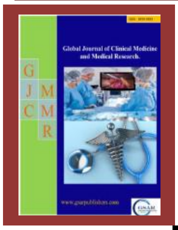

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Comparison of Vitamin D level in normal population and in patients with Chronic Liver Disease (CLD) conferring to child-Pugh classification in Northern Sindh, Pakistan

By

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Abstract

Background and Aim: *Individuals with chronic liver disease (CLD) are highly vulnerable to vitamin D deficiency, which is associated with mortality and complications such as hepatic encephalopathy and hepatic osteodystrophy in liver cirrhosis. This research aimed to evaluate the association between vitamin D levels and illness progression in CLD individuals by comparing vitamin D concentrations in the normal population and CLD cases based on the Child-Pugh Classification (Classes A, B, and C).*

Methodology: *A comparative cross-sectional study was conducted over a six-month period involving 148 participants: 73 healthy individuals and 75 CLD patients. The CLD patients were systematically divided into three groups of 25 patients each based on their Child-Pugh scores (A, B, and C). Statistical comparison of vitamin D levels employed the independent t-test (for normal vs. CLD groups and Hepatitis B vs. C groups), ANOVA (for Child-Pugh classes), and chi-square analysis (for gender correlation).*

Results: *The study revealed that 33% of those with CLD were vitamin D deficient, contrasting with 19% of the general population. The Child-Pugh classification was significantly correlated with vitamin D levels ($p<0.05$). The results demonstrated that the majority of CLD patients, particularly those in Child-Pugh Class B and C, were vitamin D deficient. Low vitamin D concentrations were predicted by the female sex (60%). Although individuals with HBV and HCV infections exhibited low vitamin D levels, there was no strong evidence of a statistically significant difference in vitamin D concentrations between these two viral groups.*

Conclusion: *CLD patients exhibit a greater prevalence of vitamin D deficiency, which is clearly associated with the female sex and increased CLD severity. It is recommended that chronic CLD patients, particularly those in Child-Pugh Classes B and C, undergo routine vitamin D testing and receive prompt supplementation to improve prognosis and management.*

Keywords: *Vitamin D Deficiency, Child Class PUGH, Liver Disease, Cirrhosis, Chronic Disease*

Introduction

Vitamin D is a fat-soluble vitamin. Beyond its crucial role in regulating Calcium and Phosphate levels, its deficiency has been historically linked to skeletal problems such as rickets, osteomalacia, falls, and fractures (Kumar S et al., 2019). More recent research has connected low Vitamin D status to several chronic conditions, including cardiovascular disease (CVD), Diabetes, Chronic Liver Disease (CLD), and various types of Cancers (Akhtar S et al., 2016).

Vitamin D deficiency is a major global health concern, affecting approximately one billion people worldwide. In South Asia, nearly 70% of the population is deficient in Vitamin D. Locally, in Pakistan, 53.5% of the population is reported to have Vitamin D deficiency (Riaz H et al., 2016).

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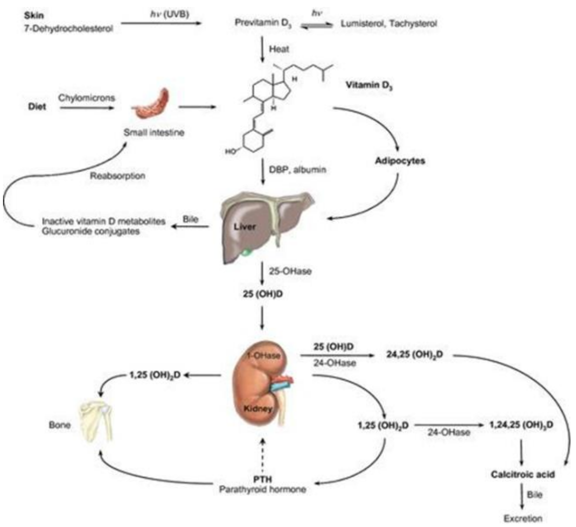

Figure 1: Epithelia biosynthesize vitamin D as a response to sunshine absorption. First, UV light in the endodermis transforms the cholesterol precursor 7-dehydrocholesterol producing pre-vitamin D3. Pre-vitamin D3 would then be converted into synthesis D3, which is later processed by sunlight, maintaining the balance between the two vitamins.

Chronic Liver Disease (CLD), characterized by widespread fibrosis leading to cirrhosis and the formation of regenerative nodules, poses a significant health burden internationally, with liver cirrhosis being identified as the fourth biggest cause of mortality in many nations and estimated to be responsible for about one million deaths globally in 2010 (Farhat M et al., 2014). In several nations, including Pakistan, Hepatitis C virus (HCV) transmission is a leading cause of liver cirrhosis.

Patients with CLD are highly vulnerable to vitamin D deficiency. The liver is a crucial organ for Vitamin D synthesis and metabolism. Specifically, severe liver illness causes lower 25(OH)D levels by reducing vitamin D hydroxylation, as well as production of albumin and Vitamin D Binding Protein (DBP) (Yousif MM et al., 2019). As a result, approximately 93% of patients having Chronic Liver Disease (CLD) frequently experience severe vitamin D deficiency to some extent.

Low Vitamin D concentrations in patients with liver cirrhosis are associated with increased mortality and several severe complications, including hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatic osteodystrophy (Abdelkarim et al., 2024). The skeletal manifestation of liver disease, known as hepatic osteodystrophy, results in a higher risk of bone breakage in cirrhotic patients, undesirably affecting their morbidity (Jamil Z et al., 2018). Vitamin D also plays a role in modulating the immune system and intestinal epithelial defense against infections, suggesting its significance in the progression and complications of CLD. Deficiency has also been linked to countless risks of complications of portal hypertension and may decrease the probability of viral response to treatment.

The severity and prognosis of cirrhosis are often assessed using the Child-Pugh (CP) score (Paternostro R et al., 2017). This system, which categorizes patients into classes A, B, and C based on parameters such as ascites, hepatic encephalopathy, serum bilirubin, serum albumin, and prothrombin time/INR, has been widely correlated with Vitamin D status. Different studies have demonstrated a strong association between Vitamin D status and Child-Pugh classification, noting that Vitamin D deficiency frequency rises as liver cirrhosis advances. Specifically, studies have shown that patients belonging to Class C (the most severe stage) were typically the most Vitamin D deficient (Iruzubieta P et al., 2014).

Although vitamin D deficiency is highly prevalent in the general population of Pakistan, very few local studies have compared Vitamin D levels in the normal population with those in CLD patients. Given that Pakistan has the world's second-highest prevalence of hepatitis C (a major causative factor for liver disease), investigating this correlation locally is critical (Stokes CS et al., 2012).

This research, conducted in Northern Sindh, Pakistan, aimed to scientifically establish the correlation between Vitamin D deficiency and liver disease severity in our clinical setting, specifically examining whether this deficiency is related to the liver condition or to other factors prevalent in the healthy population (Kitson et al., 2012).

The primary goal of this research was to evaluate the association between vitamin D levels and illness progression in CLD individuals by comparing levels of vitamin D in the normal population and CLD cases based on the Child-Pugh Class A, B, and C classification (Konstantakis C et al., 2016).

The specific objectives of the study were:

- ❖ To compare the level of Vitamin D in the normal population and CLD patients based on the classification of Child-Pugh Class A, B, and C.
- ❖ To compare Vitamin D levels on the basis of gender.
- ❖ To compare Vitamin D based on the viral cause Hepatitis B and C in patients of chronic liver disease.

We hypothesized that Vitamin D was significantly deficient in CLD patients and its deficiency is related to the severity of the condition as compared to the normal population. Identifying and promptly addressing this deficiency through supplementation in CLD patients (particularly those in Child-Pugh classes B and C) may improve functional status, prognosis, and overall outcome (Volker D et al., 2019).

Literature review

Vitamin D, recognized as a fat-soluble vitamin, plays a crucial physiological role not only in the maintenance of muscles and bones through the regulation of Calcium and Phosphate levels but also through emerging involvement in immunology, malignancy, and viral illnesses. Historically, its deficiency has been linked to skeletal problems such as rickets, osteomalacia, falls, and fractures (Nair S et al., 2010). However, recent research connects low Vitamin D status to several chronic conditions, including Cardiovascular Disease (CVD), Diabetes, Chronic Liver Disease (CLD), and various types of Cancers. Vitamin D deficiency is a major global health crisis, affecting approximately one billion people worldwide. The prevalence is particularly high in South Asia (nearly 70%) and specifically in Pakistan (53.5%) (Hammid S et al., 2017).

Chronic Liver Disease (CLD), marked by widespread fibrosis leading to cirrhosis, poses a major public health challenge internationally, with liver cirrhosis being responsible for approximately one million deaths globally in 2010 and ranking as the fourth biggest cause of mortality in many nations. In several regions, including Pakistan, Hepatitis C virus (HCV) transmission is a leading cause of liver cirrhosis. Patients suffering from CLD are highly vulnerable to vitamin D deficiency (Aftab L et al., 2018). Low Vitamin D concentrations in patients with liver cirrhosis have been strongly associated with increased mortality and severe complications such as hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatic osteodystrophy. Hepatic osteodystrophy, the skeletal manifestation of liver disease, results in a higher risk of bone breakage in cirrhotic patients, undesirably affecting their morbidity (Iqbal R et al., 2010).

The high prevalence of Vitamin D deficiency in CLD patients is fundamentally linked to the liver's critical role in Vitamin D metabolism. Vitamin D (cholecalciferol), obtained from diet or exposure to sunlight, is first hydroxylated in the liver to produce 25-hydroxyvitamin D (25(OH)D). The final activation to 1,25-dihydroxyvitamin D (calcitriol) occurs primarily in the kidneys (Joshi et al., 2016). Severe liver illness causes lower 25(OH)D levels by reducing the capacity for vitamin D hydroxylation. Furthermore, the liver produces Vitamin D Binding Protein (DBP), the main transporter of Vitamin D, and severe liver dysfunction reduces the production of DBP and albumin, further contributing to lower levels of available 25(OH)D (N Frulio et al., 2013). Additional factors contributing to vitamin D deficiency in CLD include cholestasis (which impairs the absorption of fat-soluble vitamins), anorexia (leading to nutritional shortage), and reduced sun exposure due to chronic illness limiting movement. Approximately 93% of patients with CLD frequently experience severe vitamin D deficiency to some extent (Ramos et al., 2015).

The severity and prognosis of cirrhosis are conventionally assessed using the Child-Pugh (CP) score, which classifies patients into classes A, B, and C based on five parameters: hepatic encephalopathy, ascites, serum bilirubin, serum albumin, and prothrombin time/INR. Multiple studies have demonstrated a strong association between Vitamin D status and Child-Pugh classification (Mokdad et al., 2014). The frequency of Vitamin D deficiency increases as liver cirrhosis advances. Dilshad et al. reported that Vitamin D levels gradually decline as the Child-Pugh Class rises, noting that 83.7% of cirrhotic cases studied were deficient. Similarly, research has shown that patients belonging to Class C (the most severe stage) were typically the most Vitamin D deficient. A study by Finkelmeier et al. (2015) found a negative correlation between 25(OH)D3 amounts and the MELD (Model for End-Stage Liver Disease) score, which also significantly varied across Child-Pugh ratings (Cheng et al., 1996). This evidence suggests that Vitamin D concentrations serve as an indicator of the progression of severe disease.

Vitamin D status has also been examined in the context of specific causative factors for CLD. In patients with Primary Biliary Cirrhosis (PBC), a chronic autoimmune-mediated hepatic disease, reduced serum vitamin D concentration levels have been found, with significant prevalence of insufficiency (Peng Y et al., 2016). Low vitamin D concentrations in PBC patients have been linked oppositely to indicators of disease severity, such as alkaline phosphatase and bilirubin. The introduction of Ursodeoxycholic acid (UDCA) treatment has been observed to raise vitamin D concentrations in PBC patients (Kang et al., 2014).

Regarding chronic viral infections, low vitamin D levels have been associated with high viral loads in patients with chronic Hepatitis B Virus (HBV). Studies suggest that the overwhelming majority of HBV-infected individuals suffer from vitamin D insufficiency, which is linked to poor clinical outcomes (Rosen et al., 2011). For chronic Hepatitis C Virus (HCV) infection, decreased serum vitamin D concentrations at the start of treatment were linked to an inability to achieve a sustained virological reaction after therapy with PEGylated interferon and ribavirin. Vitamin D is also thought to act as an innate antiviral agent, suppressing HCV replication (Looker et al., 2002). For both HBV and HCV, vitamin D replacement has been proposed as a helpful therapeutic option in managing chronic liver illnesses (Wu SH et al., 1996).

It is important to note that Vitamin D insufficiency is highly prevalent in the general population globally, irrespective of ongoing medical conditions. Age, sex, and race are factors in vitamin D insufficiency (Cervoni et al., 2012). Locally, research suggests that low concentrations of vitamin D are observed in a large percentage of individuals presenting to tertiary care settings, even those who are otherwise healthy. Specifically, the female sex has been identified as a researched risk factor contributing to vitamin D insufficiency in healthier populations (Targher et al., 2007). Consistent with this, studies focusing on CLD patients have also identified the female sex as a predominant determinant of decreased vitamin D levels (Zittermann et al., 2012).

Given the wide-ranging role of Vitamin D in immune modulation, intestinal defense, and bone health, low levels are linked to severe clinical consequences in CLD patients, including increased risk of infection (leucopenia/leukocytosis) and overall higher mortality rates (Venu et al., 2013). Consequently, the identification and prompt treatment of this deficiency through supplementation in CLD patients, particularly those in more advanced stages (Child-Pugh Classes B and C), are advised to potentially improve functional status, prognosis, and overall outcome (Fernández et al., 2016).

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Material and Methods

Study Setting, Design, and Duration

This research employed a comparative cross-sectional study design. The study was conducted over a period of six months, from April 2022 to September 2022, after the approval of the synopsis. The setting for the study was the Medical Unit V of the Shaheed Mohtarma Benazir Bhutto Medical University (SMBBMU) in Larkana, Northern Sindh, Pakistan. Ethical permission was granted by the hospital authorities before the trial commenced, and all participating individuals (and parents in the case of minors) provided their informed consent.

Sample Size and Sampling Technique

The total sample size recruited for the investigation was 148 participants. The sample size was determined using the Cochran formula ($n = Z^2(P\ (q)\ /\ e^2)$). The parameters used were a 95% confidence interval ($Z = 1.96$), a prevalence of Vitamin D deficiency in Pakistan ($P = 53.5$), $q = 46.5$, and $e = 8$.

The total sample consisted of 73 healthy individuals (the normal population group) and 75 patients with Chronic Liver Disease (CLD). The CLD patients were systematically divided into three groups of 25 patients each based on the severity of their condition using the Child-Pugh (CP) scores: Class A, Class B, and Class C. The sampling technique employed was non-probability convenient sampling.

Sample Selection

Normal Population Group: Attendants of patients coming to the indoor/Outpatient Department (OPD) were included after a detailed history and examination to rule out any comorbidities.

Chronic Liver Disease (CLD) Patients: Patients aged 14 to 80 years with a diagnosis of Chronic Liver Disease, regardless of etiology, were included if they exhibited one or more of the following features:

- ❖ Clinical findings indicative of CLD.
- ❖ Physiochemical disturbances implying CLD, such as deranged liver function tests present for longer than three months, in the presence of CLD risk factors.
- ❖ Ultrasonography findings of CLD (including ground nodularity, coarse and inhomogeneous liver texture, and lateral hypertrophy or atrophy).
- ❖ A Fibro Scan report suggesting CLD.
- ❖ Any previous record of liver biopsy or severe liver histological abnormalities.
- ❖ Health documents pointing to CLD (such as prior hospitalizations for variceal hemorrhage, ascites, or liver encephalopathy, or prescription drugs).

Operationally, Chronic Liver Disease refers to an incurable widespread fibrosis-related end-stage hepatic condition with the formation of regenerative nodules diagnosed on Fibro Scan.

Exclusion Criteria

Individuals were excluded if they met any of the following criteria:

- ❖ Persons who had received Vitamin D supplements in the previous two months.
- ❖ Patients who had undergone Liver transplantation.
- ❖ Patients with a diagnosis of Acute or Chronic Kidney Disease.
- ❖ Patients taking medications known to impact plasma vitamin D levels, such as antiepileptic drugs, glucocorticoids, laxatives, antituberculosis drugs, or antiretroviral drugs.

Data Collection Procedure and Classification

Full demographic profiles and thorough histories were documented for all subjects. A complete physical examination was performed on all participants.

CLD Severity Assessment (Child-Pugh Classification): CLD patients were grouped according to the Child-Pugh (CP) score using five clinical and laboratory parameters, with each factor scored 1, 2, or 3. The parameters used were: hepatic encephalopathy, ascites, serum bilirubin (mg/dl), serum albumin (g/dl), and Prothrombin Time (PT) / International Normalized Ratio (INR). The minimal possible CP score was 5, and the maximum was 15. Ascites severity (mild, moderate, or severe) was categorized via abdominal ultrasonography. The degree of encephalopathy was assessed by the researcher. Patients were categorized as Child Class A (score 5-6), Child Class B (score 7-9), or Child Class C (score >9).

Laboratory Procedures: Blood specimens (10cc) were obtained from subjects. The blood was divided into three tubes for laboratory analysis: one containing EDTA for the Complete Blood Count (CBC); a plain tube for serum albumin and bilirubin; and a tube containing sodium citrate for the INR.

Vitamin D Measurement and Status Definition: Blood samples were taken from participants to measure vitamin D levels. Vitamin D concentrations in plasma were assessed using the ELISA technique.

Vitamin D status was classified using the following concentrations:

- **Deficient:** Vitamin D concentrations below 20 ng/mL.
- **Insufficient**: Vitamin D concentrations between 20 ng/mL and 30 ng/mL.
- **Adequate (Sufficient):** Vitamin D concentrations over 30 ng/mL.

Viral Serology: Viral Hepatitis Serology (for Hepatitis B and C) was performed on all subjects (CLD patients and attendants) using the ELISA method.

Normal Population Screening: To rule out underlying disease in the normal population group, a detailed history and physical examination were performed, and an ultrasound was performed to look for any underlying liver or kidney disease.

Statistical Analysis

The gathered information was analyzed using SPSS version 26. Descriptive statistics were utilized to summarize the findings.

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The specific statistical tests used were:

1. **Independent t-test:** Used for comparing the mean level of Vitamin D between the normal population and CLD patients.
2. **One-way Analysis of Variance (ANOVA):** Used to analyze the comparison of the level of Vitamin D among the classification of Child-Pugh Class A, B, and C.
3. **Chi-square test:** Used to assess the relationship between vitamin D levels and gender.
4. **Independent t-test:** Used for comparing vitamin D levels based on the viral causes of Hepatitis B and C in chronic liver disease patients.
5. **Shapiro Wilks test:** Used to check for normality.

A P value < 0.05 was established as the threshold for statistical significance.

Results

A total of 148 participants were enrolled in the study, comprising 73 healthy individuals and 75 patients diagnosed with Chronic Liver Disease (CLD). The mean age of all participants ranged from 14 to 80 years. Among the healthy individuals (N=73), the highest proportion of participants (26.03%) fell into both the 21–30 years and 31–40 years age categories. For the CLD group (N=75), the largest proportion (28%) was observed in the 41–50 years age category. Regarding gender distribution across the total sample (N=148), there were 55 males (37.16%) and 93 females (62.84%). The further age categories and their proportion is presented in table.1.

Table.1. Age categories and their proportion in both groups

Age category (in years)	Healthy individuals (N= 73)		CLD (N=75)	
	Frequency	Percentage	Frequency	Percentage
14-20 years	5	6.85%	7	9.33%
21-30 years	19	26.03%	9	12.00%
31-40 years	19	26.03%	15	20.00%
41-50 years	18	24.66%	21	28.00%
51-60 years	6	8.22%	14	18.67%
61-70 years	5	6.85%	8	10.67%
71-80 years	1	1.37%	1	1.33%

Table.2. Comparison of vitamin D levels between two groups

Group	N	Mean	Std. Deviation	P- value
Healthy individuals	73	33.85	18.73	0.000
CLD	75	18.91	11.19	

The analysis comparing mean vitamin D levels between the two primary groups revealed a statistically significant difference as shown in table 2. The mean vitamin D level in healthy individuals was 33.85 (Standard Deviation [SD] 18.73), which was notably higher than the mean level found in the CLD group, 18.91 (SD 11.19). This resulted in a mean difference of 14.94 between the healthy population and CLD patients, with a P-value of 0.000, confirming the significant difference. Overall, the results showed that 33% of those with CLD were vitamin D deficient, compared to 19% of the general population.

The 75 CLD patients were classified into three groups (Class A, B, and C) based on the Child-Pugh score. Descriptive statistics for baseline parameters within the CLD group showed the mean Albumin was 3.55, Bilirubin was 1.82, and PT/INR was 2.62. One-way Analysis of Variance (ANOVA) was performed to assess the impact of the Child-Pugh classes on vitamin D levels. The ANOVA results indicated a significant difference among the group means (F = 19.615, p = .000), supporting the rejection of the null hypothesis as shown in table 3.

Table 3: One-way ANOVA

ANOVA

Vit. D

	nmation Squares	Df	Mean Square	F	Sig.
Between-group	3266.272	2	1633.136	19.615	.000
Within group	5994.800	72	83.261		
Total	9261.072	74			

Analysis of mean vitamin D levels by class revealed that Class A was associated with the statistically greatest mean level of vitamin D (M=27.8152), while Class C was linked with the statistically lowest average level of vitamin D (M=12.0392). Class B had an intermediate mean vitamin D level (M=16.8752).

Analyses further explored these differences:

- A substantial and significant mean difference of 10.940 (p < .001) was observed when comparing Group A with Group B.
- Group A also showed a significant mean difference of 15.776 (p < .001) compared to Group C.
- The comparison between Group B and Group C yielded a mean difference of 4.836, which was not statistically significant (p = .065).

These findings indicate that the majority of CLD patients, particularly those in Child-Pugh Class B and C, are vitamin D

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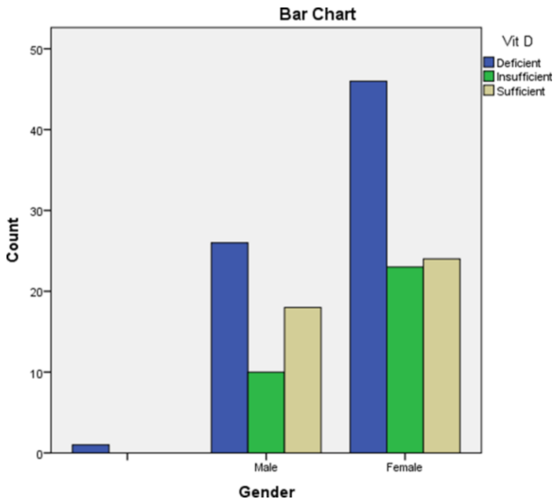
deficient, and that the Child-Pugh classification was significantly correlated with vitamin D levels (p<0.05).

A chi-square test was conducted to examine the association between gender and vitamin D status. The results indicated that 46% of females were vitamin D deficient, compared to 26% of males. Furthermore, 23% of females were found to be insufficient for vitamin D, while 10% of males were insufficient. The female sex was concluded to be a predictor for low vitamin D concentrations, with 60% deficiency predicted by chi-square analysis as shown in below in Graph 1.



For the analysis of viral etiology among the CLD patients (N=75), the maximum number of patients were suffering from Hepatitis C (HCV) at 50.67%, followed by Hepatitis B (HBV) at 36.00%. The remaining 13.33% suffered from both Hepatitis B and C. An independent samples t-test was used to compare vitamin D concentrations based on HBV and HCV infection.

- Hepatitis B (N=27) was associated with a mean Vitamin D level of 17.0141 (SD 10.14416).
- Hepatitis C (N=38) was associated with a numerically larger mean Vitamin D level of 20.2589 (SD 11.84303).
- The mean difference was 3.2448.

The t-test for Equality of Means yielded a two-tailed P-value of 0.253 (assuming equal variances), which does not reach statistical significance at the conventional alpha level of 0.05. Therefore, there is no strong evidence to conclude that Vitamin D levels significantly differ between the two groups (HBV and HCV patients).

Table 4: Descriptive statistics of the variable for child class PUGH

	N	Min	Max	Mean	Std. Deviation
Albumin	75	1.70	5.60	3.5597	.80605
Bilirubin	73	.1	14.2	1.823	2.2647
PT/INR	46	1	16	2.62	3.717
Valid N	46				

All patients in CLD group were divided into three classes according to child class PUGH. Several common basis parameters' dispersions between the CLD patients for the classification of child class PUGH were shown in table 4. The mean of Albumin was 3.55, while the mean of Bilirubin and PT/INR was 1.82 and 2.26 correspondingly.

Table 5: Descriptive statistics with a confidence interval

Group	N	Mean	Std. Deviation	d. Error Mean	Mean 95% confidence inter	
					Lower Limit	Upper Limit
Class A	25	27.8152	12.37189	2.47438	22.7083	32.9221
Class B	25	16.8752	8.72394	1.74479	13.2741	20.4763
Class C	25	12.0392	4.54011	.90802	10.1651	13.9133
Total	75	18.9099	11.18703	1.29177	16.3360	21.4838

Table 5 presents the descriptive data for vitamin D confidence levels throughout the three-child class PUGH groupings. It is clear that Class A was connected with the statistically greatest mean level of vitamin D (M=27.8152) and Class C was linked with the statistically lowest average level of vitamin D (M=12.0392).

Table 6: Fisher's LSD post hoc tests Multiple Comparisons

Dependent Variable
Vit.
LSD

	Mean Differences	Std. Error	Sig	95%confidence Interval Lower limit	95%confidence Interval Upper limit
GroupA **Group B** **Group C**	10.940 15.776	.17263 .17428	.000 .000	5.7951 10.6311	16.0849 20.9209
GroupB **Group A** **Group C**	- 10.940 4.836	.17263 .17083	.000 .065	-16.0849 -.3089	-5.7951 9.9809
GroupC **Group**	-	.17428	.000	-20.9209	-

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A Group B	15.776 - 4.4.83 6	.17083	.065	-9.9809	10.6311 .3089

Three Fisher's LSD post hoc analyses were conducted after the statistically meaningful ANOVA to thoroughly assess the significance of the differences between the three means (Hayter, 1986). In comparing Group, A with Group B, a substantial and significant mean difference of 10.940 ($p < .001$, 95% CI [5.7951, 16.0849]) is observed. Similarly, Group A compared to Group C reveals a significant mean difference of 15.776 ($p < .001$, 95% CI [10.6311, 20.9209]). Finally, Group B versus Group C shows a mean difference of 4.836 ($p = .065$, 95% CI [-0.3089, 9.9809]) which is not statistically significant.

Table 7: Frequency of gender

Gender	Frequency	Percent %	Cumulative %
Male	55	37.16	37.16
Female	93	62.84	100.0
Total	148	100.0	100 %

The above showed the occurrence based on gender. There were 55 males i.e. 37% and 93 i.e. 63% females out of 148 participants.

Table 8 Frequency of hepatitis

		Frequency	Percent	Cumulative Percent
Valid	Hepatitis B	27	36.00	36.0
	Hepatitis C	38	50.67	86.67
	Hepatitis B+C	10	13.33	100.0
	Total	75	100	100

The table showed the frequencies of occurrence of hepatitis B, Hepatitis C, and both hepatitis B and C in patients with chronic liver disease. The maximum number of CLD patients was suffering from hepatitis C (50.67%), followed by hepatitis B (36%). While there were only 13.33% of CLD patients suffering from both hepatitis B and C.

Graph 2:

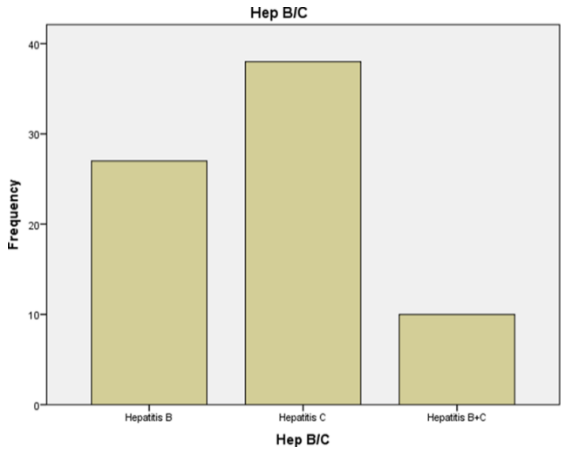

Table 9 Group statistics of Hepatitis Band C

Vitamin D				
Hep B/C	Mean	N	Std. Deviation	Std. Error of Mean
Hepatitis B	17.0141	27	10.14416	1.95225
Hepatitis C	20.2589	38	11.84303	1.92119
Total	18.9111	65	11.20214	1.38945

Hepatitis B (N=27) was associated with Vitamin D M 17.0141 (SD 10.14416). By comparison, Hepatitis C (N=38) was associated with a numerically larger Vitamin D M 20.2589 (SD 11.84303). There was a mean difference of 3.2448 among Hepatitis B and C patients for Vit D concentration. An independent sampling t-test was carried out to assess the comparison of vitamin D depending on the virus origins of HBV and HCV in an individual with CLD.

Discussion

The objective of this research was to evaluate the prevalence of vitamin D deficiency in patients suffering from Chronic Liver Disease (CLD) in Northern Sindh, Pakistan, and to correlate these levels with the severity of the disease as categorized by the Child-Pugh classification (Dietrich et al., 2016).

The current study found a marked difference in vitamin D status between the two primary groups: 33% of individuals with CLD were vitamin D deficient, compared to only 19% of the general population surveyed (Zhao et al., 2016). The mean vitamin D concentration in CLD patients (18.91 ng/mL) was substantially lower than that in healthy controls (33.85 ng/mL), demonstrating that many CLD patients possess insufficient vitamin D reserves. Overall, 73% of CLD cases lacked appropriate vitamin D reserves, while only 42% had adequate reserves (>30 ng/mL) (Arteh J et al., 2010).

These findings align with extensive international literature. For instance, Crawford et al. reported that 52.1% of patients studied had vitamin D shortage (51 nmol/l). Looker et al. observed insufficient serum 25(OH)D values in 90% of cases, with deficiency (51 nmol/L) present in 67% and inadequacy (50-80 nmol/L) in 24%. Further supporting the results,

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numerous extensive investigations confirm the absence of vitamin D in CLD sufferers (Heuman et al., 2007). Zhao et al. observed very low levels in 345 cirrhotic sufferers, and a Spanish study by Fernandez et al. reported 88% deficiency in 95 cirrhotic individuals.

However, it is important to contextualize these differences. Although the mean vitamin D concentrations in CLD patients were statistically significantly lower than in the comparison group ($p<0.05$), vitamin D deficiency is highly prevalent in the overall population, even among healthy individuals in the region (Targher et al., 2007). For example, studies in Pakistan have reported high rates of low concentrations, such as Kiani et al. finding 84% low concentrations in patients presenting to an outpatient clinic with various disorders, and Mehboob et al. reporting 56% deficiency among healthy people in third-world nations. This suggests that while baseline vitamin D lack is widespread, Chronic Liver Disease clearly contributes to the subsequent progress and severity of vitamin D insufficiency in affected individuals (Lange et al., 2011).

The study confirmed a clear and significant association between the Child-Pugh class and vitamin D deficiency ($p<0.05$). The concentrations worsen as the condition deteriorates. Analysis of mean vitamin D levels across the three classes showed that Child-Pugh Class A was associated with the statistically greatest mean level (M=27.8152), while Class C was linked with the statistically lowest average level (M=12.0392) (Malik et al., 2025). Post hoc analyses demonstrated substantial and significant mean differences when comparing Group A with Group B (10.940, $p < .001$) and Group A with Group C (15.776, $p < .001$). This supports the hypothesis that vitamin D deficiency is related to the severity of the condition (Abu-Mouch et al., 2011).

The association between increasing CLD severity and deficiency is biologically plausible. The primary mechanism by which liver cirrhosis causes vitamin D insufficiency is the restriction of vitamin D hydroxylation. The liver is essential for vitamin D's enzymatic activity as it is where 25-hydroxylation occurs (Nimer et al., 2012). Additional probable factors contributing to vitamin D deficit in CLD include: reduced manufacturing of albumin and Vitamin D Binding Protein (DBP), lack of food consumption (anorexia), inadequate sunlight exposure, reduced vitamin D uptake due to cholestasis-induced biliary interruption or digestive abnormality supplementary to portal hypertension, and the degradation of vitamin synthesizing on the epidermal layer due to hyperbilirubinemia (Gal-tanamy et al., 2011).

In addition to disease severity, the study investigated secondary factors influencing vitamin D status. The results indicated that the female sex was concluded to be a predictor for low vitamin D concentrations. A chi-square test revealed that 46% of females were vitamin D deficient, compared to 26% of males (Matsumura et al., 2011). This supports external research that recognizes the female gender as a hazard factor contributing to vitamin D insufficiency in healthier populations in these nations, often due to factors like limited sunlight exposure, illiteracy, and insufficient supplementation.

Regarding the viral causes of CLD, the maximum number of patients were suffering from Hepatitis C (HCV) at 50.67%, followed by Hepatitis B (HBV) at 36.00% (Nguyễn LT et al., 2013). While the mean vitamin D level in HBV sufferers was numerically lower (M 17.01) than that in HCV sufferers (M 20.25), the independent samples t-test found no strong evidence to conclude that Vitamin D levels significantly differ between the two groups ($p=0.253$). Despite the lack of statistical significance, this finding aligns with meta-analyses suggesting a negative correlation between serum vitamin D levels and HBV contagious loads. Low vitamin D concentrations have also been associated with high levels of HBV replication (Agmon et al., 2015).

The clinical significance of vitamin D deficiency in this population is high, as it has been linked to increased mortality, bacterial invasions, and complications from portal hypertension, and the stage of fibrosis in individuals with CLDs. Vitamin D insufficiency in CLD sufferers is also associated with earlier exacerbations and higher fatality rates (Guo GY et al., 2015).

Given that supplementation with vitamin D has been shown in various studies to potentially improve functional capacity, survival, Child-Pugh rating, and cumulative mortality in patients with liver problems, the timely addressing of this deficiency is crucial (Malham et al., 2011). Consequently, it is strongly advised that individuals with chronic CLD, particularly those in Child-Pugh classes B and C, undergo routine vitamin D testing so that sufficient replenishment with supplementation can be started promptly as a medical therapy in managing their condition.

Conclusion

The study concluded that Chronic Liver Disease (CLD) patients had a greater prevalence of vitamin D deficiency compared to healthy individuals. Specifically, 33% of those with CLD were vitamin D deficient, compared to 19% of the general population. Crucially, there was a clear and significant association between the severity of CLD, as determined by the Child-Pugh classification, and vitamin D deficiency. Vitamin D concentrations worsened as the condition deteriorated, with patients in Child-Pugh Class B and C experiencing more pronounced deficiency compared to Class A. Furthermore, the female sex was found to be a predominant factor affecting vitamin D deficiency in CLD patients.

The primary clinical contribution of this research is the strong recommendation for timely medical intervention in managing CLD patients. Immediate Clinical Impact: It is advised that all individuals with chronic CLD, particularly those categorized into Child-Pugh Classes B and C, must undergo routine vitamin D testing. Prompt and sufficient replenishment with vitamin D supplementation should be initiated as a medical therapy in handling these patients. This approach is intended to improve the patient's functional status, prognosis, Child-Pugh Score, overall indisposition, and death rates in this clinical setup. Further investigation is required to better understand how much the underlying CLD condition contributes to the progression of vitamin D insufficiency,

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given that deficiency is already highly prevalent in the apparently healthy regional population.

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