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Assessment of Vitamin D Levels in Patients of Chronic Liver Disease with Hepatic Encephalopathy

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Abstract

Introduction: Patients with chronic liver disease (CLD) manifest various nutritional abnormalities associated with progression and severity of liver disease. Hepatic encephalopathy is a manifestation of disease progression with worsening liver function. The pathogenesis of hepatic encephalopathy is considered multi factorial and the exact mechanisms are yet to be deciphered.

25 hydroxyvitamin D (25 OHD) is a fat-soluble seco-steroid hormone associated with regulation of calcium, magnesium, and phosphate homeostasis. It is also associated with various other biological effects as an anti-inflammatory and immune-modulatory agent.

Levels of 25 OH vitamin D progressively declines with deteriorating liver function. Vitamin D deficiency is reported in patients with chronic liver disease and at least one- third of these have severe vitamin D deficiency. Vitamin D deficiency is also linked with severity of Child Pugh score in cirrhosis. Although uncertain is the role of vitamin D as a causative factor for hepatic encephalopathy, studies have demonstrated significant lower levels of vitamin D with worsening hepatic encephalopathy.

This study was therefore undertaken with the aim to access the vitamin D levels in patients of chronic liver disease and to draw its correlation with the degree of severity in hepatic encephalopathy.

Materials and Method: This Hospital based observational study was done in the Department of Medicine, Assam Medical College and Hospital, Dibrugarh. One year (1st June 2020 to 31st May 2021).

Chronic liver disease with hepatic encephalopathy with patients >12 years of age included in the study. Acute Hepatitis, Acute liver failure, Cardiac disease, Chronic kidney disease, Cerebrovascular accidents, Malignancy, Active tuberculosis, Steroids, bisphosphonates therapy, Vitamin D supplementation in past 3 months, Pregnancy and lactation, Malabsorption syndromes were excluded.

Results: Out of 88 patients, the majority of the subjects in the study group belonged to the age group of 51-60 years (51. 14%).The mean age was 54.44 ± 7.09 years.

Male were more common (71.5%, n=63) than female (28.4%, n=25). The sex ratio was 2.52:1.

The mean vitamin D levels in covert and overt subgroups were 24.11 ± 6.46 ng/ml and 11.72 ± 4.84 ng/ml respectively.

In the overt group, 47.92% (n=23) of subjects were having serum levels of vitamin D lower than 10ng/ml i.e. severe deficiency. The number of subjects in the deficiency level of vitamin D (10 - < 20 ng/ml) in the covert group were 30% (n=12) and in the overt group were 45.83% (n=22) respectively. 57.50% (n=23) in the covert group and 6.25% (n=3) in the overt group were having serum levels of vitamin D between 20-30 ng/ml. 12.5% (n=5) of the subjects in the covert group were having sufficient serum levels of vitamin D.

The mean serum vitamin D values in CTP Class A, B, C were 27.69 ± 6.66 ng/ml, 19.91 ± 3.76 ng/ml, 11.64 ± 5.06 ng/ml respectively. The results were statistically significant after One Way ANOVA analysis (p< 0.001).

The mean serum vitamin D level in patients with grade 1 Hepatic encephalopathy is 24.11 ± 6.46 ng/ml followed by grade 2 encephalopathy (13.61 ± 4.73 ng/ml), grade 3(8.41 ± 2.84 ng/ml) and grade 4 encephalopathy (8.00 ± 2.66 ng/ml). Statistical analysis with One Way ANOVA showed a significant p value (<0.001). There was a statistically significant negative correlation between serum vitamin D levels and hepatic encephalopathy (r = -0.731, p <0.001)

There was a statistically significant negative correlation between CTP score and serum vitamin D levels. (r = -0.767, p <0.001).

Conclusion: Our study showed that there was a significant negative correlation between serum levels of vitamin D and the grades of hepatic encephalopathy.

Keywords: Chronic Liver Disease, Hepatic Encephalopathy, Vitamin D

Introduction

Hepatic encephalopathy is the term used to describe the complex and variable changes in neuropsychiatric status that complicate liver disease[1]. Patients with chronic liver disease (CLD) manifest various nutritional abnormalities associated with progression and severity of liver disease. The manifestation is thought to be due to alteration in absorption of nutrients with associated altered metabolism as well as deterioration in synthetic function of liver[2].

Hepatic encephalopathy is thus a manifestation of disease progression with worsening liver function. In hepatic encephalopathy, a spectrum of neuropsychiatric abnormalities exists, ranging from clinically indiscernible changes in cognition to clinically obvious changes in intellect, behavior, motor function and consciousness. Hepatic encephalopathy is classified into covert and overt hepatic encephalopathy.[1] Hepatic encephalopathy has been observed in nearly 55% of patients with cirrhosis with overt hepatic encephalopathy occurring in about 30 – 45 % of patients with cirrhosis.[3]

The pathogenesis of hepatic encephalopathy is considered multifactorial and the exact mechanisms are yet to be deciphered. There are, however, a wide array of precipitating factors, which includes abnormal ammonia metabolism, upper gastrointestinal bleed, spontaneous bacterial peritonitis, intestinal dysbiosis[4], decrease in serum levels of branched chain amino acids[5], abnormalities of electrolytes[6], and micronutrient abnormalities like zinc and manganese.[7] Most importantly features of hepatic encephalopathy are

reversible with adequate treatment, thereby ascerting it to be a functional cause.[8] However, repeated episodes of overt hepatic encephalopathy can have persistence and cumulative defects in working memory, response inhibition and learning [9]implicating the fact that prevention of overt hepatic encephalopathy is paramount to preservation of mental integrity in patients with cirrhosis.[10]

25 hydroxyvitamin D (25 OHD) is a fat-soluble seco-steroid hormone associated with regulation of calcium, magnesium, and phosphate homeostasis. It is increasingly recognized that apart from being involved in homeostasis of calcium and phosphate, vitamin D is involved in a wide range of multiple biological targets mediated by the Vitamin D receptors, evidenced by the presence of these receptors in tissues including brain, cardiac tissue, skeletal muscle to name a few. Thus, vitamin D is also associated with various other biological effects as an anti-inflammatory and immune-modulatory agent. Interestingly, studies have demonstrated its role to be associated with various neurological deficits like loss of cognitive function, dementia, and Alzheimer‘s disease.[11] And similar studies have shown association between vitamin D deficiency and depression and schizophrenia.[12]

Obtained from dietary sources and indigenous production in the body after exposure to ultraviolet light, the first step in the hydroxylation of vitamin D occurs in the liver. Levels of 25 OH vitamin D progressively declines with deteriorating liver function.[10] Vitamin D deficiency is reported in 92% of patients with chronic liver disease and at least one- third of these have severe vitamin D deficiency.[13] Vitamin D deficiency is also linked with severity of Child Pugh score in cirrhosis.[14]

Although uncertain is the role of vitamin D as a causative factor for hepatic encephalopathy, studies have demonstrated significant lower levels of vitamin D with worsening hepatic encephalopathy.[10] Studies have shown lower levels of vitamin D in patients with encephalopathy than their non- encephalopathy counterparts.[15]This study was therefore undertaken with the aim to access the vitamin D levels in patients of chronic liver disease and to draw its correlation with the degree of severity in hepatic encephalopathy.

Aims and objectives

Aims: To study vitamin D level in chronic liver disease with hepatic encephalopathy.

Objectives: To access vitamin D levels in patients of chronic liver disease. To compare levels of vitamin D between patients of chronic liver disease with covert and overt hepatic encephalopathy.

Materials and methods

This Hospital based observational study was done in the Department of Medicine, Assam Medical College and Hospital, Dibrugarh. One year (1st June 2020 to 31st May 2021). All patient of chronic liver disease with hepatic encephalopathy above 12 years of age who were admitted in the wards of Department of Medicine in Assam Medical College and Hospital.

Inclusion criteria

1. Male and female patients >12 years of age with patient/guardian written consent.
2. All patients who have been diagnosed as chronic liver disease with hepatic encephalopathy.

Exclusion criteria

Acute Hepatitis, Acute liver failure, Cardiac disease, Chronic kidney disease, Cerebrovascular accidents, Malignancy, Active tuberculosis, Steroids, bisphosphonates therapy, Vitamin D supplementation in past 3 months, Pregnancy and lactation, Malabsorption syndromes and Not giving informed consent.

Sample size: Considering 95% confidence interval with a margin of error of 10% and taking the findings of study by Vidot H et al.[10] as reference the sample size is calculated to be 88 for the present study.

Consent: Informed written consent was taken from the patients or their attendants after explaining about the purpose of the study.

Ethical clearance: Ethical clearance was taken from the Institutional Ethics Committee

Diagnostic criteria: Garcia – Tsao criteria[16]: A Non-Histopathological diagnostic criterion for cirrhosis of liver. A case of cirrhosis of liver was defined as a patient having the following:

A) Clinical signs of hepatocellular dysfunction.

B) Clinical findings of portal hypertension.

C) USG finding suggestive of cirrhosis of liver.

A) Clinical signs of hepatocellular dysfunction: Jaundice, Neurological changes (Hepatic encephalopathy), Skin changes: spider angiomas, palmar erythema and Endocrine changes: breast atrophy, gynaecomastia, testicular atrophy.

B) Clinical Findings of portal hypertension: Hematemesis, melena or gastroesophageal varices by UGI endoscopy, Splenomegaly and Ascites

Findings in USG abdomen suggestive of cirrhosis of liver: Coarse echotexture, Nodular surface, Increased caudate to right lobe (C/RL) ratio and Features of portal hypertension: ascites, splenomegaly, varices, portal venous flow rate <16 cm/sec.

Etiology of chronic liver disease

Hepatitis B: Presence of HBsAg antigen

Hepatitis: Presence of anti HCV antibody

Alcoholic CLD: The diagnosis of Alcohol related CLD was made on the basis of:

History of Alcohol Abuse- Intake of 40-80 grams ethanol/day by males and of 20-40 grams/day by females for 10 to 12 years, AST: ALT ratio usually exceeding 2:1, Rise in serum GGT and Macrocytosis (The combination of a raised mean cell volume and raised serum GGT identifies 90% of alcohol - dependent patients).

Autoimmune Hepatitis

There are 2 scoring systems that had been used for the diagnosis of Autoimmune hepatitis: Revised Original Scoring System for diagnosis of Auto Immune Hepatitis (more sensitivity) and Simplified diagnostic scoring system (more specificity and accuracy)

In our study, we are using the revised original scoring for the diagnosis of Autoimmune Hepatitis.

Method of data collection

Data was collected from patients of chronic liver disease with hepatic encephalopathy admitted in AMCH after written informed consent. Patients were selected according to inclusion and exclusion criteria. Demographic and clinical data was collected for each patient.

Methodology

Detailed history of the patient was taken including present illness, past illnesses, comorbid conditions, alcohol intake, intake of herbal medications and drugs. A thorough clinical examination was done for all patients included in the study including general examination and abdominal examination. Routine blood and urine examinations done. Liver function tests including Serum total protein and fractions, Serum bilirubin and fractions, AST, ALT, GGT, ALP was done. Prothrombin with INR was done. Random Blood sugar, serum urea, serum creatinine was done. Viral markers including Hepatitis B and Hepatitis C was done. USG whole abdomen was done in all cases to look for presence of features suggestive of cirrhosis of liver, portal hypertension and splenomegaly. Upper Gastrointestinal endoscopy

(Esophagogastroduodenoscopy) was done for presence and grading of gastroesophageal varices. Etiology specific tests was done only in those patients where it was indicated based on high index of suspicion. (Anti-nuclear antibody (ANA), Anti-mitochondrial antibody (AMA), Anti-smooth muscle antibody (ASMA), Anti-Liver Kidney Microsomal 1 antibody (Anti LKM 1), Serum ceruloplasmin, 24 hr urinary copper, Serum Ferritin, Transferrin saturation, HbA1c, Lipid Profile. Diagnostic abdominal paracentesis was done before the first dose of antibiotic and ascitic fluid examined for cell count, neutrophil count, protein, gram stain, direct microscopy for fungus, bacterial culture and sensitivity. Child Turcotte Pugh scoring was used to classify each patient. Mental status was graded according to West Haven criteria in patients of Hepatic encephalopathy.

Laboratory investigations

Routine tests: The tests were done in Pathology, Microbiology and Biochemistry Laboratories of Assam Medical College and Hospital, Dibrugarh.

CBC, **ESR, Blood Urea, Serum Creatinine and Blood Sugar:**

Liver function tests

Serum bilirubin: By dual wavelength end point colorimetric method.

Normal reference interval: Total bilirubin: 0.2-1.3mg/dL; Unconjugated:0-1.1 mg/dL; Conjugated: 0-0.3mg/dL.

Serum protein: The method of analysis was based on the biuret reaction, which produces a violet complex when protein reacts with cupric ion in an alkaline medium. The amount of coloured complex formed was proportional to the amount of total protein in the sample and was measured by reflectance spectrophotometry. Normal reference interval: Protein: 6.3-8.2gm/dl.

Serum Albumin: Albumin binds with bromocresol green dye and this binding result in a shift in wavelength of the reflectance maximum of the free dye. The colour complex formed was measured by reflectance spectrophotometry. The amount of albumin-bound dye was proportional to the concentration of albumin in the sample.

Normal reference interval- Albumin: 3.5-5g/dL; Globulin: 2.5-3.5g/dL.

AST: In the assay for aspartate aminotransferase, the amino group of L-aspartate was transferred to α-ketoglutarate in the presence of pyridoxal-5-phosphate to produce glutamate and oxaloacetate. The oxaloacetate was converted to pyruvate and carbon dioxide by oxaloacetate decarboxylase. Pyruvate was oxidized to acetyl phosphate and hydrogen peroxide by pyruvate oxidase. The final reaction step involved the peroxidase catalysed oxidation of a leuco dye to produce acoloured dye. The rate of oxidation of the leuco dye was monitored by reflectance spectrophotometry. The rate of change in reflectance density was proportional to enzyme activity in the sample.

Normal reference interval: 15-46 U/L

ALT: Alanine aminotransferase catalyses the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutamate. Lactate dehydrogenase then catalyses the conversion of pyruvate and NADH to lactate and NAD+. The rate of oxidation of NADH was monitored by reflectance spectrophotometry. The rate of change in reflection density was proportional to enzyme activity.

Normal reference interval: 13-69 U/L

Alkaline phosphatase: The ALP in the sample catalyses the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline pH. The p-nitrophenol was monitored by reflectance spectrophotometry. The rate of change in reflection density was converted to enzyme activity.

Normal reference interval: 38-126 U/L

GGT: GGT catalyses the transfer of the γ-glutamyl portion of L-γ-glutamyl-p-nitroanilide to glycylglycine, simultaneously producing p-nitroaniline. The rate of change in reflection density was measured and was used to calculate the enzyme activity of GGT.

Normal reference interval: 12-58 U/L

Prothrombin time (PT & INR)

0.2ml of UNIPLASTIN reagent was added to normal citrated plasma and a stop watch was started simultaneously. The clotting mechanism is initiated, forming solid gel clot within specified period of time. The watch was stopped as soon as the first fibrin clot was visible and gel/clot was formed. The results may be reported as ratio or mean of the double determination of PT of the test in seconds or as a ratio (R)

reagent for MNPT

$$R = \frac{\text{Mean of patient plasma PT in seconds}}{\text{MNPT for reagent}}$$

Or it can be expressed as internationalized normalized ratio (INR) = (R) ISI. Usually plasma of at least 20 normal healthy individuals should be used to establish the Mean PT (MNPT).

Estimation of vitamin d levels:

25 OH Vitamin D levels were estimated by Affimedix MicrO 25OH vitamin D Sandwich ELISA test.148 The test was carried out in Multi-Disciplinary Research Unit, Assam Medical College and Hospital.

Defining optimal vitamin D status

Most studies define Vitamin D insufficiency as a 25OH Vitamin D level below 75 nmol/L (30 ng/ml) and deficiency as levels below 50 nmol/L (20 ng/ml) .[17] Reference range was 30–100 ng/ml. Jamil Z et al.[18] conducted a study in Pakistan where he used the reference level as deficiency was defined as vitamin D levels <20 ng/ml ; between 21 ng/ml to 30 ng/ml was considered insufficient vitamin D and above 31 ng/ml was considered sufficient. The Institute of Medicine (IOM) of the National Academies in the United States suggests a serum Vitamin D concentration of 20 ng/ml as adequate. Contrary to the stipulation of IOM, the Endocrine Society of Maryland, USA recommends Vitamin D levels of 30 ng/ml (i.e. 75 nmol/L) and even suggests that concentrations between 40 and 60 ng/ml (i.e. between 100 and 150 nmol/L) may be advantageous as a precautionary measure because of the variability of assays in 25OH Vitamin D determination.143 The classification of the Vitamin D status were as follows:

Severe deficiency: < 10 ng/ml; deficiency: 10-20 ng/ml; insufficiency: 20-29 ng/ml; sufficiency: 30-100 ng/ml; (To convert results into SI units: ng/mL × 2.5 = nmol/L)149.

Affimedix MicrO 25OH vitamin D Sandwich ELISA test showed the reference values of severe deficiency as < 10 ng/ml, deficiency as 10-20 ng/ml, insufficiency as serum levels between 20-30 ng/ml and sufficiency as >30- 100, excess levels as 100-150 ng/ml and toxicity as >150 ng/ml.

For the purpose of this study Vitamin D deficiency has been defined as serum 25OH Vitamin D levels lower than 20ng/mL (i.e.50 nmol/L) and severe deficiency as < 10 ng/ml and vitamin D insufficiency has been defined as serum levels between 20 and 30 ng/ml (i.e.50-75nmol/L). Sufficiency levels described as > 30 ng/ml.

Principle of the test

Test kit employed is a sandwich based Enzyme linked immunosorbent assay. The test employs a very ―Exclusive‖ pair of anti 25OH Vitamin D Monoclonal Antibodies, first antibody is mobilized on solid phase (microtitre wells) and second antibody is in the liquid phase. The test will selectively detect 25OH Vitamin D with a high degree of sensitivity and specificity. In the

assay procedure, Calibrators and test specimen are incubated with first and second antibodies in the microtitre wells for 20 minutes. Following a wash step, the resultant Vitamin D antibody immunocomplex is detected with a third antibody conjugated with horseradish peroxidase (HRP). After 10 minutes incubation, the unbound antibody HRP conjugate is washed away. Next a solution of TMB is added for 10 minutes development of blue color. The color development is stopped by addition of stop solution and the resulted yellow color is measured at 450nm/630nm using a microtitre plate reader. The color intensity will be positively proportional to the concentration of Vitamin D in the sample. The time for assay completion is 40 minutes.

1. **Collection of blood samples**: Blood was drawn using standard venipuncture techniques and the serum separated as soon as possible. Serum samples were stored at -80oC. Affimedix MicrO 25OH vitamin D Sandwich ELISA test kit was used for estimation of vitamin D. Thawed samples are mixed prior to testing.

2. **Assay procedure:** The desired number of coated wells are secured in the holder. 10ul of serum and controls are dispensed into the appropriate wells.200ul of sample diluent are dispensed into each wells and mixed gently for 30 seconds and incubated at room temperature for 20 minutes. The plate contents are emptied into a waste container. The microtitre wells are rinsed and emptied with diluted wash buffer for 5 minutes.100ul of enzyme conjugate reagent is dispensed into each well, mixed well and incubated at room temperature for 10 minutes. The plate contents are emptied into a waste container. The microtitre wells are rinsed and emptied with diluted wash buffer for 5 minutes.100ul of TMB substrate is dispensed into each well and incubated for 10 minutes at room temperature in dark. The reaction is stopped by adding 100ul of stop solution into each well and gently mixing until the blue color completely changes to yellow. The optical density is read at 450nm/630nm with microtitre plate reader within 15 minutes.

3. **Standard curve and calculation of results**

A standard curve was generated by plotting the absorbance obtained from each reference calibrator against the corresponding concentration. A typical standard curve with optical density reading at 450 nm/630 nm in the Y axis against 25 OH Vitamin D in the X axis is plotted. The standard curve is generated for each testing. The absorbance value for each sample is used to determine its corresponding concentration of vitamin D from the standard curve.

West Haven's Criteria for Hepatic Encephalopathy[19] and The Ishen Criteria

Grade	Intellectual function	Neuromuscular function
0	Normal	Normal
Minimal	Normal examination findings, subtle changes in work or driving.	Minor abnormalities of visual perception or on psychometric or number tests.
1	Personality changes, attention deficits, irritability, depressed state	Tremor and incoordination
2	Changes in sleep-wake cycle, lethargy, mood and behavioural changes, cognitive dysfunction	Asterixis, ataxic gait, speech abnormalities (slow and slurred).
3	Altered level of consciousness (somnolence),	Muscular rigidity, nystagmus, clonus, Babinski

	confusion, disorientation, and amnesia	sign, hyporeflexia.
4	Stupor and coma	Oculocephalic reflex, unresponsiveness to noxious stimuli.

Child Turcotte Pugh Score (CTP Score) [20,21]

The CTP score of each patient was calculated on the basis of 2 qualitative (Ascites and Hepatic Encephalopathy) and 3 quantitative variables (Albumin, Bilirubin, and INR).

Parameter	Points		
	1 point	2 points	3 points
Hepatic encephalopathy	None	Grade 1-2	Grade 3-4
Ascites	None	Mild	Moderate to severe
Bilirubin(mg/dL)	<2	2-3	>3
Bilirubin in PBC	<4	4-10	>10
Albumin(g/dL)	>3.5	2.8-3.5	<2.8
Prothrombin time prolongation (sec prolonged) or INR	< 4s or INR<1.7	4-6s or INR 1.7-2.3	>6 or INR >2.3

The Child Pugh score was calculated by adding the scores of the five factors and can range from 5 to 15.

Child Pugh class is A when score is 5-6.

Child Pugh class is B when score is 7-9.

Child Pugh class is C when score is 10-15.

Imaging: Imaging studies such as ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) are sensitive tests to diagnose cirrhosis, however final diagnosis still relies on histology. Ultrasound scan of liver, widely used as a sensitive test for early detection of liver cirrhosis. It is highly useful in picking up the nodularity, round border and hypo echoic nodules of liver**.**[22,23]

The ultrasound features associated with cirrhosis include the following:

Volume Redistribution: In early stages the liver may enlarge, whereas in advanced disease it is often small, however with a relative enlargement of the caudate lobe, left lobe, or both, in comparison with the right lobe. A caudate lobe width to the right lobe width (C/RL) value of 0.65 is considered indicative of cirrhosis**.**[24]

Coarse Echotexture: In diffuse liver disease the features of increased echogenicity and coarse echotexture are seen. These findings are however subjective and have a relatively low accuracy in distinguishing diffuse liver disease.[25]

Nodular Surface: Surface irregularity of the liver is taken as a sign of cirrhosis when the appearance is gross or when ascites is present.102 The nodularity represents the regenerating nodules and fibrosis.

Regenerating Nodules: The regenerating hepatocytes are surrounded by fibrotic septae. Because regenerative nodules have a similar architecture to the normal liver, ultrasound and CT have limited ability in their detection. Regenerating nodules are isoechoic or hypoechoic with a thin echogenic border that corresponds to fibrofatty connective tissue.[26]

Dysplastic Nodules: Dysplastic nodules or adenomatous hyperplastic nodules are larger than regenerating nodules

(diameter of 10 mm) and are considered premalignant.[27] They contain well-differentiated hepatocytes, a portal venous blood supply, and atypical malignant cells.

USG findings of portal hypertension include the secondary signs of splenomegaly (>13cm), ascites, and portosystemic venous collaterals.

Five major sites of portosystemic venous collaterals are visualized by ultrasound.[28,]

1. Gastroesophageal junction
2. Paraumbilical vein
3. Splenorenal and gastrorenal
4. Intestinal-retroperitoneal anastomoses
5. Hemorrhoidal- perianal region

Other modalities of imaging include Conventional CT and MRI, although both are not helpful to determine the severity of cirrhosis.[29]

Upper GI Endoscopy in Cirrhosis: Upper GI endoscopy is the most commonly used method to detect varices which is a sign of portal hypertension. Various criteria have been proposed till date to standardize the grading of esophageal varices. The most accepted of these criteria are those given by the Japanese Research Society for Portal Hypertension.[30]

It includes –

(a) Red color signs:

Red ―wale markings: these are longitudinal whip-like marks on the varix.

Cherry-red spots: these are usually 2 to 3 mm or less in diameter.

Hematocystic spots: these are blood-filled blisters 4 mm or greater in diameter.

Diffuse redness.

(b) Color of the varix: The color of the varix can be white or blue.

(c) Form (size) of the varix: It includes three grades:

Grade I- Small and straight.

Grade II - Tortuous and occupying less than one third of the esophageal lumen.

Grade III- Large and occupying more than one third of the esophageal lumen.

(d) Location of the varix: Esophageal varices may be present in the lower third, middle third, or upper third of the esophagus. Other findings of upper GI endoscopy in cirrhosis are- portal gastropathy, peptic ulcer, erosive gastritis, reflux esophagitis etc.

Statistical Analysis: The data collected was tabulated in Microsoft Excel Worksheet 2010 and computer-based analysis was performed using the Statistical product and service solutions (SPSS) 20.0 software (SPSS, Chicago, Illinois, USA). Results on continuous measurements are presented as mean ± standard deviation and are compared using Analysis of Variance (ANOVA). Discrete data are expressed as number (%) and analysed using Chi square test and Fischer's exact test. Pearson's correlation coefficient (r) was used to measure the associations among continuous variables. For all analyses, statistical significance was fixed at 5% level (p value < 0.05).

Results and observations

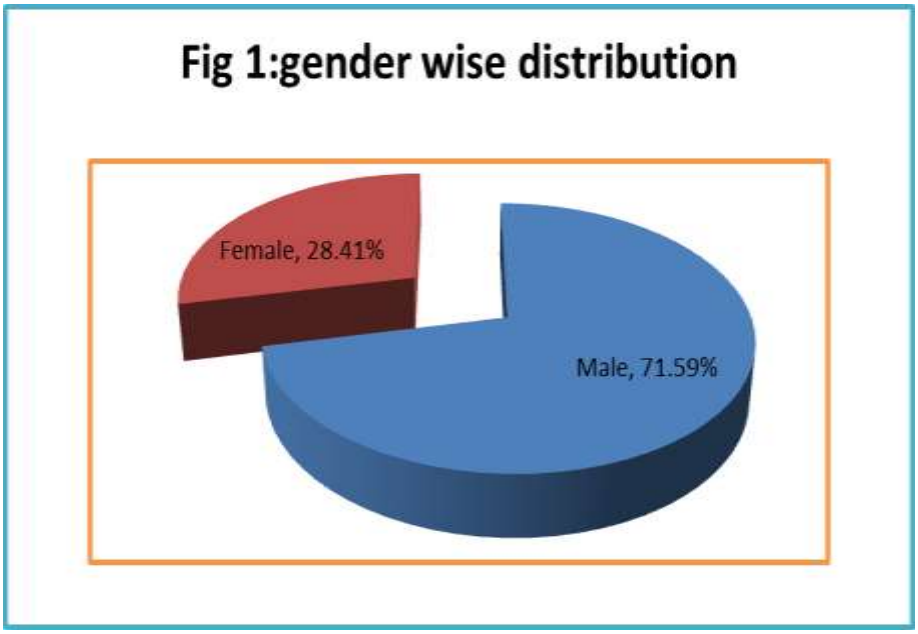
Total of 88 patients were included in our study. The results and observations are illustrated in the following tables and figures:

Table 1: age wise distribution of the study population

Age Group (In Years)	Number (N)	Percentage (%)
12–20	0	0.00
21-30	0	0.00
31–40	0	0.00
41–50	27	30.68
51–60	45	51.14
61–70	14	15.91
>70	2	2.27
Total	88	100.00
Mean ± S.D. = 54.44 ± 7.09 years		

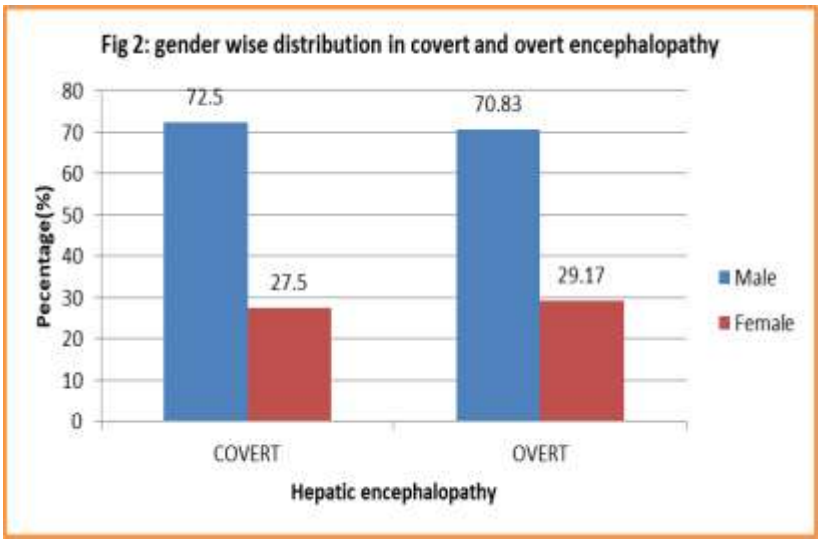
The above table 1 shows that the majority of the subjects in the study group belonged to the age group of 51-60 years (51.14%) followed by the subjects in the age group of 41-50 years (30.68%), 61-70 years (15.91%) and >70 years (2.27%). The mean age of the whole study group was 54.44 ± 7.09 years.

Figure 1: Gender wise distribution



The above fig 1 shows that male are more common (71.5%, n=63) than female (28.4%, n=25) in the study group. The sex ratio was 2.52:1.

Figure 2: Gender wise distribution in covert and overt encephalopathy



Above fig 2 shows that, in the covert encephalopathy group male subjects constituted 72.50% (n=29), while in the overt subgroup 70.83% (n=34) were male subjects. The female distribution in the two groups of covert and overt encephalopathy were 27.5 % (n=11) and 29.17% (n=14) respectively.

Table 2: Age wise distribution in covert and overt encephalopathy

Age group (in years)	Covert		Overt	
	number (n)	Percent (%)	Number (n)	Percent (%)
12–20	0	0.00	0	0.00
21-30	0	0.00	0	0.00
31–40	0	0.00	0	0.00
41–50	13	32.50	14	29.17
51–60	22	55.00	23	47.92
61–70	4	10.00	10	20.83
>70	1	2.50	1	2.08
TOTAL	40	100.00	48	100.00
Mean ± S.D.	53.98 ± 6.64 years		54.83 ± 7.49 years	

The above table 2 shows that the majority of the subjects belonged to the age group of 51-60 years (55% in the covert subgroup and 47.92% in the overt subgroup), followed by the subjects in the age group of 41-50 years (32.5% in covert and 29.17% in overt subgroups). 10% and 20.83% of the study population fall in the age group of 61-70 years in covert and overt subgroups. The mean age in covert group is 53.98 ± 6.64 years, and in the overt group is 54.83 ± 7.49 years.

Table 3: Etiology of CLD in the study population

Etiology	Number (n)	Percentage (%)
Alcoholic	59	67.05
Hepatitis B	8	9.09

Hepatitis C	2	2.27
Autoimmune	4	4.55
Unknown	15	17.05
Total	88	100.00

Above table 3 shows that the most common cause of CLD was alcohol (67.05%) followed by Unknown (17.05%). Other causes of CLD included Chronic hepatits B, Chronic hepatitis C and Autoimmune causes accounting for 9.09%, 2.27% and 4.55% respectively.

Table 4: Baseline laboratory parameters in covert and overt encephalopathy

Parameter	Covert		Overt		p value*
	Mean	±S.D.	Mean	±S.D.	
25-OH Vitamin D	24.11	24.11	24.11	24.11	0.002
Total Bilirubin	2.91	2.20	4.41	3.22	0.0115
Albumin	3.07	0.48	2.60	0.52	0.003
INR	2.03	1.15	2.68	1.21	0.0121
AST	96.93	96.93	92.60	92.60	0.6126
ALT	63.63	32.00	58.04	25.75	0.3767
ALP	110.10	30.87	98.90	27.64	0.0793

*Student t Test; The p-value is significant at 5% level of significance.

The above table (Table 4) shows the laboratory findings in the study group. The mean vitamin D levels in covert and overt subgroups were 24.11 ± 6.46 ng/ml and 11.72 ± 4.84 ng/ml respectively. The mean albumin was 3.07 ± 0. 48 g/dl and 2.68 ± 0.52 g/dl, mean total bilirubin was 2.91 ± 2.20 mg/dl and 4.41 ± 3.22 mg/dl, mean SGOT / AST was 96.93 ± 96.93 U/L and 92.60 ± 92.60 U/L, mean SGPT/ ALT was 63.63 ± 32 U/L and 58.04 ± 25.75 U/L, mean ALP was 110.1 ± 30.87 and 98.9 ± 27.64 U/L mean INR was 2.03 ± 1.15 and 2.68 ± 1.21.

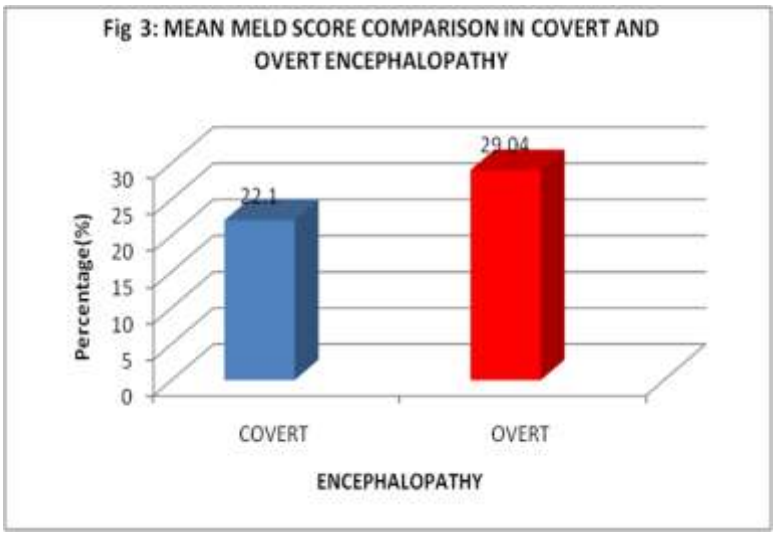
Table 5: CTP class distribution in subgroups of covert and overt encephalopathy

CTP Score Class	Covert		Overt		p value*
	Number (N)	Percent (%)	Number (N)	Percent (%)	
A	20	50.00	0	0.00	<0.001
B	12	30.00	10	20.83	
C	8	20.00	38	79.17	
Total	40	100.00	48	100.00	

*Fisher Exact Test; The p-value is significant at 5% level of significance.

Above Table 5 depicts the distribution of cases in the study group based on the Child Turcotte Pugh score. In the covert group majority of the patients belong to class A, 50% (n=20), followed by class B, 30% (n=12), followed by class C, 20% (n=8). Whereas in the overt encephalopathy group majority of the patients belong to CTP class C 79.17 % (n=38) and 20.83% (n=10) belong to CTP class B.

Figure 3: Mean MELD score comparison in covert and overt encephalopathy



The above Fig 3 shows the values of mean MELD scores among the subgroups of covert and overt hepatic encephalopathy. The mean MELD score in covert group was 22.10 ± 8.89 and in the overt group was 29.04 ± 7.03. The difference in mean MELD score was statistically significant ($p < 0.001$).

Table 6: Comparison of vitamin d level in covert and overt encephalopathy

Vitamin D Level (Ng/Ml)		Covert		Overt		p value*
Severe Deficiency	<10	0	0.00	23	47.92	<0.001
	10 - <20	12	30.00	22	45.83	
Insufficiency	20–30	23	57.50	3	6.25	
Sufficiency	>30	5	12.50	0	0.00	
Total		40	100.00	48	100.00	

Table 6 the distribution of serum vitamin D levels among the groups of covert and overt hepatic encephalopathy. In the overt group, 47.92% (n=23) of subjects were having serum levels of vitamin D lower than 10ng/ml i.e. severe deficiency. The number of subjects in the deficiency level of vitamin D (10 - < 20 ng/ml) in the covert group were 30% (n=12) and in the overt group were 45.83% (n=22) respectively. 57.50% (n=23) in the covert group and 6.25% (n=3) in the overt group were having serum levels of vitamin D between 20-30 ng/ml. 12.5% (n=5) of the subjects in the covert group were having sufficient serum levels of vitamin D.

Table 7: Comparison of vitamin D level in different etiology of hepatic encephalopathy

Etiology	Number (N)	Percentage (%)	Vitamin D Level (ng/ml) (Mean ± S.D.)
SBP	17	19.32	15.63 ± 5.44
HRS	8	9.09	14.31 ± 3.41
H/M	38	43.18	18.12 ± 9.68

Hypokalemia	47	53.41	16.59 ± 8.58
Others	26	29.55	16.06 ± 7.15

Table 7 above shows the distribution of mean values of vitamin D among different etiology of hepatic encephalopathy. The mean vitamin D levels were 15.63 ± 5.44 ng/ml, 14.31 ± 3.41 ng/ml, 18.12 ± 9.68 ng/ml, 16.59 ± 8.58 ng/ml, 16.06 ± 7.15 ng/ml in the etiologies of sub-acute bacterial peritonitis, Hepato-renal syndrome, Haematemesis and maelena, hypokalemia and others respectively.

Figure 4: Comparison of vitamin d level with CTP class

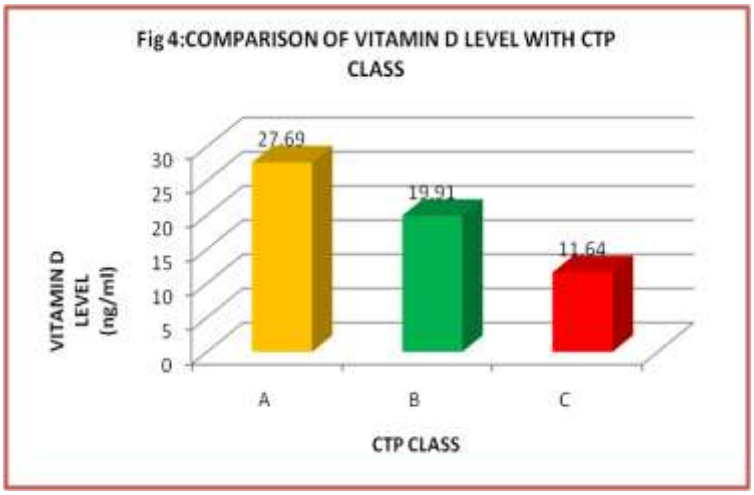


Fig 4 shows the mean Vitamin D values among the CTP Class. The mean serum vitamin D values in CTP Class A, B, C were 27.69 ± 6.66 ng/ml, 19.91 ± 3.76 ng/ml, 11.64 ± 5.06 ng/ml respectively. The results were statistically significant after One Way ANOVA analysis ($p < 0.001$).

Table 8: Comparison of vitamin D level with MELD score

Meld Score	Number (N)	Percentage (%)	Vitamin D Level (ng/ml) (Mean ± S.D.)	P value*
≤9	2	2.27	26.34 ± 9.59	<0.001
10—19	21	23.86	23.54 ± 6.92	
20—29	34	38.64	18.58 ± 7.82	
30—39	23	26.14	11.37 ± 5.03	
≥40	8	9.09	10.87 ± 6.24	
Total	88	100.00	17.36 ± 8.35	

*One Way ANOVA; The p-value is significant at 5% level of significance

Table 8 shows the mean values of serum vitamin D levels with MELD scores in the study population.

A MELD score of ≤9 had a mean serum vitamin D level 26.34 ± 9.59 ng/ml, followed by 23.54 ± 6.92 ng/ml in patients with a score of 10—19. Patients with a score of 20—29 had serum vitamin D levels of 18.58 ± 7.82 ng/ml, followed by patients with a score of 30—39 had vitamin D levels of 11.37 ± 5.03 ng/ml. Patients with a MELD score of ≥40 had serum vitamin D levels between 10.87 ± 6.24 ng/ml. On One Way ANOVA analysis the results were statistically significant with a p value of < 0.05.

Table 9: Comparison of vitamin d level in grades of hepatic encephalopathy

Hepatic Encephalopathy Grade	Number (N)	Percentage (%)	Vitamin D Level (Ng/Ml) (Mean ± S.D.)	P value*
0	0	0.00	–	
1	40	45.45	24.11 ± 6.46	
2	31	35.23	13.61 ± 4.73	
3	12	13.64	8.41 ± 2.84	<0.001
4	5	5.68	8.00 ± 2.66	
Total	88	100.00	17.36 ± 8.35	

Table 9 shows the mean serum vitamin D level in patients with grade 1 Hepatic encephalopathy is 24.11 ± 6.46 ng/ml followed by grade 2 encephalopathy (13.61 ± 4.73 ng/ml), grade 3(8.41 ± 2.84 ng/ml) and grade 4 encephalopathy (8.00 ± 2.66 ng/ml). Statistical analysis with One Way ANOVA showed a significant p value (<0.001).

Table 10: Comparison of vitamin d level according to outcome

Outcome	Number (N)	Percentage (%)	Vitamin D Level (ng/ml) (Mean ± S.D.)	p value*
Discharged	77	87.50	18.34 ± 8.30	0.003
Death	11	12.50	10.48 ± 4.94	
Total	88	100.00	17.36 ± 8.35	

*One Way ANOVA; The p-value is significant at 5% level of significance.

Table 10 shows the comparison of levels of vitamin D according to the outcome. The mean serum vitamin D levels in patients who were discharged was 18.34 ± 8.30 ng/ml, while the mean value in expired patients were 10.48 ± 4.94 ng/ml. On statistical analysis the results were not significant.

Table 11: Correlation between grade of hepatic encephalopathy and serum vitamin D levels

	Vitamin D Level (ng/ml)	
	r value	p value
Hepatic Encephalopathy (Grade)	–0.731	<0.001

Table 11 shows the correlation between the grades of hepatic encephalopathy and serum vitamin D levels. There was a statistically significant negative correlation between serum vitamin D levels and hepatic encephalopathy (r = -0.731, p <0.001).

Table 12: Correlation between CTP score and vitamin D

	CTP Score	
	r value	p value
Vitamin D Level (ng/ml)	–0.767	<0.001

Table 12 shows the correlation between the severity of CTP score and the serum levels of vitamin D. There was a statistically significant negative correlation between CTP score and serum vitamin D levels. (r = -0.767, p <0.001).

Table 13: Correlation between meld score and vitamin D

	MELD Score	
	r value	p value
Vitamin D Level (ng/ml)	–0.578	<0.001

Table 13 shows the correlation between severity of MELD Score and the serum levels of vitamin D. There was a statistically significant negative correlation between MELD score and serum vitamin D levels. (r = -0.578, p <0.001).

Discussion

The present hospital based observational study was carried out among 88 patients of cirrhosis of liver with overt and covert hepatic encephalopathy.

Age Distribution in the Study Population:

Our study shows that the majority of the subjects belonged to the age group of 51-60 years (51.14%) followed by age group of 41-50 years (30.68%),61-70 years (15.91%), and >70 years (2.27%). The mean age of the whole study group was 54.44 ± 7.09 years.

On further division of the study population into overt and covert encephalopathy groups, majority of the subjects in belonged to the age group of 51-60 years (55%) in the covert subgroup and (47.92%) in the overt subgroup followed by the age group of 41-50 years (32.5%) in covert and (29.17%) in overt subgroups.10% and 20.83% of the study population fall in the age group of 61-70 years in covert and overt subgroups respectively. Further, 2.5% and 2% in the covert and overt subgroups respectively fall in the age group of >70 years. The mean age in covert group is 53.98 ± 6.64 years, and in the overt group is 54.83 ± 7.49 years.

Kumar P et al. [2] observed that the age of the enrolled subjects in the study (chronic liver disease patients with hepatic encephalopathy) and control groups (chronic liver disease patients without hepatic encephalopathy) the mean age in the groups were 46.77 ± 13.76 years and 43.64 ± 13.89 years respectively.

Vidot H et al. [10] found the mean age in the overt encephalopathy group was 52 ± 7 years and in the non-hepatic encephalopathy group was 54 ± 8 years.

Afifi MAE et al. [31] observed that the age group in the hepatic encephalopathy and non-hepatic encephalopathy groups were 51.1 ± 6.2 years and 50.6 ± 7.2 years respectively.

Jha AK et al. [32] found that the mean age of the patients in the treatment group and control were 46.2 years (± 14.93) and 43.28 years (± 12.53), respectively in his study.

Jamil Z et al. [18] encountered in his study that the minimum age was 18 years and the maximum was 84 years with mean age of 56.88 years.

Gender Distribution in The Study Population:

We encounterd 71.5% male and 28.4% female with the sex ratio 2.52: 1.

Also, in the covert encephalopathy group male subjects constituted 72.50% while in the overt subgroup 70.83% were male subjects. The female distribution in the two groups of covert and overt encephalopathy were 27.5 % (n=11) and 29.17% (n=14) respectively.

Kumar P et al. [2] encountered 80% males in the study group.

Vidot H et al. [10] found that out 165 study subjects 119 were male and 46 were female with sex ratio 2.5:1. The number of male subjects in the covert encephalopathy group were 68 and female subjects were 20.

Afifi MAE et al. [31] encountered 38 males and 12 females in group 1 (patients with chronic liver disease with hepatic encephalopathy present), while 33 males and 17

females in group 2 (chronic liver disease without hepatic encephalopathy) with a sex ratio of 3.1: 1 and 1.9:1 respectively in both the groups.

Jha AK et al. [32] observed the male: female ratio in the treatment and control group were 3.6:1 and 2.8:1 respectively.

Etiology of Chronic Liver Disease in The Study Population:

In our study we observed that the most common cause of CLD was alcohol (67.05%) followed by unknown (17.05%), Chronic hepatitis B (9.09%,), Chronic hepatitis C (2.27%) and Autoimmune (4.55%) respectively.

Kumar P et al. [2] found that the alcohol ingestion was the most common etiology of cirrhosis. In the control group Alcoholic cirrhosis was seen in 58% and in the study group 62%, followed by hepatitis B which comprised 18% and 17% in the control and study group respectively.

Vidot H et al. [10] found that the most common etiology among the study groups of overt encephalopathy and non-hepatic encephalopathy was viral etiologies which include 59.5% and 50.6% subjects in each group respectively. Followed by Alcoholic cirrhosis in the overt encephalopathy group 20.5% and Cholestatic disease 25.9% in the non-encephalopathy group. In the overt encephalopathy group Cholestatic disease, NASH, and other causes include 11.5%, 6.3% and 2.2% subjects respectively. On the other hand, in the non-encephalopathy group alcohol, NASH and other causes constituted 6.4%, 1.2% and 15.5% of the study group respectively.

Jha AK et al. [32] observed that most common etiology of CLD was ethanol in both control (n = 24; 48%) and treatment (n = 19; 38%) groups, followed by HBV (control = 20%; treatment = 24%), cryptogenic CLD (control = 14%; treatment = 20%), and hepatitis C virus (control = 8%; treatment = 8%). Other uncommon etiologies were NASH and Wilson disease.

Jamil Z et al. [18] found that most common etiology of CLD was chronic hepatitis C (93.6%) which was followed by hepatitis B infection (4.8%) and seronegative liver disease (1.6%).

Sharma D et al.[33] encountered the etiologies of liver cirrhosis were alcohol (70%), viral infection (20%), autoimmune (2%), and cryptogenic (8%) of the patients.

Thus, the etiology of Chronic liver disease varies according to the geographical distribution and the racial characteristics. In our study and in the majority of the studies mentioned above the most common etiology was alcoholic cirrhosis.

Labarotory Parameters in The Study Popultion:

In our study we found that the mean vitamin D level in covert and overt subgroups were 24.11 ± 6.46 ng/ml and 11.72 ± 4.84 ng/ml respectively.

The mean albumin was 3.07 ± 0. 48 g/dl and 2.68 ± 0.52 g/dl, mean total bilirubin was 2.91 ± 2.20 mg/dl and 4.41 ± 3.22 mg/dl, mean SGOT/AST was 96.93 ± 96.93 U/L and 92.60 ± 92.60 U/L, mean SGPT/ ALT was 63.63 ± 32 U/L and 58.04 ± 25.75 U/L, mean ALP was 110.1 ± 30.87 and 98.9 ± 27.64 U/L mean INR was 2.03 ± 1.15 and 2.68 ± 1.21.

The study showed a negative correlation between serum albumin levels and grades of hepatic encephalopathy. There was a statistically significant negative correlation ($r = -0.507$, $p < 0.001$).

Kumar P et al. [2] found that the baseline laboratory parameters in the study and the control groups the mean level of serum 25-hydroxyvitamin D was found to be 25.62±21.94 nmol/l and 37.44±18.61 nmol/l respectively. The serum bilirubin, SGOT and SGPT, and INR were 3.6±2.32 mg/dl and 3.86±3.77mg/dl,84.33±70.4IU/L and 93.82±71.4 IU/L,73.98±49.73 IU/L and 75.66±37.48

IU/L,1.7±0.49 and 1.81±0.45 in the study and control group respectively. There were significantly higher levels of ALP (IU/L) in the study group (160.85 ± 48.65 vs 112.78 ± 38.65; P = 0.0001).

Jha AK et al. [32] observed that the mean bilirubin in the control and treatment groups were 8.95 (6.62-11.29) mg/dl and 6.89 (5.15-8.64) mg/dl respectively. Further mean parameters between the control and the treatment groups in the study of INR, ALT, AST, Albumin were 1.50 (1.41-1.59) and 1.43 (1.37-1.48),92 (61-119) IU/L and 84 (62-106) IU/L,157 (98-215) IU/L and 127 (106-147) IU/L,2.41 (2.32-2.50) g/dl and 2.38 (2.28- 2.49) g/dl,

respectively.

Afifi MAE et al. [31] showed that the baseline laboratory parameters in the hepatic encephalopathy and the non-hepatic encephalopathy groups as mean vitamin D levels of 27.2 ± 11.9 nmol/l and 41.0 ± 15.2 nmol/l, serum bilirubin levels of 3.7 ± 1.3 mg/dl and 2.2 ± 0.9 mg/dl, mean albumin levels of 2.9 ± 0.3 mg/dl and 3.5 ± 0.4 mg/dl, mean INR 1.9 ± 0.3 and 1.2 ± 0.3 respectively.

The mentioned studies showed lower vitamin D levels in the study groups with hepatic encephalopathy when compared with the non-encephalopathy counterparts.

CTP Score Distribution in The Study Population

We observed that the majority of the patients belong to class A (50%) followed by class B (30%) and class C (20%) in the covert group. Whereas in the overt encephalopathy group majority (79.17 %)of the patient belong to CTP class C followed by CTP class B (20.83%). On Fisher Exact analysis the results were statistically significant with a p value of < 0.001.

Vidot H et al.[10] found that majority of the patients belong to class A (53%) followed by class B(32%) and class C(14%) in the non-hepatic encephalopathy group. Whereas in the overt encephalopathy group majority of the patients belong to CTP class C (75%) followed by CTP class B (25%).

Afifi MAE et al. [31] found that none of the HE group was classified as CTP class A, while 54% of the non-HE group were classified as A, and 17% of this group were classified as C while 74% of the HE group were classified as C, and this difference was statistically significant.

Distribution of Meld Score in the Study Population

In our study we found that the mean MELD score in covert group was 22.10 ± 8.89 and in the overt group was 29.04 ± 7.03. The difference in mean MELD score was statistically significant (p < 0.001).

Vidot H et al. [10] observed that the mean MELD score in the overt encephalopathy was 19.9 ± 6. 5, whereas the mean score in the group without encephalopathy was 13.9 ± 5.7.

Afifi MAE et al.[31] found that the mean MELD score in the hepatic encephalopathy group was 21.8 ± 5.6 and the mean score in the non- encephalopathy group was 12.6 ± 5.9.

The results on statistical analysis were significant.

Serum Vitamin D Levels in The Study Population

In our study on comparing the levels of serum vitamin D in the two groups of overt and covert encephalopathy, we found that in the overt group, 47.92% (n=23) of subjects were having serum levels of vitamin D lower than 10ng/ml i.e. severe deficiency.

The number of subjects in the deficiency level of vitamin D (10 - < 20 ng/ml) in the covert group were 30% (n=12) and in the overt group were 45.83% (n=22) respectively.

57.50% (n=23) in the covert group and 6.25% (n=3) in the overt group were having serum levels of vitamin D between 20-30 ng/ml.

12.5% (n=5) of the subjects in the covert group were having sufficient serum levels of vitamin D.

Kumar P et al. [2] showed that the serum vitamin D levels were found to be sufficient only in 4% in study and 2% in control groups. Severe deficiency was seen in 38% in study group as compared to only 6% in the control group. Moderate deficiency was found in 26% in the study group compared to 22% in the control group, followed by mild deficiency of 17% and 40% in both the groups respectively. Insufficiency was reported in 15% in the study group compared to 30% in the control group. The results were statistically significant.

Vidot H et al. [10] found that serum vitamin D levels in the overt encephalopathy group were insufficient in 6.8%, mildly deficient in 52%, moderately deficient in 34%, and severely deficient in 6.8% whereas in the non-overt hepatic encephalopathy group the serum levels were sufficient in 2.5%, insufficient in 27.2%, mildly deficient in 53.2%, moderately deficient in 15.5% and severely deficient in 1.6% of the study subjects.

Afifi MAE et al. [31] observed severe Vitamin D deficiency (16%) in the HE group as compared to 6% in the other group, and the moderate deficiency was 24% as compared to 10% in the other group. The insufficient Vitamin D level represented 46% of the non-HE group while none of the HE group falls in this category.

Comparison of Vitamin D Levels with The Etiology of Hepatic Encephalopathy

In our study we found that the mean vitamin D levels were 15.63 ± 5.44 ng/ml, 14.31 ± 3.41 ng/ml, 18.12 ± 9.68 ng/ml, 16.59 ± 8.58 ng/ml, 16.06 ± 7.15 ng/ml in the etiologies of sub-acute bacterial peritonitis, hepato-renal syndrome, hematemesis and melena, hypokalemia and others respectively.

Afifi MAE et al. [31] observed that the patients with HE due to SBP and HRS showed the least serum Vitamin D levels, 18.4 ± 6.1 nmol/L and 16.8 ± 8.2 nmol/L respectively, followed by 25-O Vitamin D levels in HE patients due to H/M (29.3 ± 3.7 nmol/l) and hypokalemia (44.9 ± 2.5 nmol/l).

Comparison of Vitamin D Level with CTP Class and Meld Score in the Study Population

In our study we found that there was decrease in serum mean vitamin D concentration with increase in the severity of liver disease. The mean serum vitamin D values in CTP Class A, B, C were 27.69 ± 6.66 ng/ml, 19.91 ± 3.76 ng/ml, 11.64 ± 5.06 ng/ml respectively. The results were statistically significant after One Way ANOVA analysis ($p< 0.001$).

Also in our study on comparing with the severity of MELD score we found that the mean levels of serum vitamin D decreases with increasing values of MELD score. A MELD score of ≤9 had a mean serum level 26.34 ± 9.59 ng/ml, followed by 23.54 ± 6.92 ng/ml in patients with a score of 10—19. Patients with a score of 20—29 had serum vitamin D levels of 18.58 ± 7.82 ng/ml,

followed by patients with a score of 30—39 had vitamin D levels of 11.37 ± 5.03 ng/ml. Patients with a MELD score of ≥40 had serum vitamin D levels between 10.87 ± 6.24 ng/ml. On One Way ANOVA analysis the results were statistically significant with a p value of < 0.001.

Afifi MAE et al. [31] found that the level of Vitamin D in Child A was higher than Child B and Child B was higher than Child C. The mean vitamin D level in Class A was 53.3 ± 4.4 nmol/L, followed by 35.9 ± 7.2 nmol/L in class B and 21.2 ± 9.1 nmol/L in Class C. Results were statistically significant on analysis. The study also demonstrated that there was a strong negative correlation ($r = -0.91$) between the Vitamin D level and the MELD score.

Jamil Z et al. [18] observed that in CTP class C, 76.3% were vitamin D-deficient, 23.6% were insufficient, while none had sufficient vitamin D stores. In contrast to this,

in CTP class A 25% had sufficient vitamin D levels, 60.4% had insufficiency and 14.5% had deficiency. In CTP Class the number of deficient, insufficient, and sufficient vitamin D were seen in 17.9%, 71.7%, 10.2% subjects respectively.

The results of this study also found that there is decrease in the serum levels of vitamin D with increase in MELD score. The mean MELD score in the groups of deficient, insufficient and sufficient vitamin D levels were 23.19, 18.43, 14.94 respectively. The results on statistical analysis were significant.

Sharma D et al. [33]observed that the mean vitamin D level were 28.2 ng/ml, 21.6 ng/ml, 12.6 ng/ml in CTP class A, B and C respectively. It was found that the Vitamin D levels were significantly lower in Child-Pugh class C compared to Child-Pugh class A and B (P<0.05).

Vidot H et al. [10] showed a strongly negative correlation between MELD score and vitamin D levels ($p < 0.0001$) in all patients. Patients with Overt HE had significantly worse liver disease with a MELD score of 19.9 ± 6.5 whilst those who were not encephalopathic had significantly lower MELD score of 13.9 ± 5.7 ($p < 0.0001$).

Comparison of Serum Vitamin D Level with Grades of Hepatic Encephalopathy in The Study Population

In our study we found that the mean serum vitamin D level in patients with Hepatic encephalopathy of grade 1, grade 2, grade 3 and grade 4 were 24.11 ± 6.46 ng/ml, 13.61 ± 4.73 ng/ml, 8.41 ± 2.84 ng/ml and 8.00 ± 2.66 ng/ml respectively.

Statistical analysis with One Way ANOVA showed a significant p value (<0.001).

Kumar P et al. [2] found that the mean levels of serum Vitamin D in various grades of hepatic encephalopathy were 30.64 ± 21.64 nmol/L,38.16±24.83 nmol/L, 12.03 ± 11.05 nmol/L and 18.8 ± 16.88 nmol/L in grade 1, grade 2, grade 3 and grade 4 HE respectively with a $P < 0.0001$. Correlation between serum Vitamin D level and hepatic encephalopathy was assessed by Pearson 's correlation coefficient and a negative correlation was found. A lower level of serum Vitamin D was associated with increasing presence of hepatic encephalopathy ($r = -0.354$; $P = 0.003$).

Vidot H et al. [10] showed that mild Vitamin D deficiency was not associated with an increase in Overt Hepatic Encephalopathy. However, moderate and severe Vitamin D deficiency was significantly associated with the development of Overt Hepatic Encephalopathy ($p < 0.0001$).

The relationship between 25-OH vitamin D and OHE accessed using χ 2 analysis which showed lower 25-OH vitamin D levels were associated with a significant trend towards increasing levels of OHE ($p < 0.0001$). A significant difference between Vitamin D levels in patients with Overt Hepatic encephalopathy and those without Overt Hepatic encephalopathy was identified, 30 ± 13 nmol/L and 42 ± 16 nmol/L respectively, ($p < 0.0001$). Furthermore, the study showed a significant correlation between increasing Overt Hepatic encephalopathy in patients with lower 25-OH Vitamin D levels at the same level of disease severity as measured by the MELD scores.

Afifi MAE et al. [31] showed that the level of Vitamin D in Grade 1 was higher (43.8 ± 2.8 nmol/L) than that in Grade 2 (29.6 ± 3.3 nmol/L) which was higher than that in Grades 3 to 4 (15.3 ± 5.0 nmol/L), and this difference was found to be statistically significant.

Limitations of the Study

The limitation of the study was that in this hospital based cross-sectional study normal population was not taken for the comparative analysis of serum vitamin D levels and follow-up was also not done to assess effect of

supplementation of vitamin D. Therefore, cause effect and benefit of Vitamin D supplementation in hepatic encephalopathy after adjusting for various other causative factors could not be ascertained.

Conclusion

Our study showed that there was a significant negative correlation between serum levels of vitamin D and the grades of hepatic encephalopathy. The vitamin D deficiency also increases with the severity of liver disease as evidenced by negative correlation with the increasing CTP score and MELD score. However, it is to be noted that further studies are necessary to establish vitamin D as a causative role in the pathogenesis of hepatic encephalopathy.

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