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ORIGINAL ARTICLE

ROLE OF PLASMA AMINO ACIDS AND GABA IN ALCOHOLIC AND NON-ALCOHOLIC FATTY LIVER DISEASE- A PILOT STUDY

S Mukherjee, K Vaidyanathan, D M Vasudevan and S K Das*

*Department of Biochemistry, Amrita Institute of Medical Sciences, Elamakkara P.O., Cochin 682 026, Kerala, *Agartala Govt Medical College, Kunjaban P.O., Agartala 799 006, Tripura, India*

ABSTRACT

Alcohol appears to affect brain function, primarily by interfering with the action of gamma-aminobutyric acid (GABA) and other neurotransmitters. As alcohol is mainly metabolized in the liver, therefore we undertook this pilot study to monitor the patterns of changes in plasma amino-acid concentrations due to alcoholic and non-alcohol fatty liver disease and their relation with plasma GABA level. Plasma amino-acid concentrations were measured in 25 alcoholic liver disease (ALD) patients, 18 non-alcoholic fatty liver disease (NAFLD) patients, and 24 age and sex matched control subjects by HPLC. GABA concentration was elevated, while isoleucine and leucine levels reduced significantly in ALD patients compared to the control subjects. Methionine and phenylalanine levels elevated and valine content reduced significantly in ALD patients compared to other two groups, and GABA level was significantly correlated with methionine and phenylalanine. Plasma concentration of lysine was significantly reduced in both groups of liver disease patients compared to the control group, but was not correlated with GABA level. Glycine and tyrosine levels reduced significantly in NAFLD patients compared to other two groups and were significantly correlated with GABA. Interestingly, though amino acids such as alanine, histidine, proline and serine were not affected by liver diseases, but were significantly correlated with GABA level. This pilot study indicated that alcoholic liver disease presented a more deranged plasma amino acid pattern than nonalcoholic, and the amino acid imbalances. More studies are necessary to identify the role of any particular amino acid on brain function and on neurotransmitter(s).

KEY WORDS

Alcohol, Amino acids, Gamma aminobutyric acid, Liver, Non-alcoholic fatty liver disease.

INTRODUCTION

Alcoholic beverages have been used in human societies since the beginning of recorded history. The patterns of alcohol intake around the world are constantly evolving and alcohol is ubiquitous today. Research has contributed substantially to our understanding of the relation of drinking to specific disorders and has shown that the relation between alcohol consumption and health outcomes is complex and multidimensional. In addition, alcohol is linked to categories of disease whose relative impact on the global burden is predicted to increase (1). Chronic heavy drinking and alcoholism can have serious consequences for the functioning of the entire nervous system, particularly the brain (2) by interacting with multiple neurotransmitter systems, and thereby disrupting the delicate balance between inhibitory and excitatory neurotransmitters (3). At the cellular level, alcohol appears to affect brain function, primarily by interfering with the action of glutamate, gamma-aminobutyric acid (GABA) and other neurotransmitters (2). Alcohol being soluble both in water and lipids can diffuse rapidly through the mucous membrane of the oesophagous and stomach. After its absorption ethanol appears in both expired air and in urine. It is not stored in the body as whatever is ingested is metabolized mainly in the liver (4).

Address for Correspondence :

Dr. Subir Kumar Das
Department of Biochemistry
Agartala Government Medical College,
Kunjaban P.O., Agartala 799 006, Tripura
E-mail : drsubirkdas@gmail.com

The liver plays a pivotal role in nutrient and hormone metabolism. Metabolic abnormalities are common in liver disease (5). Liver damage is also associated with characteristic changes in the plasma amino acids (6-7). Although certain work done in animals suggests that changes in plasma amino-acids after liver damage may be specifically linked to the nature of the damaging agent, few attempts have been made to relate this work to liver disease in man (8-10). Therefore, we monitored the plasma amino-acid concentrations in alcoholic and non-alcohol fatty liver disease patients to look for patterns of change related to the nature of the damage present and their relation with plasma GABA level in this pilot study.

MATERIALS AND METHODS

All analytical grade chemicals from Sigma Chemical Co., USA. were used. The study protocol was in keeping with the ethical guidelines of the 1975 Declaration of Helsinki and all the patients gave written informed consent to the study. Patients were selected from those who had visited Gastroenterology Department, Amrita Institute of Medical Sciences, Cochin. Institutional ethics Committee approved the procedures. As age and sex both influence plasma amino-acid concentrations (11, 12), therefore only age- matched male subjects were considered in this study.

The patients were broadly classified into two groups: Alcoholic Liver Disease (ALD) and Non-Alcoholic Fatty Liver Disease (NAFLD); on the basis of oral questionnaire, laboratory investigations, clinical findings, and ultrasound/ CT scan imaging or biopsy, where applicable. Inclusion criteria were: i) elevated liver aminotransferases, ii) no findings on investigations suggestive of viral, metabolic or other specific etiologies of liver diseases. Exclusion criteria were: i) recent gastrointestinal surgery, ii) older age (>65 yrs), iii) usage of drugs known to result in steatosis, including glucocorticoids, synthetic esterogens, aspirin, tamoxifen, amiodarone, calcium channel blockers, and methotrexate; or iv) malignancy.

Twenty five ALD, patients, who had been drinking average more than 80g alcohol (2-3 drinks) per day for at least five years, and 18 NAFLD patients were selected in this study. Most ALD patients were steady rather than “spree” drinkers and all were thought to be actively drinking until shortly before presentation. 24 subjects who attended routine health check up programs without any specific problem and found no abnormality in clinical and laboratory findings were considered ‘Control’. All regular alcohol consumers were excluded from ‘NAFLD’ and ‘Control’ groups. Venous blood collected after over night fast was centrifuged to separate the plasma. 400 μl plasma was mixed with 200 μl 10% sulphosalicylic acid at 4°C for 30 min and centrifuged.

100 μl 100 m*M* phenyl isothiocyanate (PITC) and 100 μl 1*M* Triethyl amine (TEA) were added to 200 μl of the supernatant or standard, and allowed to stand at room temperature for 2 h. 400 μl hexane was mixed with 200 μl PITC-TEA-Supernatant mixture and vortexed. After separating the hexane layer, 20 μl membrane filtered (0.45 μm) aqueous layer was injected to HPLC system

HPLC system (332 UV detector model, Shimadzu, Japan) is consists of a 20-μl injection loop and LUNA C18 column (25 cm) fitted with a guard column (1 cm) housed in an incubator oven set at 40°C constant temperature. Amino acids were separated using mobile phase 10 m*M* phosphate buffer, pH 7.0 (solvent A) and acetonirile (solvent B), and gradient was programmed using concentration of B as 5% (0 min), 35% (28 min), 80% (28.01 min), 80% (33 min), 5% (33.01 min), and 5% (39 min). Isocratic HPLC separations were performed with gradient elution at a flow rate 1 μl/min, at 254 nm absorption wave length. 20 ml 0.625 μmole/μl of amino acid standard was used for quantitation of different amino acids. Amino acids were detected based on the retention time established for the individual amino acid under defined experimental conditions. Linearity of the peak areas for different concentrations, ranging from 0.125-0.625 μmoles, of individual amino acids was determined. Calculation was based on the area under peak established for a given amino acid of known standard concentration.

Results are expressed as mean ± SD (standard deviation). All data were analysed using the SPSS statistical package (version 11.0; SPSS Inc., Chicago, IL, USA). The differences were considered significant at $P<0.05$.

RESULTS

The described HPLC method is able to resolve and quantify most of the amino acids present in biological fluids within a total run period of 30 min (Figs 2-5). In this study, GABA concentration was elevated in ALD patients compared to other two groups (Fig 1). Among the branched chain amino acids (BCAA), isoleucine and leucine levels reduced significantly in ALD patients compared to the control subjects, whereas plasma valine concentration reduced significantly in ALD patients compared to other two groups (Table 1) and none of the BCAA significantly correlated with GABA concentration (Table 2). Methionine and phenylalanine levels elevated significantly in ALD patients compared to other two groups

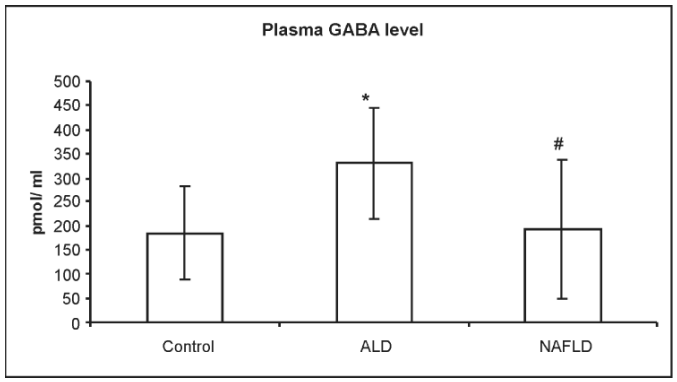

Fig 1: Plasma GABA concentration of control subjects, alcoholic liver disease and non-alcoholic fatty liver disease patients. P values: *<0.01 compared to control group; #<0.01 compared to ALD group

Table 2: Correlation of GABA with different amino acids

Amino acid	r(p)	Amino acid	r(p)
Alanine	0.501(<0.001)*	Arginine	0.03 (0.837)
Cystine	0.073 (0.595)	Glycine	0.507(<0.001)*
Histidine	0.617(<0.001)*	Isoleucine	-0.123 (0.406)
Leucine	-0.05 (0.718)	Lysine	-0.149 (0.277)
Methionine	0.424 (0.002)*	Phenyl alanine	0.444 (0.001)*
Proline	0.409(0.001)*	Serine	0.41 (0.002)*
Threonine	0.09 (0.532)	Tyrosine	0.448 (0.001)*
Valine	-0.241 (0.076)		

*Correlation is significant at <0.01 (2-tailed).

(Table 1) and were significantly correlated with GABA level (Table 2). Plasma concentration of lysine was significantly reduced in both groups of liver disease patients compared to the control group (Table 1) but was not correlated with GABA (Table 2). Glycine and tyrosine levels reduced significantly in NAFLD patients compared to other two groups (Table 1) and were significantly correlated with GABA level (Table 2). Interestingly, though amino acids such as alanine, histidine, proline and serine were not affected by liver diseases (Table 1), but were significantly correlated with GABA level (Table 2). However, arginine, cystine and threonine were neither affected by liver diseases (Table 1) nor significantly correlated with GABA level (Table 2).

DISCUSSION

Amino acids are lipid insoluble and their passage across biological membranes depends on carrier-mediated transport systems with varying affinities for different amino acids (13). In contrast to the finding in whole blood, the plasma level of aspartate is increased (14). This indicates that a derangement of transport could be responsible for the changes in aspartate.

Table 1: Plasma amino acid concentrations in control subjects, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) patients (μ mol/ L)

	Control (n=24)	ALD (n=25)	NAFLD (n=18)	F-variance	Significance	Normal range*
Alanine	361.1 ± 190.7	391.9 ± 103.3	323.5 ± 117	1.432	0.248	240-482
Arginine	102.6 ± 23.8	101.5 ± 42.9	118.8 ± 18.9	1.36	0.266	68-128
Cystine	6.75 ± 3.33	8.5 ± 3.8	6.3 ± 2.6	2.657	0.08	36-61
Glycine	261.2±124.8	254.3 ± 56.8	180.1± 86.3[cf]	4.915	0.011	183-322
Histidine	50.2 ± 21.4	63.9 ± 28.4	43.3 ± 28.8	3.138	0.052	77-107
Isoleucine	65.4 ± 6.4	46.3 ± 22.1[c]	59.7 ± 16.8	4.459	0.017	47-74
Leucine	138.6 ± 17.6	93.2 ± 37.7[b]	120.8± 36.9	7.994	<0.001	101-159
Lysine	207 ± 31.5	157.7 ± 42.6[b]	153.8 ± 35.1[b]	8.424	<0.001	157-242
Methionine	28.7 ± 3.6	51.3 ± 31.7[c]	21.3 ± 4.9[d]	10.057	<0.001	20-34
Phenyl alanine	72.7 ± 14.8	88.1 ± 19.4[c]	65.4 ± 13.4[d]	10.181	<0.001	47-74
Proline	179.7 ± 14.7	203.2 ± 69.5	163.3 ± 60.1	2.409	0.1	113-271
Serine	107.5 ± 90.6	118.8 ± 46.7	83.3 ± 77.2	1.389	0.258	101-177
Threonine	84 ± 5.4	105.4 ± 42.8	85.9 ± 21.9	2.442	0.098	104-188
Tyrosine	81.2 ± 24.1	88.2 ± 46.6	48 ± 19.4[ce]	7.135	0.002	46-87
Valine	227.1 ± 45.9	135.1 ± 62.5[a]	188.3± 39.7[e]	13.59	<0.001	178-275

P values: [a]<0.001, [b]<0.01, [c]<0.05 compared to control group; [d]<0.001, [e]<0.01, [f]<0.05 compared to ALD group. NA: not available; *(Ref. No. 27)

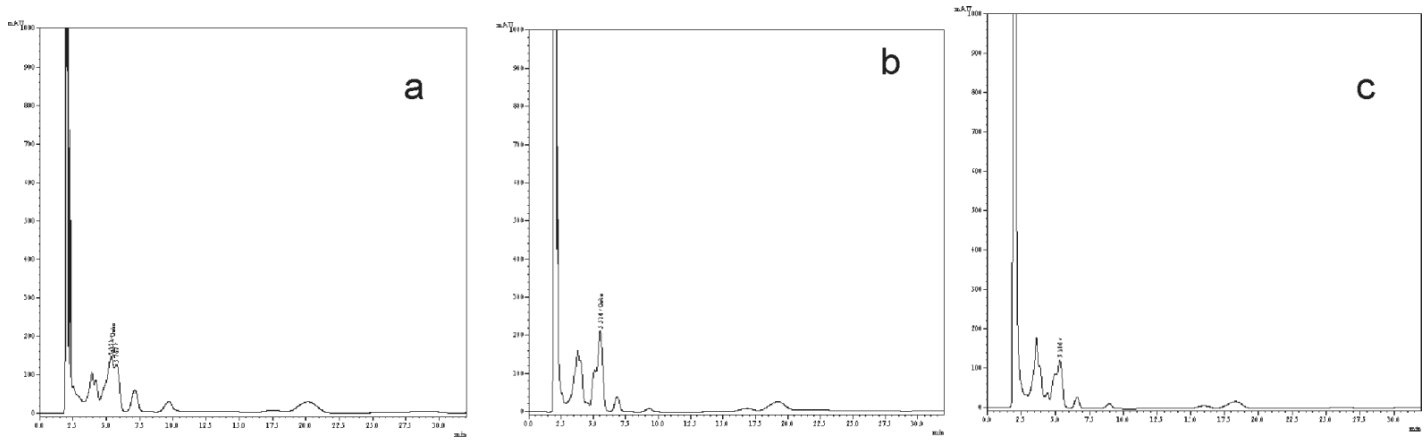

Fig 2: HPLC chromatogram for GABA of control subjects (a), alcoholic liver disease patients (b), and non-alcoholic fatty liver disease patients (c)

Intracellular aspartate levels are reported to be forty- to fifty folds greater than the plasma concentrations (15). In addition, there is difficulty in obtaining reliable results from analysis for tryptophan, glutamic acid and glutamine (12). Therefore, these amino acids have been omitted from this study.

Gamma amino butyric acid (GABA) is an inhibitory neurotransmitter; it decreases the activity of neurons. Excess GABA may cause decreased attention, memory alterations, mood changes and drowsiness (3). It was suggested that alcohol facilitates GABA function (16). In one study GABA serum levels showed a significant 10-fold increase in liver cirrhosis patients (17) and in this study, plasma GABA concentration increased in ALD patients compared to the other two groups (Fig 1).

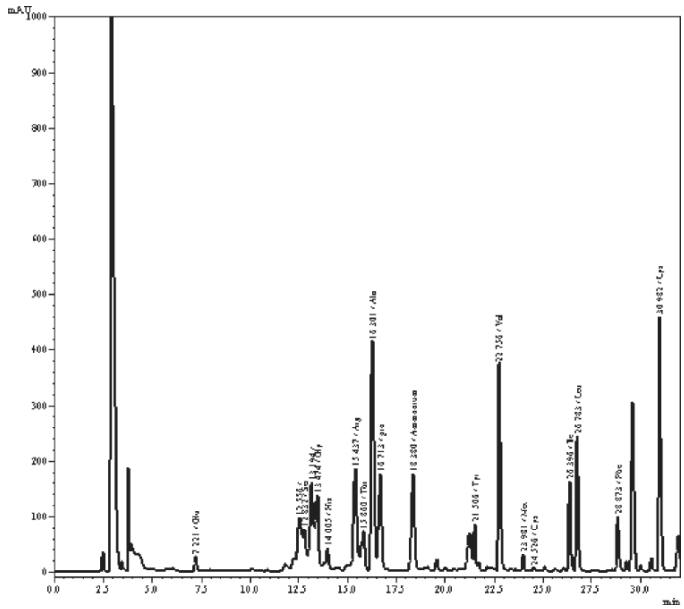

Fig 3: HPLC chromatogram of amino acids for control subjects

Glycine accomplishes several functions as a transmitter in the CNS (3). Though the patients with alcoholic liver disease showed reduced concentrations of plasma glycine level in one study (14), our study showed that the plasma glycine level reduced insignificantly in ALD subjects compared to the control group, but significantly in NAFLD subjects compared to other two groups (Table1). Moreover, this glycine level was significantly correlated with plasma GABA level (Table 2).

Proline is used in collagen synthesis, and hyperprolinemia has been proposed as a marker of fibrogenesis. While, some studies reported elevated level of plasma proline in ALD patients (10, 18-19), other studies reported decreased (20) or

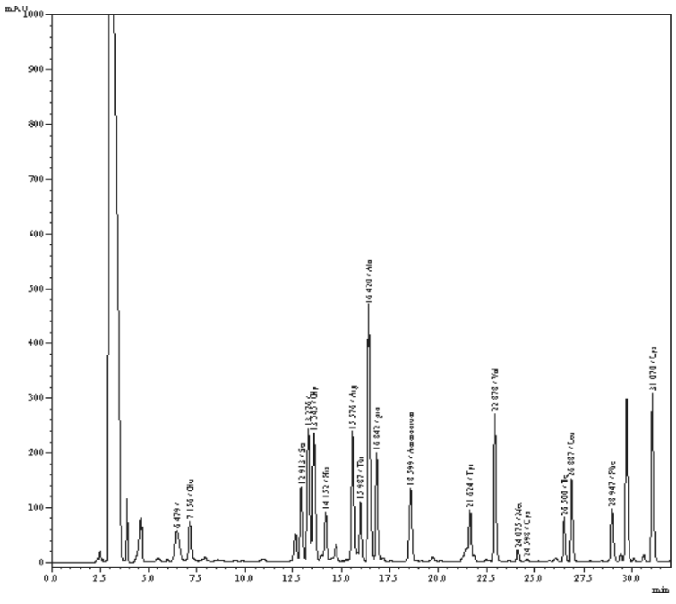

Fig 4: HPLC chromatogram of amino acids for alcoholic liver disease patients

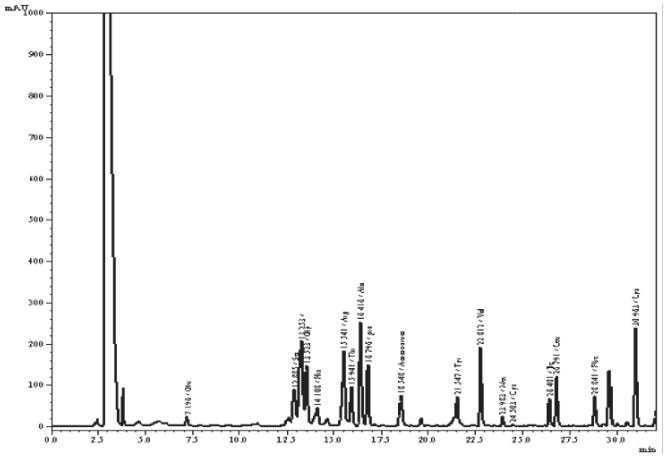

Fig 5: HPLC chromatogram of amino acids for non-alcoholic fatty liver disease patients

normal level (21) compared to the control group. It was also reported that patients with nonalcoholic cirrhosis or chronic active liver disease had serum proline value similar to those of normal subjects (10). In another study, plasma proline level was found significantly higher in alcoholic than nonalcoholic liver cirrhosis (22). However, in this study, mean plasma proline level elevated insignificantly in ALD patients compared to other two groups (Table 1) and was significantly correlated with GABA level (Table 2). In general, the plasma concentration of hydroxy amino acids and alanine decreases in alcoholics with liver damage (20). Though these amino acids did not change significantly (Table 1), GABA was significantly correlated not only with alanine and serine but also with histidine in this study (Table 2).

While one study showed that patients with alcoholic liver cirrhosis had significantly increased serum levels of phenylalanine and tyrosine compared with controls (18), another study did not find any statistically different plasma aromatic amino acids concentrations in alcoholic patients compared to those of the control subjects (23). In a third study, plasma tyrosine was found higher in alcoholic than nonalcoholic liver cirrhosis (22). In the present study, phenylalanine level elevated significantly in ALD patients, while tyrosine level decreased significantly in NAFLD subjects compared to other two groups (Table 1) and both phenylalanine and tyrosine were significantly correlated with GABA (Table 2).

Impairment of hepatic transsulfuration reactions is suggested to be critically linked with alcoholic liver injury (24). Alcoholic liver disease patients showed significantly increased plasma levels of methionine compared to other two groups in this study (Table 1) is also in agreement with other studies (14, 18) and probably result from impaired hepatic metabolism and portal systemic shunting of blood (8). Ethanol feeding impairs several of the multiple steps in methionine metabolism that leads to progressive liver injury (25). Moreover, GABA is significantly correlated with methionine level (Table 2). Though continual alcohol ingestion alters sulfur-containing amino acid utilization (26), however, there was no significant change in cystine level in this study and more interestingly it was found constantly low in our subjects compared to western population (Table 2) (27). Decreased concentrations of the three branched chain amino acids in alcoholic liver disease patients compared to the control group in this study is consistent with other findings (14). In other study, alcoholic liver cirrhosis patients showed significantly decreased valine concentration compared with controls (18). Lower plasma valine concentration in ALD patients compared to NAFLD patients in this study is also in agreement with other study (22).

These results indicated that alcoholic liver disease patients presented a more deranged plasma amino acid pattern than non-alcoholic fatty liver disease patients and the amino acid imbalances. Thus, the unraveling of the myriad connections between nutrients and alcohol and their metabolic and disease implications continues to challenge our critical thinking. Though the main risk factors for NAFLD are the metabolic abnormalities commonly observed in metabolic syndrome, such as insulin resistance, visceral obesity, dyslipidemia and altered adipokine profile (28); insulin resistance affects amino acid metabolism (29). However, the contribution of other factors of NAFLD on amino acid metabolism is not known. Moreover, the influence of any particular amino acid on GABA is inconclusive from this pilot study. The major limitation of this study is sample size. Other limiting factor is wide normal range of amino acids in population. Therefore, more studies with larger sample size are necessary in this area. Further the role of individual amino acid and also their combined effect(s) need to addressed. Finally quantification of individual amino acids is of importance in monitoring the therapeutic intervention by way of dietary manipulations and/or vitamin supplement therapy and therefore normal reference range for our population should be determined

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