ASSOCIATION OF HYPERHOMOCYSTEINEMIA TO ALCOHOL WITHDRAWAL IN CHRONIC ALCOHOLICS

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ABSTRACT

This study is conducted in chronic alcoholics to assess the association of an Individual's level of serum homocysteine with the success of achieving alcohol deaddiction in the patient. The patients' nutrition status is also assessed. 50 chronic alcoholics admitted to a deaddiction center were inducted into the study. Patients underwent an 8 weeks holistic program to promote rehabilitation from alcoholism. All the patients were addicted to alcohol for 8 – 10 years. Of the 50 patients enrolled, 39 of them completed the 8 weeks program including complete abstinence from alcohol during this period. Fasting blood samples were collected on admission and again after 8 weeks of alcohol abstinence for analyses of serum homocysteine and serum prealbumin, transferrin, total proteins and albumin, gammaglutamyl transferase (GGT) and alanine transaminase (ALT). Of the 50 patients enrolled in the study, 39 completed the 8 weeks rehabilitation program. 11 patients discontinued within 2 weeks of admission. During the 8 weeks of complete alcohol abstinence, patients were given a balanced diet and multivitamin supplements. A significant improvement in their nutritional status was noted by the elevation of serum levels of prealbumin, transferrin, total proteins and albumin. Serum homocysteine levels decreased significantly ($p < 0.002$) to normal levels from previous hyperhomocystenemia. This was accompanied by decrease in serum GGT and ALT levels indicating improved liver functions. Serum estimation of homocysteine in chronic alcoholics is important to assess whether the patient will have a successful rehabilitation. Normal homocysteine levels are achieved after dietary changes and abstinence from alcohol. Timely correction of hyperhomocysteinemia also provides successful rehabilitation.

KEY WORDS

Chronic alcoholics, Homocysten, Liver enzymes, Transferrin.

INTRODUCTION

Homocysteine has received increasing attention as a risk factor for atherosclerosis (1) and recently, evidence is accumulating that it may be involved in the pathogenesis of alcoholic liver injury (2,3). Homocysteine plays a pivotal role in the metabolism of the essential amino acid methionine, which is the precursor of S-adenosylmethionine (SAM), the methyl donor in hundreds of methylation reactions including RNA,

DNA, proteins, and lipids. Homocysteine is formed upon demethylation of SAM and subsequent hydrolysis of S-adenosylhomocysteine (SAH) and lies at the junction of two intersecting pathways, the transsulfuration pathway - the pathway that converts the sulfur atom of methionine to cysteine and glutathione, as well as its ultimate removal as sulfate - and the remethylation pathway - the pathway that conserves homocysteine as methionine, and that is coupled to cobalamin, folate and betaine metabolism. The coenzyme forms of vitamin B₆, 5-methyltetrahydrofolic acid and methyl cobalamine are required for its metabolism (4). As chronic alcoholics suffer from micronutrient deficiencies including vitamins, they are prone to developing hyperhomocysteinemia (5,6,7). Recently, it has been shown that excitatory amino acid neurotransmitters and homocysteine levels are elevated in alcoholic patients (1).

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Furthermore, it has been found that homocysteine induces neuronal cell damage by stimulating N-methyl-D-aspartate (NMDA) receptors as well as by producing free radicals (7). Homocysteine neurotoxicity via over stimulation of NMDA receptors may contribute to the pathogenesis of both, brain shrinkage and withdrawal seizures linked to alcoholism (8,9). There are, however, two other biochemical reactions specific for homocysteine that may be involved in homocysteine-mediated liver injury. The first is the synthesis of SAH, a potent inhibitor of methyltransferases (10) in equilibrium with homocysteine. Consequently, increased homocysteine may result in inhibition of multiple methylation reactions, a key component of many signaling pathways. The second reaction is the formation of homocysteine thiolactone (11) that can react with primary amines of proteins such as lysine.

Homocysteine thiolactone can react with lysine residues and free amine groups on numerous cellular proteins, leading to altered biological activity and premature degradation (12). All of these aforementioned mechanisms may be involved in mediating the adverse effect of hyperhomocysteinemia.

The aim of this study was to assess serum homocysteine levels in patients of chronic alcohol abuse and their association to alcohol withdrawal. We studied serum levels of homocysteine and nutritional parameters like prealbumin, transferrin, total protein and albumin in two groups of patients of chronic alcohol abuse who were admitted into an alcohol rehabilitation unit for 8 weeks. The first group completed the 8 weeks program successfully. The participants in the second group discontinued the program within 2 weeks of admission after experiencing excessive alcohol withdrawal symptoms and crave for alcohol.

MATERIALS AND METHODS

Patients : Patients of chronic alcohol abuse (n=50) admitted to the "Deaddiction Center for Substance Abuse, Kamineni Foundation", Kamineni Institute of Medical Sciences, (Narkatpally, Andhra Pradesh, India) were inducted into the study. Patients participated in a holistic 8-week program to promote rehabilitation from alcoholism. All the patients were addicted to alcohol for 8- 10 years. The demographic and clinical findings on admission are given in Table-1. Patients with pre-existing liver diseases and gross malnutrition were excluded from the study.

During the hospital stay, patients received a balanced nutritious diet and multivitamin tablets of B₆, B₁₂ and folic acid. A qualified counsellor provided psychological counselling for the patient and the patients' family. Other than treatment for minor

ailments, no other medications were given to the patient during the 8-week period.

Of the 50 patients enrolled in the study, 39 patients completed the 8 weeks program during which time, the patients abstained completely from alcohol. These were Group I patients, which were investigated twice, Ia (sample on admission), and Ib (sample on discharge) Eleven patients dropped out of the program and were placed in Group – II. The study was conducted in accordance with the requirements of the Kamineni Institutional Review Board.

Methods : Blood samples were obtained under fasting conditions on admission and after 8 weeks of alcohol abstinence. For eleven patients who discontinued the program, samples were not collected at the time of discontinuation of the program. Serum homocysteine was estimated by ELISA. Serum prealbumin, transferrin, total protein and albumin, gamma glutamyl transferase (GGT) and alanine transaminase (ALT) were estimated on autoanalyser.

Statistics : Results are expressed as Mean±SD Levels of parameters in group I, Ia (sample on admission), Ib (sample on discharge) and group II were compared by students 't'-test. All analysis were performed with one tailed test, with $p \leq 0.05$ as the level of significance. Correlation was studied using Pearson's correlation matrix and significance was calculated.

RESULTS

Among the 50 patients who joined the program, 39 completed the 8-week rehabilitation program (group 1). Eleven patients left within 2 weeks of joining the program (group II). The chief complaints and symptoms exhibited by group II patients were anxiety, restlessness, intense craving for alcohol, aggressive behavior, headache and nausea.

During the 8 weeks of alcohol abstinence, patients were given a balanced diet and vitamin B complex supplementation. A significant improvement was observed in their nutritional status as evinced by elevation of serum levels of prealbumin, transferrin, total protein and albumin (Table 2). Serum levels of homocysteine decreased significantly ($p < 0.002$) to normal levels from previous levels of moderate hyperhomocysteinemia. This was accompanied by decrease in serum GGT and ALT levels indicating improved hepatocellular integrity and liver function (Table 2).

The more important finding in this study was that group II

Table 1 : Demographic and clinical findings on admission in 50 patients admitted to an alcohol withdrawal unit. All patients were males

Age:	39 ± 12 years
Years of alcoholism:	9 ± 3
Nausea and vomiting:	3
Chest pain, breathlessness cough:	18
Headache, confusion memory loss:	4
Burning in palms and feet:	8
Abdominal pain:	7
Generalized body pains:	7
Blurred vision:	6
Tremors:	5
Appetite Loss:	10
Weakness in Limbs:	4
Sleep disturbances:	5
Ear pain / discharge:	6
Pedal edema:	3
Paralytic stroke:	1
General seizures:	1
UTI:	4
Diabetes:	4
Hypertension:	2

patients who were unable to tolerate abstinence from alcohol, had significantly higher serum homocysteine levels ($p < 0.001$) and poorer nutritional status when compared to group I patients who completed the scheduled program (Table 3). At the time of admission, the average serum homocysteine levels in group II patients indicated severe hyperhomocysteinemia whereas moderate hyperhomocysteinemia was indicated in group I patients.

Table 2 : Levels of serum homocysteine, prealbumin, transferrin, total protein and albumin in patients of chronic alcoholism before and after completion of an 8-week alcohol abstinence program. Blood samples were drawn from the patient after an overnight fast. The first sample was taken within 2 days of admission and the second sample was taken before discharging the patient, 8 weeks later. Values are expressed as Mean ± SD

Parameter	Levels at admission (n=50)	Levels 8 weeks later (n= 39)	Reference Range	P-Value
Homocysteine (μmol/L)	26.00 ± 13.36	14.31 ± 5.7	4 – 12	p < 0.002
Transferrin (mg/dl)	263.58 ± 72.19	303.67 ± 83.50	215 – 380	p < 0.01
Prealbumin (mg/dl)	29.14 ± 11.35	25.76 ± 8.50	10- 40	p < 0.05
Total protein (g/dl)	6.8 ± 0.72	7.06 ± 0.48	6 – 8	p < 0.05
Albumin (g/dl)	3.8 ± 0.57	4.02 ± 0.40	3.5 – 4.5	p < 0.02
GGT (IU/l)	43.72 ± 37.03	25.9 ± 8.97	2 – 30	p < 0.03
ALT (IU/l)	37.29 ± 27.04	33.07 ± 17.33	10 – 40	NS*

*Not significant

Table 3 : Serum levels of homocysteine, prealbumin, transferrin, total protein and albumin at the time of admission in patients who completed the 8 weeks alcohol rehabilitation program (Group – Ia, n =39) and in patients who discontinued the program (Group-II, n =11)

Parameter	Group – Ia (n =39) (Mean ± S.D)	Group – II (n =11) (Mean ± S.D)	P
Homocysteine	22.4 ± 11.7	42.3 ± 12.4	<0.001
Transferrin (mg/dl)	268.2 ± 73.7	236.8 ± 49.4	<0.01
Prealbumin (mg/dl)	29.5 ± 11.5	23.31 ± 9	<0.07
Total protein (g/dl)	6.84 ± 0.77	6.85 ± 0.31	NS*
Albumin (g/dl)	3.8 ± 0.6	3.7 ± 0.5	NS*

*Not significant.

No correlation was observed between serum homocysteine levels and nutritional parameters with levels of liver enzymes in both group 1 and group II patients. Hence, the hyperhomocysteinemia observed in these patients is independent of the degree of liver dysfunction.

DISCUSSION

The possible link between homocysteine and alcoholism stems from the fact that homocysteine metabolism is closely linked to the metabolism of the three B complex vitamins (5). Due to these relationships, nutritional deficiency of any one of these vitamins, as a consequence of chronic alcohol intake, could lead to metabolic impairment and potentially to hyperhomocysteinemia (13). Another study performed in chronic alcoholics in whom hyperhomocysteinemia was observed along with disturbed vitamin status, demonstrated DNA hypomethylation in peripheral lymphocytes as well (1). Since all these alterations were observed in the absence of

clinically overt liver disease, one might speculate whether these metabolic abnormalities could be involved in the pathogenesis of withdrawal symptoms associated with chronic alcoholism.

It was postulated that hyperhomocysteinaemia and excitatory amino acid neurotransmitters, by their agonism at the N-methyl-D-aspartate receptor, may partly mediate alcohol-associated withdrawal symptomatology (12,13). In this study, hyperhomocysteinemia was associated with the inability to tolerate abstinence from alcohol. Patients who successfully completed the 8 weeks program had lower homocysteine levels, which reduced further by the end of the program.

REFERENCES

1. Bonsch D, Lenz B, Reulbach U, Kornhuber J, Bleich S. Homocysteine associated genomic DNA hypermethylation in patients with chronic alcoholism. *J Neural Transm* 2004 Dec; 111(12): 1611-6.
2. Refsum H, Ueland P, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998; 49: 31-62
3. Ji C, Kaplowitz N. Hyperhomocysteinemia, endoplasmic reticulum stress, and alcoholic liver injury. *World J Gastro* 2004; 10 : 1699-1708.
4. Bayes B, Pastor MC, Bonal J, Romero R. New cardiovascular risk factors in patients with chronic kidney disease: Role of folic acid treatment. *Kidney Int* 2005;67: S39-S43.
5. Cravo ML, Camilo ME. Hyperhomocysteinemia in chronic alcoholism relations to folic acid and vitamins B₆ and B₁₂ status. *Nutrition* 2000;16: 296-302.
6. Cravo ML, Gloria LM, Selhub J, Nadeau MR, Camilo ME, Resende MP, Cardoso JN, Leitao CN, Mitra FC. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B₁₂ and Vitamin B₆ status. *Am J Clin Nutr* 1996;63: 220-24.
7. Joline WJ, Aafje S, Gertjan S, Kok FJ, Struys EA, Jakobs C, Hendriks FJ. Kinetics of homocysteine metabolism after moderate alcohol consumption. *Alcohol Clin Exp Res* 2005; 29:739-45.
8. Bleich S, Degner D, Javaheripour K, Kurth C, Kornhuber J. Homocysteine and alcoholism. *J Neural Transm Suppl* 2000;60:187-96.
9. Bleich S, Degner D, Bandelow B, Von Ahsen N, Ruther E, Kornhuber J. Plasma homocysteine is a predictor of alcohol withdrawal seizures. *Neuroreport* 2000;11: 2749-52.
10. Baernstein HD. A modification of the method for determining methionine in proteins. *J Biol Chem* 1934; 106: 451-6.
11. Clarke S, Banfield K. S-Adenosylmethionine-dependent methyltransferases. In: Carmel R, Jacobsen DW, eds. *Homocysteine in Health and Disease*. UK: Cambridge University Press, 2001: 63-78.
12. Bleich S, Degner D, Wiltfang J, Maler JM, Niedmann P, Cohrs S, Mangholz A, Porzing J, Sprung R, Ruther E, Kornhuber J. Elevated homocysteine levels in alcohol withdrawal. *Alcohol Alcoholism* 2000;35:351-54.
13. Bleich Stefan, Carl Marco, Bayerlein Kristina, Reulbach Udo, Biermann Teresa, Hillemacher Thomas, Bonsch Dominikus, Kornhuber Johannes. Evidence of Increased Homocysteine Levels in Alcoholism: The Franconian Alcoholism Research Studies (FARS). *Alcoholism: Clinical & Experimental Research*. 29(3): 334-336, March 2005.