount exceeds antioxidant capacity and leads to tissue damage in the nasal and paranasal mucosa (27). Oxidative and immune-active products may continue inflammation even after the infection has been treated (28).

The main function of immune reactions is to fight micro-organisms and other foreign bodies (26). When the natural immune response is activated, it leads to the degranulation of inflammatory cells, the rapid release of reactive oxygen species (ROS) including the superoxide anion radical and the hydrogen peroxide radical. A balance exists between ROS production and elimination in most cellular processes of the organisms (29,30). This balance is preserved by endogenous antioxidant mechanisms (31). Hemostasis is impaired in the ROS over-expression resulting in oxidative stress, and tissue damage occurs through triggered inflammation (29,30). Additional protective mechanisms that restrict the severity of tissue damage like antioxidant defense mechanisms contribute to the control of the disease (31).

In various studies, it has been reported that taurine shows a cyto-protective effect due to its antioxidant activity (32,33). Taurine, whose antioxidant, antimicrobial, and anti-inflammatory activity is well known, was found to be lower in the serum of the patients with CRS in our study compared to the healthy control group. This suggests that taurine may be effective in the physiopathology and/or treatment of CRS, one of the chronic inflammatory diseases.

When neutrophil granulocytes, which are the dominant immune cells in inflammation, are activated, the myeloperoxidase enzyme is released into the extracellular environment. Taurine reacts with the enzyme products hypohalous acids (HOCl, HOBr) to form TauCl and TauBr products (34,35). Since TauCl is a less potent oxidant than HOCl, neutralization of HOCl represents one of the important antioxidant mechanisms of taurine. This reaction, which is catalyzed by myeloperoxidase, is also responsible for the anti-inflammatory activity of taurine since TauCl inhibits

the production of pro-inflammatory cytokines, inhibits the increase of nitric oxide and prostaglandin E2, reduces the activity of matrix metalloproteinases, and initiates leukocyte apoptosis to terminate acute inflammation (16,35).

The antimicrobial, antioxidant, and anti-inflammatory effects of TauCl and TauBr have enabled these agents to be used clinically, especially in the local treatment of infectious and inflammatory diseases (35). Nagl et al. reported that a 1-2% Lyc topical solution of N-chlorotaurine (NCT), a N-chloral derivative of taurine, was well tolerated by patients with bacterial conjunctivitis and was effective within 3-5 days (36). In the study by Neher et al., it was reported that NCT is a very well tolerated and highly effective drug for external otitis (37). In another study conducted by Neher A. et al., irrigation of nasal and paranasal sinuses was performed three times a week for a month in patients with chronic sinusitis, and NCT was applied. No toxic side effects were observed in the patients, and positive results including an increase in nasal breathing and ability to smell were obtained. It is not clear whether the positive results were due to NCT or irrigation (38). In the study by Nagl M. et al., the use of 1% NCT for five days was sufficient to eliminate the clinical signs of infection in infected leg ulcers, and NCT has also been reported to significantly suppress pain and granulation tissue formation and to accelerate re-epithelization (39). In addition, studies are available reporting that it is effective against the HIV27 virus (40).

Marcinkiewicz et al reported that the use of topical TauBr in acne vulgaris patients resulted in clinical improvement and could be a new treatment option for inflammatory acne (41).

In addition to successful topical treatments for various diseases, the rapid degradation of TauCl and TauBr in the systemic circulation limits the systemic use of these agents (35). The use of 5-aminosalicyltaurine (5-ASA-Tau), a colon-specific pro-drug administered orally in previous experimentally induced colitis (42) and dietary taurine supplementation in experimentally induced colitis mice in another study (43) yielded successful treatment outcomes. Based on these data, taurine supplementation can be considered a systemic treatment option when necessary in the treatment of diseases.

5. CONCLUSION

The result of this study showed that decreased serum taurine levels are associated with CRS. Identification of this relationship has shown that serum taurine levels may be another factor in the aetiology of CRS. In addition, both in vitro and clinical studies have shown that the use of taurine and taurine derivatives in the treatment of many infections and inflammatory diseases showed positive results. Based on this, we consider that taurine supplementation and/or nasal topical administration in CRS can be a supportive treatment option.

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