DOI: 10.7860/JCDR/2019/42661.13311

Original Article

Biochemistry Section

Comparison of Homocysteine Levels in Various Liver Diseases

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ABSTRACT

Introduction: Homocysteine (Hcy) is an intermediate product formed in methionine metabolism. Disturbances in liver function are likely to affect the metabolism of both methionine and Hcy which leads to increased serum Hcy levels. Hyperhomocysteinemia might be a risk factor for cirrhotic patients in addition to its known role for cardiovascular diseases. Hcy metabolism is dependent on B complex vitamins, particularly folate and vitamin B12 are associated. Individuals with low folate status along with mutated methylenetetrahydrofolate reductase have an increased levels of Hcy. The decreased levels of Vitamin B12 and folic acid in the cells, increases the serum levels of Hcy thus causing hyperhomocysteinemia.

Aim: To estimate Hcy levels in patients with Chronic Liver Disease (CLD).

Materials and Methods: This hospital based cross-sectional comparative study was conducted on a total of 100 individuals which included 50 patients of CLD with sub groups- alcoholic liver disease-fibrosis (30), cirrhosis (10), viral hepatitis patients (10) and 50 age and sex matched healthy individuals. Three mL of blood sample was collected under all aseptic conditions for investigations. The samples was allowed to clot and the serum was separated and analysed for estimation of Hcy levels and was estimated on chemiluminiscence machine based on competitive immunoassay. Statistical analysis was done using SPSS version 16. All the tests were 2 tailed and the p-value <0.05 was considered significant.

Results: The study groups included patients with age between 25-65 years, which comprised of 44 males and 6 females. Hcy levels were statistically increased in CLD patients in comparison with controls.

Conclusion: While evaluating a patient of CLD, the physician should keep in mind the possibility of increased Hcy levels as one of its risk factors.

Keywords: Alcoholic liver disease, Cirrhosis, Hepatitis, Liver damage, Methionine

INTRODUCTION

Several toxic effects are known to be exhibited by Hcy, which is a breakdown product of methionine. Hcy is an intermediate in Methionine metabolism, which takes place mainly in the liver [1]. Since liver is the main store for many nutrients, particularly water soluble vitamins. Loss of storage capacity can therefore exacerbate micronutrient deficiencies caused by low or unbalanced dietary intakes [2]. Metabolism of homocysteine is a complex process which involves several enzymes and folate and vitamin B as co-enzymes. Improper Functioning or deficient supply of any of these supplements can effect the homeostasis. The extend by which Hyc is raised depends on the severity of underlying defect and it can be controlled by dietary intervention to some extend [3]. When there is a deficiency of these vitamins, Hcy levels in the blood rise. High levels of Hcy have been associated with atherosclerosis and ischemic heart disease. Alcoholics have elevated Hcy levels and increased vascular risk [4].

Hcy gets accumulated in cells and reaches the circulation either due to deficiency of some cofactors or any defect in the enzyme. Factors like renal failure, impaired catabolic liver function, and hypoalbuminemia influence genesis of homocysteinemia, in case of liver cirrhosis [5-7]. A previous study reported that majority (85%) of the infected patients develops chronic infection, 10-20% of which progress to cirrhosis. And of these cirrhosis patients around 7% develop Hepatocellular Carcinoma (HCC). The total Hcy is inversely associated with dietary folate and B vitamins and positively associated with alcohol consumption [8].

This study was conducted to estimate the levels of Hcy in patients of CLD and compared with healthy controls to assess the importance of Hcy levels as one of the diagnostic marker in patients of CLD.

MATERIALS AND METHODS

This hospital based cross-sectional comparative study was conducted on total of 50 clinically diagnosed patients of CLD attending the OPD or admitted in wards of Department of Medicine, Guru Gobind Singh Medical College & Hospital, Faridkot, Punjab, India, from January 2016 to August 2016. The hospital ethical committee approved the study vide letter number ECR/836/Inst/PB/2016.

The average duration of illness of CLD patients was 2-3 years. The control group comprised of 50 age and sex matched healthy persons free from any systemic illness. The controls were the relatives or the accompanying persons of the patients.

Inclusion Criteria

- All the patients with CLD.
- All patients with age between 25-65 years of either sex.

Exclusion Criteria

- Patients taking supplements of vitamin B-complex.
- Patients on drugs that decrease folic acid measurements.

Based on the aetiological agent involved, the CLD patients (study group) were divided into 3 sub-groups-

1(a)– Alcoholic Liver Disease (Fibrosis)

1(b)– Alcoholic Liver Disease (Cirrhosis)

1(c)– Viral Hepatitis

Further informed consent was obtained for venipuncture. Before taking samples, a detailed history was taken regarding age, sex and related information to know duration of the disease and any other associated complications. A Three mL of blood was taken in plain

vial after cleaning the venipuncture site under all aseptic conditions. Serum was then separated by centrifugation at 3000 rpm for 10 minutes. The serum was collected in aliquots and stored in refrigerator at 2-8°C till analysis was done. Afterwards, Hcy levels were measured on chemiluminiscence machine (Immulite 1000) based on competitive immunoassay.

The reference values were; Homocysteine- 5-15 µmol/L [9], (Catalogue number- LKHO1) [10].

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS (Statistical Package for the Social Sciences) version 16. For comparing the two variables t-test was used. All the tests were two tailed and the p-value <0.05 was considered significant. The sample size was calculated by $4pq/d^2$. The power of study was 0.8 and confidence interval was 0.05.

RESULTS

The samples were analysed for Hcy in patients of CLD and following results were obtained as shown in [Table/Fig-1,2].

Parameter	Group	N	Mean±S.D	t value	p-value
Homocysteine (µmol/L)	Study group	50	16.02±4.74	10.75	<0.05*
	Controls	50	8.15±2.08		

[Table/Fig-1]: Homocysteine levels in the study groups.
*Paired t-test

Group	N	Mean±S.D	t value	p-value
1(a)-Ald-fibrosis	30	15.49±4.38	10.11	0.0001*
1(b)- Ald- cirrhosis	10	18.2±6.04	9.5	0.0001*
1(c)- Viral hepatitis	10	15.42±4.17	8.3	0.0001*
Controls	50	8.15±2.08		

[Table/Fig-2]: Homocysteine levels in various liver diseases.
*Paired t-test

DISCUSSION

In the present study, the levels of Hcy in CLD patients were found to be highly significant. This could be due to the reason that the liver is the centre for methionine and Hcy metabolism. So, any disturbance in liver function can affect these metabolisms. Defect in the metabolism leads to the increased serum Hcy levels. The present study was in accordance with the following studies.

Study done by Essam F et al., found that, the Hcy concentrations were elevated in all patient groups, (cirrhosis, chronic hepatitis and HCC). There was a trend towards higher Hcy concentrations in more severe stages of liver disease [1].

Ben-Ari Z et al., found that the increased levels of Hcy were seen in patients with liver cirrhosis and HCC. This could be because a part of tissue damage occurs directly through raised Hcy leakage or indirectly by initiated cell repair [11].

In another study, Hcy levels in chronic alcoholics were found to be 21.2±8.0 µmol/L, twice as that of controls (p<0.05) [12]. García-Tevijano ER et al., found that, the mean Hcy concentration was significantly higher for all cirrhotics (14.1±1.3 µmol/L) than for the control group (8.1±0.9 µmol/L, p<0.03). It has been suggested that impairment of Hcy metabolism in cirrhosis can be also related to decreased availability or utilisation of vitamins B6, B12 or folates [13].

In a study from Slovak republic, higher levels of serum Hcy were seen in various groups of patients with CLD: steatosis 12.1±7.3, (p<0.01), mild fibrosis/cirrhosis, 14.1±10.8, (p<0.01), up to severe cirrhosis, 16.9±10.9, (p<0.001) [14]. 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. Hcy level in alcoholic cirrhosis patients were high with a range of 5.4 to 58.3 µmol/L, in active alcohol drinkers the range was 5.8 to 23 µmol/L, and among controls the range was 4.1 to 10 µmol/L with p<0.0001 [15].

A study by Gibson A et al., has shown that Hcy increases and vitamin B12 as well as folic acid decreases with alcohol consumption [16]. Kazimierska E et al., studied that, Vitamin B12 and folic acid are cofactors for Hcy metabolism. A vitaminosis is often presented by alcoholics. A study stated that hyperhomocysteinemia was seen in 50% patients and mean Hcy concentration was significant with p<0.05, when compared to the controls. Mean concentration was 13.29±8.16 mmol/l in patients with 11.03±1.6 mmol/l in controls. A negative correlation was found between Hcy and folic acid concentration in patients of hyperhomocysteinemia. However, vitamin B12 levels were found to be significantly higher [17]. 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 [18].

A study by Blasco C et al., showed significant Hcy levels in chronic alcoholics with liver injury in comparison to the normal liver and in controls (9.66±8.1 vs 6.93±2.33 mumol/l, p<0.025). The prevalence of hyperhomocysteinemia was also significantly higher 12.17±10.14 mumol/l in alcoholics with liver damage than in those with normal liver and in controls [19]. Halifeoglu I et al., observed that raised serum Hcy can induce liver diseases and plays a role in hepatic disorders [20].

Gill JS et al., 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 Hcy 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 [21].

LIMITATION

The cost of Hcy estimation per test is very high which restricts the physicians to prescribe this test in the routine investigation protocol of CLD patients. This was the reason we had to choose a small sample size.

CONCLUSION

Hcy is associated with Vitamin B12 and folic acid, as they serve as coenzymes in methionine-Hcy metabolism. Deficiency of this vitamin causes Hcy 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.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 20, 2019
- Manual Googling: Oct 12, 2019
- iThenticate Software: Oct 30, 2019 (16%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: No
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 20, 2019**
Date of Peer Review: **Sep 06, 2019**
Date of Acceptance: **Oct 14, 2019**
Date of Publishing: **Nov 01, 2019**