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ORIGINAL RESEARCH

Study Of Serum Vitamin B12 Level in Chronic Liver Disease of Adult Patients and Its Correlation with Child- Turcotte- Pugh Score

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ABSTRACT

Aim: Assessing serum vitamin B12 levels in patients with chronic liver disease and comparing them to the Child-Turcotte Pugh score was the study's goal. **Material and Methods**: From May 2023 to November 2024, 100 patients over the age of eighteen patient with chronic liver disease (CLD) who were enrolled in the OPD/IPD of General Medicine at Sharda University's School of Medical Sciences and Research in Greater Noida participated in this cross-sectional study. **Results**: The majority of participants in this study were between the ages of 41 and 50 (39%) and 51 and 60 (31%). The study participants' most frequent cause of CLD was alcohol. The majority of subjects had Child Pugh grade C. The research participants with Child Pugh Grades A, B, and C had mean vitamin B12 levels (pg/ml) of 530±14.142, 732.91±202.614, and 912.02±176.143, respectively. As a result, vitamin B levels rose as liver disease severity increased. The Pearson correlation test revealed a significant positive link between Vitamin B12 level (pg/ml) and Child Pugh Grade ($r=0.59$; $p=<0.01$), meaning that when Child Pugh Grade rises, so does Vitamin B12 level. **Conclusion**: The study concludes that a significant number of patients with severe CLD had unusually elevated serum B12 concentrations.

Keywords: Vitamin B12, Chronic Liver Disease, Child- Turcotte- Pugh Score

INTRODUCTION

In India, liver problems are quickly becoming acknowledged as public health issues. India bears a heavy burden of liver disease, accounting for 18.3% of the two million liver disease-related deaths worldwide in 2015 [1]. In contrast to China, another populous Asian nation, where the contribution of cirrhosis and its complications—collectively known as chronic liver diseases, or CLDs—as causes of death has been steadily rising since 1980, India's CLDs have been on the decline [2]. Patients with cirrhosis have a significantly shorter life expectancy and are more vulnerable to many problems. Numerous liver disease conditions can cause cholestasis or persistent hepatic inflammation, which can lead to cirrhosis. In India, hepatitis B, hepatitis C, alcohol-associated liver disease, and non-alcohol-associated liver disease are the most prevalent causes of chronic liver disease, accounting for over 80% of all patients who need liver transplantation [4-5]. Given that it accounted for 18.3% of all liver diseases worldwide in 2015, India bears a heavy burden of chronic liver disease [4, 6-9].

A variety of liver diseases are on the rise in India as a result of the country's current cultural lifestyle shift, which includes a gradual adoption of a western diet, sedentary lifestyles, and a sense of liberation from long-standing social taboos surrounding alcohol [2]. In addition to viral causative elements, this shift involves the growing overall significance of alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) as causes of liver disease. India's liver disease epidemiology is changing. Improved awareness of preventative measures in an integrated manner, better screening methods, and more successful connection to care for early-stage liver disease could all be beneficial interventions [6].

A group of Harvard University doctors found in 1926 that most patients could avoid pernicious anemia by

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consuming half a pound of liver every day. Since then, scientists from all across the world have worked to identify the component in the liver that prevents anemia [10]. Folkers and his colleagues produced small, vivid red crystals of vitamin B12 (cobalamin) in 1947. A patient with pernicious anemia was cured after this novel medication was tested on them the next year [11].

Hepatitis and cirrhosis have been linked to vitamin B12, which mostly manifests in two forms in humans: methyl cobalamin and 5′-deoxyadenosylcobalamine [12]. Furthermore, methyl malonyl CoA mutase, which controls the rate at which long-chain fatty Acyl-CoA enters mitochondria and affects lipid metabolic pathways, required vitamin B12 as a cofactor [13].

Cell division and one-carbon metabolism depend on vitamin B12, which is mostly stored in the liver. In mammalian systems, it serves as a cofactor for two enzymatic reactions: the synthesis of succinyl-CoA from methylmalonyl-CoA and the production of methionine from homocysteine [11]. Elevated serum levels of vitamin B12 may signify a dangerous and perhaps fatal illness, according to some research. Acute hepatitis, severe alcoholic liver disease, cirrhosis, and myeloproliferative illness are all associated with abnormally elevated serum vitamin B12 levels [14–15].

S-adenosylmethionine (SAM), the only methyl donor utilized by DNA, RNA, histone, and protein methyltransferases, is produced by enzymes in one-carbon metabolism, which is mostly dependent on vitamin B12 as a cofactor. As a result, vitamin B12 consumption needs to be carefully considered, particularly in relation to its effects on CLD [16]. Assessing serum vitamin B12 levels in patients with chronic liver disease and comparing them to the Child-Turcotte Pugh score are the goals of the current investigation.

MATERIAL & METHOD

After getting the approval of Ethics Committee, the present cross-sectional study was conducted at Department of Internal Medicine, this study was conducted among the Cirrhosis of liver patients attending the OPD/IPD of General Medicine of School of Medical Sciences and Research, Sharda University, Greater Noida from May 2023 to November 2024.

Sample size

Hundred patients fulfilling inclusion criteria was selected for the study. Informed written consent was obtained. Prevalence of CLD patient among hospital attendant patients-40% (From epidemiology of liver disease in India) [17]. Sample size was 100.

Sample size has been calculated using Cochran's formula-

$N_0 = z^2pq/e^2$

where e is the desired level of precision (margin of error) p is the (estimated) proportion of the population which has the attribute in question, q is 1-p. Using this formula with z=1.96, prevalence- 40%, precision around- 10%, sample size was 100.

Inclusion Criteria

1. All patients aged more than 18 years of both genders.
2. Patients diagnosed with Chronic liver disease
3. Clinical criteria-H/O episode of jaundice at least once, for more than six months and signs of cirrhosis of liver e.g.- hematemesis or melena or ascites or splenomegaly or hepatomegaly.
4. Biochemical criteria-Altered liver function test report for more than six months
5. Imaging criteria- USG or Computed Tomography showing shrunken or nodular liver features of portal hypertension)

Exclusion Criteria

1. Age below 18 years.
2. Pregnant women.
3. Patients with hepatocellular carcinoma.
4. Patients on hepatotoxic medications.
5. Patients already taking Vitamin B12 supplement.

Study tool

All participants were explained about the study. Willing participants were asked to give written consent. All the patients meeting inclusion criteria underwent detailed assessment of history and through general physical and systemic examination as following protocol and diagnosis of liver Cirrhosis was made on the basis:

Detailed history of the patients.

Clinical examination.

Bio chemical tests.

General and systemic examination

Patients underwent a thorough systemic examination, which included a cardiovascular, respiratory, central nervous system, and abdominal examination. Vital signs were regularly monitored, and the proforma was followed as needed to determine the patient's status.

Every blood test was performed, including PT/INR, HIV, HCV, HBsAg, liver and renal function tests, and complete blood counts.

Urine analysis, ECG, chest X-ray, USG whole abdomen (liver has nodular/irregular borders and splenomegaly), and, if necessary, CT abdomen were further tests.

Data was collected and subjected to statistical analysis.

Statistical analysis

Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for

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windows; SPSS inc, Chicago, USA). For each assessment point, data were statistically analyzed using one way ANOVA and the level of significance was set at $p < 0.05$. Pearson correlation test was used to analyse correlation between the two variables.

RESULTS

Eighty-six percent of the patients were male and fourteen percent were female. As a result, males predominated in this study. Of the subjects in this study, 7%, 13%, 39%, 31%, and 10% were in the 19–30, 31–40, 41–50, 51–60, and >60 age groups, respectively. Most common cause of CLD among the study subjects was alcohol (table 1).

Table 1: Baseline characteristics among the study subjects

Gender	N=100	%
Male	86	86
Female	14	14
Age Group (in years)		
19-30	7	7
31-40	13	13
41-50	39	39
51-60	31	31
>60	10	10
Cause		
Alcohol Related CLD	67	67
HEP B Related CLD	9	9
NASH Related CLD	6	6
IDIOPATHIC CLD	4	4
Alcohol and HCV Related CLD	3	3
HCV Related CLD	5	5
HEP B and HEP C Related CLD	3	3
AKT Induced CLD	1	1
HBV and Alcohol Related CLD	2	2

Mean vitamin B12 level (pg/ml) among the study subjects was 847.06±205.63pg/ml with minimum and maximum of 161 and 1000 respectively. Mean Na and K among the study subjects was 131.38±4.98 and 4.13±.44 respectively (table 2).

Table 2: Descriptive analysis of investigative profile

Variables	Minimum	Maximum	Mean	SD
HB	3.6	12.5	8.5	1.89
TLC	1.11	38.75	7.47	6.50
MCV	75	112	99	6.45
Platelet	2.59	220	86.73	36.23
Serum Albumin	1.4	4.90	2.53	.55
Total Bilirubin	.17	32.06	4.75	5.68
Direct Bilirubin	.11	19	3.03	3.99
Indirect Bilirubin	.02	14.18	1.71	2.39
Na	120	142	131.38	4.98
K	3	5.5	4.13	.44
Urea	8	139	32.41	21.17
Creatinine	.20	5	1.26	.63
Serum Vitamin B12 level (pg/ml)	161	1000	847.06	205.63

Mild, moderate and severe ascites was reported among 18%, 18% and 63% of the subjects respectively. Encephalopathy was not present in 69% of the subjects. Grade 1, 2 and 3 was revealed among 15%, 7% and 9% of the subjects respectively. Mean Child Pugh score among the study subjects was 10.71±2.22. Child Pugh grade A, B and C was found in 2%, 32% and 66% of the subjects respectively. Hence maximum subjects were suffering from Child Pugh grade C (table 3).

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Table 3: Ascites, encephalopathy and Child Pugh Grade among the study subjects

Ascites	**N=100**	**%**
Absent	1	1
Mild	18	18
Moderate	18	18
Severe/Gross	63	63
Encephalopathy		
No	69	69
Grade1	15	15
Grade2	7	7
Grade3	9	9
Child Pugh Grade		
A	2	2
B	32	32
C	66	66
Mean±SD	10.71±2.22	

The study participants with Child Pugh Grades A, B, and C had mean vitamin B12 levels (pg/ml) of 530±14.142, 732.91±202.614, and 912.02±176.143, respectively. As a result, vitamin B levels rose as liver disease severity increased. A statistical comparison of the severity of liver disease based on vitamin B12 levels revealed a significant difference ($p<0.05$) (table 4).

Table 4: Mean comparison of Serum Vitamin B12 level (pg/ml) according to Child Pugh Grade

Child Pugh Grade	**Mean Serum Vitamin B12 level (pg/ml)**	**SD**
A	530.00	14.142
B	732.91	202.614
C	912.02	176.143
p value	<0.01*	

*: statistically significant

The results of the Pearson correlation test showed a strong positive association between the levels of vitamin B12 (pg/ml) and child Pugh grade ($r=0.59$; $p=<0.01$), meaning that as child Pugh grade increases, so does the level of vitamin B12 (table 5).

Table 5: Correlation between Serum Vitamin B12 level (pg/ml) and Child Pugh Grade

Variables	**Value**
r value	0.59
p value	<0.01*

*: statistically significant

DISCUSSION

From May 2023 to November 2024, 100 patients over the age of 18 with chronic liver disease who were enrolled in the OPD/IPD of General Medicine at Sharda University's School of Medical Sciences and Research in Greater Noida participated in this cross-sectional study. Assessing serum vitamin B12 levels in patients with chronic liver disease and comparing them to the Child-Turcotte Pugh score was the study's goal.

Eighty-six percent of the patients were male and fourteen percent were female. Males predominated in the current study as a result. Maximum individuals in this study were between the ages of 41 and 50 (39%) and 51 and 60 (31%), while minimum subjects were between the ages of 19 and 30 (7%). In their study, Takaaki Sugihara et al. [9] reported a similar male dominance. In their research, JiuFeng Dou et al. [5] found that the patients' ages ranged from 31 to 66 years (mean±SD, 47.2±11.2 years; 29 women and 76 males). The current study is comparable to this. In their study, Gatram Pavan Kumar et al. [18] found that the majority of the patients were in their fourth or fifth decade, and that the majority of the study group was male (69%) with a mean age of 50.80±10.35. This aligns with the current investigation. In their study, V Gurudatta Murthy et al. [19] found that 88% of the patients were men and 12% were women. One possible explanation for this male dominance is that men are more likely to drink alcohol. According to Sorensen et al. [20], the severity of alcoholic liver disease increases with age, most likely as a result of increased alcohol intake.

Alcohol was the most frequent cause of CLD in the research participants. In their study, Gatram Pavan Kumar et al. [18] also demonstrated that alcohol was the most common cause of CLD.

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The average Child Pugh score for the participants in this study was 10.71±2.22. 2%, 32%, and 66% of the subjects had Child Pugh grades A, B, and C, respectively. As a result, the majority of subjects had Child Pugh grade C. The study participants' mean vitamin B12 levels (pg/ml) ranged from 161 to 1000, with a mean of 847.06±205.63 pg/ml. The study participants with Child Pugh Grades A, B, and C had mean vitamin B12 levels (pg/ml) of 530±14.142, 732.91±202.614, and 912.02±176.143, respectively. As a result, vitamin B levels rose as liver disease severity increased. The results of the Pearson correlation test showed a strong positive association between the levels of vitamin B12 (pg/ml) and child Pugh grade ($r=0.59$; $p=<0.01$), meaning that as child Pugh grade increases, so does the level of vitamin B12.

In a similar study, Takaaki Sugihara et al. [9] found that Child-Pugh C had a significantly higher mean total serum vitamin B12 concentration (1308 ± 599 pg/mL) than those with chronic hepatitis (655 ± 551 pg/mL), Child-Pugh A (784 ± 559 pg/mL), and Child-Pugh B (660 ± 464 pg/mL) ($P = 0.036$). According to a study by Gatram Pavan Kumar et al. [18], when compared to normal individuals (650±300 pg/ml), patients with chronic liver disease had a considerably higher mean total serum vitamin B12 content (1639±504 pg/ml). B12 levels were greater in Child-Pugh C (1858±359 pg/ml) than in Child-Pugh B (1076±370 pg/ml). The current study is comparable to this. According to a study by JiuFeng Dou et al. [5], patients with acute-on-chronic liver failure (AoCLF) had considerably greater B12 levels than HCs, and higher B12 was linked to a more severe illness. Furthermore, the 3-month death rate in patients with AoCLF can be independently predicted by B12 levels.

It is not surprising that liver illnesses are linked to significant variations in serum vitamin B12 concentrations because the liver is crucial for the storage of vitamin B12. Despite normal serum vitamin B12 concentrations, Cylwik et al. [21] found that vitamin B12 concentrations in alcohol misuse individuals are substantially greater than those of healthy subjects. According to Fragasso et al. [22], certain alcohol-dependent patients with megaloblastic anemia may have lower serum vitamin B12 concentrations, however these nevertheless fall within the reference range. According to Ermens et al. [23], depending on the severity of the disease, plasma vitamin B12 concentrations in liver cirrhosis can reach high levels. According to a prospective observational study by Muro N et al. [24], vitamin B12 plasma levels were greater in cirrhotic patients (1151 ± 568 pg/ml) versus controls (440±133 pg/ml) ($p<0.05$).

Alcoholics have high plasma levels of vitamin B12 and low liver levels, according to Kanazawa S et al. [25]. These results point to the way that cobalamin is retained in peripheral tissues before building up in plasma. In addition, Harsharan Kaur et al. [26] found that cirrhotic patients had higher vitamin B12 levels than the control group. In their investigation, Essam F. Al-Jumaily et al. [27] also found that patients with liver cancer and cirrhosis had considerably higher mean serum concentrations of vitamin B12 than did the control group and patients with chronic hepatitis symptoms. In their study, V Gurudatta Murthy et al. [19] shown that higher B12 levels were linked to a higher 3-month mortality rate and more severe liver disease. B12 levels and the endstage liver disease score model were shown to be independent predictors of death by multivariate analysis.

But in their investigation, Holdsworth et al. [28] found that a sizable portion of cirrhotic individuals had reduced serum vitamin B12 concentrations.

Cobalamin is thought to be released from the liver as a result of inflammation-induced hepatocyte degradation in viral infections and/or pathological hepatocyte destruction in liver cirrhosis. Increased plasma cobalamin has been linked to tissue B12 depletion in liver cirrhosis, according to multiple investigations [15,25]. Rats with hepatoma had livers with a lower total B12 level than control animals [29].

Limitations

A few limitations warrant consideration.

1. To begin with, this study was conducted at a single center, and our sample size might have presented a challenge. Prospectively planned, multi-center investigations are required to validate the results.
2. Second, it is unknown whether RDW readings are gradually raised when a patient's condition gradually deteriorates because vitamin B12 was not dynamically observed.

CONCLUSION

The study concludes that a significant number of patients with severe CLD had unusually elevated serum B12 concentrations. Liver damage was linked to the factors that led to elevated serum B12 levels. When combined with other traditional clinical criteria like the CHILD PUGH score, the molecular marker of elevated B12 concentrations offers predictive value that may be prognostically helpful for early illness progression detection and improved treatment approaches.

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