
Serum Homocysteine Levels in Alcoholic Liver Diseases

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ABSTRACT: *Serum Homocysteine is an intermediate compound of Methionine metabolism. Its effect in liver function is likely to affect the metabolism of both methionine and Homocysteine. These alterations in metabolism; leads to increased serum Homocysteine levels. Hyperhomocysteinemia is known for its role in cardiovascular diseases; in addition, it's one of the important risk factors in alcoholic patients. Homocysteine metabolism is dependent on B-complex vitamins like pyridoxine, folate, vitamin-B12. Individuals with mutated methylenetetrahydrofolate reductase (MTHFR) and low folate trap status shows increase in Homocysteine levels. The reduced levels of B12 and folic acid in the cells, increases the serum Homocysteine levels causing Hyperhomocysteinemia. The aim of present study is to estimate homocysteine levels in alcoholic liver disease (ALD) patients to assess their clinical presentations and to compare and correlate the findings with various types of ALD. In this study 100 alcoholic liver disease subjects and 50 healthy controls were investigated for serum homocysteine (S. Hcy) along with other biochemical parameters. Serum homocysteine levels were marginally increased in all the three groups of alcoholic liver disease subjects in comparison with controls. According to the present study, there are more chances of increased Homocysteine levels in ALD.*

KEY WORDS: Methionine, Alcoholic liver disease, Homocysteine, Hyperhomocysteinemia.

INTRODUCTION

Toxic and non toxic effects are known to be exhibited by homocysteine; it's a thiol- (sulfhydryl-) containing amino acid, intermediate metabolized product of essential amino acid- Methionine; which take place mainly in the liver. 'The liver is the main stored organ for many nutrients compounds, water soluble vitamins. If liver loses its storage capacity, it can exacerbate micronutrient deficiencies caused by low or unbalanced dietary intakes nutrients compounds. ^[1,2] Sulfur Metabolism of Homocysteine is a multiple complex process which involves several enzymes and folate, vitamin B12 and pyridoxine as co-enzymes and one carbon metabolism is

involved. Insufficient supply or improper functioning of any of these supplements can affect the homeostasis and impaired metabolism. Homocystinuria is raised depends on the severity of underlying defect and it can be controlled by dietary intervention to some extent.^[3] When there is a deficiency of these vitamins, homocysteine levels will be raised. Increased levels of homocysteine cause atherosclerosis and ischemic heart disease. Those who are having more alcohol have elevated homocysteine levels; have increased vascular risk along with Liver impairment.^[4]

Homocysteine is accumulated in cells and reaches the circulation either due to deficiency of some cofactors like B-complex vitamins or any defect in the enzyme.^[5] Renal failure, impaired catabolic liver function, and hypoalbuminemia influence genesis of Homocystinemia, in case of liver diseases.^[6,7] One old study was reported that majority (75%) of the infected patients develops chronic infection, 15-20% of which progress to fibrosis. And of these fibrosis patients around 9% develop Hepatocellular Carcinoma. The total Homocysteine level is inversely associated with dietary folate trap and B vitamins and positively associated with alcohol consumption.^[8]

The Study was conducted to estimate of Homocysteine levels in patients of alcoholic liver diseases and compared with healthy controls to assess the importance of homocysteine levels as one of the diagnostic marker in patients of ALD.

MATERIALS AND METHODS:

This is a hospital based cross-sectional comparative and observational study carried out at Dr. Kiran C. Patel Medical College and Research centre, Bharuch, Gujarat, India. Study include 100 diagnosed patients of ALD with the age group of 27-60 years attending the OPD or IPD in Department of Medicine and surgery from Dec 2019 to May 2021 as Subject. Patients suffering average duration of ALD patients were 3-4 years. The control group comprised of 50 in healthy persons free from any systemic illness. The controls were the relatives or the accompanying persons of the patients.

Patients taking supplemented vitamin B complex or drugs that decrease folic acid measurements were excluded from the study group.

The study group (Patients of Alcoholic Liver diseases) is divided into 3 sub-groups-

- 1) Alcoholic fatty liver
- 2) Alcoholic hepatitis
- 3) Alcoholic fibrosis

After the oral informed consent taken from the patient by communicating in either English or local language, 2 ml of venous blood drawn from the antecubital vein after an overnight fast with all antiseptic precautions in EDTA vials, while 3 ml is collected in plain vials for biochemistry parameters. Serum was separated by centrifugation at 3000-4000 rpm for 10-13 minutes. The serum sample was collected in aliquots and stored in refrigerator at 2-8°C till analysis was done. Afterwards, serum homocysteine levels were measured on chemiluminiscence machine based on competitive immunoassay^[9], Serum AST- IFCC without PLP method^[10], Serum ALT – IFCC without PLP method^[11], Serum GGT- Kinetic IFCC method^[12], Serum uric acid- Uricase-PAP, End-point method^[13], Serum Mg- Colorimetric xylidyl blue method^[14], Electrolyte – Potentiometric Method, and MCV.

Ethical consideration:

The protocol for this study was approved by the Institutional Ethics and Research Committee (IERC) in accordance with the ethical standards of the committee on human institutional experimentation and with the Helsinki Declaration of 1975 that was revised in 2000.

Statistical Analysis

The results obtained were statistically analyzed by using SPSS, with version 20.0. The variables were presented as mean with standard deviations and then compared between different groups of the study by applying Independent 't' test. Then values were taken as significant when the probability ($p < 0.05$), ($p < 0.001$) as percentage of the observing values of 't' at a particular degree of freedom.

RESULTS AND OBSERVATION:

In the present study, 100 known subjects of alcoholic liver disease from Dept. of Medicine and Dept. of Surgery were compared with 50 healthy controls were studied.

Table 1: Comparison of biochemical parameters in subjects and controls

Parameters	Subjects (ALD patients) N=100	Controls N=50	P-Value
Serum Homocysteine ($\mu\text{mol/L}$)	18.01 ± 5.74	9.15 ± 2.09	<0.05
Serum AST (U/L)	125.58 ± 57	23.78 ± 5.56	<0.01
Serum ALT (U/L)	49.32 ± 20.99	24.22 ± 7.31	<0.01
AST/ALT ratio	2.54 ± 0.77	1.04 ± 0.32	<0.05
Serum GGT (U/L)	55.12 ± 17.02	24 ± 7.16	<0.01
Serum Uric Acid (mg/dL)	4.27 ± 0.51	4.13 ± 0.47	NS
Serum Electrolyte Sodium (mEq/L) Potassium (mEq/L)	145 ± 4.65 3.1 ± 0.14	130 ± 5.65 4.1 ± 0.7	<0.01
Serum Magnesium (mg/dL)	1.64 ± 0.30	2.33 ± 0.37	<0.05
MCV	97.62 ± 21.3	89.13 ± 6.48	<0.01

$P < 0.01$: highly significant

Table 2 : Homocysteine levels in various alcoholic liver diseases

Group	N	Mean \pm S.D	t- Value	P
Alcoholic Fibrosis	60	19.49 ± 0.19	12.11	0.001
Alcoholic hepatitis	20	17.2 ± 6.04	9.9	0.001
Alcoholic fatty liver	20	14.42 ± 3.17	8.9	0.001
Control	50	8.12 ± 1.09		

$P < 0.001$: highly significant

DISCUSSION

In the present study, the levels of homocysteine in ALD patients were found to be highly significant. This could be due to the reason that the liver is the central point for methionine, sulfur metabolism. So, any dysfunction in liver can affect the methionine metabolism. Any Defect in the metabolism leads to the increased serum levels of homocysteine. The present study was in accordance with the following studies.

Ben-Ari Z. et al., (2001) found that the increased levels of Homocysteine were seen in patients with liver fibrosis, cirrhosis and HCC. This could be because a part of tissue damage occurs directly through raised Homocysteine leakage or indirectly by initiated cell repair. ^[15]

Essam F. et al., (2009) found that, the Homocysteine concentrations were elevated in all patient groups, (cirrhosis, chronic hepatitis and HCC). There was a trend towards higher in Homocysteine concentrations in more severe stages of liver disease. ^[2]

García-Tevijano ER et al., (2001) found that, the mean Homocysteine concentration was significantly higher for all fibrosis/cirrhosis ($14.1 \pm 1.3 \mu\text{mol/L}$) than for the control group ($8.1 \pm 0.9 \mu\text{mol/L}$, $p < 0.03$). It has been suggested that impairment of Homocysteine metabolism in cirrhosis can be also related to decreased availability vitamins B6, B12 or folate. ^[16]

In a study from USA, 40 alcoholic cirrhosis patients, 26 active alcohol drinkers without clinical evidence of liver disease and 28 healthy controls were included. Homocysteine level in alcoholic cirrhosis patients were higher range of 5.4 to 58.3 $\mu\text{mol/L}$, in active alcohol drinkers the range was 5.7 to 23 $\mu\text{mol/L}$, and among controls the range was 4.1 to 10 $\mu\text{mol/L}$ with $p < 0.0001$. ^[17]

In a study from Slovak republic, higher levels of serum Homocysteine were seen in various groups of patients with ALD: statuses 12.1 ± 7.3 , ($p < 0.01$), mild fibrosis/cirrhosis, 14.1 ± 11.8 , ($p < 0.01$), up to severe cirrhosis, 16.9 ± 10.9 , ($p < 0.001$). ^[18]

A study by Gibson A et al., (2008) has shown that Homocysteine increases and vitamin B₁₂ as well as folic acid decreases with alcohol consumption. ^[19] Kazimierska E et. al., (2003) studied that, Vitamin B₁₂ and folic acid are cofactors for Homocysteine metabolism. A vitaminosis is often presented by alcoholics. A study stated that Hyperhomocysteinemia was seen in 50% patients and mean Homocysteine concentration was significant with $p < 0.05$. When compared to the controls. Mean concentration was $13.29 \pm 8.16 \text{ mmol/l}$ in patients with $11.03 \pm 1.6 \text{ mmol/l}$ in controls. A negative correlation was found between Homocysteinemia and folic acid concentration in patients of Hyperhomocysteinemia. However, vitamin B₁₂ levels were found to be significantly higher. ^[20] Since, folate deficiency impairs methionine metabolism, it can lead to Hyperhomocysteinemia, even, depletion of S-adenosylmethionine (SAM) and methionine, which are important characteristics of alcoholic liver disease. ^[21]

Halifeoglu I et al., (2004) observed that raised serum Homocysteine can induce liver diseases and plays a role in hepatic disorders. ^[22] A study by Blasco C et al., (2005) showed significant Homocysteinemia levels in chronic alcoholics with liver injury in comparison to the normal liver and in controls (9.66 ± 8.1 vs $6.93 \pm 2.33 \text{ mmol/l}$, $p < 0.025$). The prevalence of Hyperhomocysteinemia was also significantly higher $12.17 \pm 10.14 \text{ mmol/l}$ in alcoholics with liver damage than in those with normal liver and in controls. ^[23]

Gill JS et al., (1991) found that Hyperhomocysteinemia, associated with chronic alcohol abuse, is a result of alcohol or its metabolites which interferes with the metabolism of vitamins like folic acid, vitamin B₁₂. The lack of correlation between serum homocysteine and vitamins- folic acid and B₁₂ studied is due to multiple deficiencies occur simultaneously, each of them contributing individually to the Homocysteinemia in the alcoholics. ^[24] One more study has showed by

Homocysteine levels in chronic alcoholics were found to be $21.2 \pm 8.0 \mu\text{mol/L}$, twice as that of controls ($p < 0.05$).^[25]

CONCLUSION

Homocysteine is associated with folic acid, Vitamin B12 and folic acid, as they serve as coenzymes in methionine-Homocysteine metabolism. Deficiency of this vitamin causes Homocysteine accumulation and undergoes increase in the circulation. Therefore, Hyperhomocysteinemia besides being a risk factor for coronary artery disease is a major risk for CLD particularly alcoholic liver disease. So it should be added in the investigation protocol of CLD patients by the physicians.

Ethical approval and consent of Participate: - Not applicable.

Consent for Publication: - All contributors agreed and given consent to Publish.

Competing Interest: - The authors declare no competing interests.

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