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Serum vitamin d levels in Nigerian patients with chronic hepatitis B- related liver disease

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Abstract
Background: Vitamin D refers to a group of seco-steroid compounds and synthesized predominantly in the liver. Liver function is crucial for physiologic vitamin D metabolism. Vitamin D itself is considered inactive and is hydroxylated to 25-hydroxyvitamin D {25(OH)D_3} in the liver and is the main circulating vitamin D. Low 25(OH)D_3 levels may indicate reduced liver function and might contribute to liver damage possibly mediated by increased inflammation and fibrosis as well as reduced antiviral response. It has been demonstrated that replication of hepatitis B virus is high in chronic hepatitis B infection when the serum level of vit D is low.
*Objective***:** This study was aimed at assessing the level of serum vitamin D in patients with HBV-related chronic liver disease (CLD) compared with healthy controls.
Methods: This was a cross-sectional comparative study involving consenting patients with HBV-related CLD and age and sex-matched healthy controls. 25(OH)D_3 was analyzed in the sera of both groups using electrochemiluminis centimmunometric method with Roche Cobas e411 chemistry analyzer. CHB was diagnosed with serum positive hepatitis B surface antigen (HBsAg) of $\leq$ 6 months duration or the presence of immunoglobulin G (IgG) core antibody (HBcAb) and HBsAg sero-positivity in the absence of immunoglobulin M against Hepatitis B Core antigen (IgM-anti-HBc) and the antibody to the HBsAg (anti-HBs). Liver cirrhosis was diagnosed using abdominal ultrasound while diagnosis of HCC was made using abdominal ultrasound, alpha feto-protein (AFP), triphasic abdominal computer tomography (CT) scan and liver biopsy where necessary. Data obtained were entered into SPSS (version 20) and analyzed using inferential and descriptive statistics.
Results: The groups consisted of 96 patients with CHB-related liver diseases and 96 controls. The CHB-related CLD group was comprised of uncomplicated CHB (30), liver cirrhosis (30) and hepatocellular carcinoma (36). Controls were healthy blood donors. Seventy-three (76%) of the CHB-related liver disease group had low levels of serum 25(OH)D_3 while only 22 (22.9%) of the controls had low serum vitamin D levels. Among the HBV-related CLD group, 11 (36.7%) of the CHB, 30 (100%) of the liver cirrhosis (LC), and 32 (88.9%) of the hepatocellular carcinoma (HCC) patients had low serum vitamin D levels. The mean serum level of 25(OH)D_3 among the control group was 25.77±8.59ng/ml, and that among the CLD groups that was 13.11±10.54ng/ml (P<0.001) {95% CI (9.919-15.393)}. The mean serum vitamin D values of: CHB, Cirrhosis and HCC were 21.53±4.88ng/ml, 8.13±3.16ng/ml and 10.25±13.53ng/ml, respectively. However, there was no statistically significant difference found between the mean serum vitamin D levels of LC and HCC patients (P-value=0.058).
*Conclusion***:** Serum vitamin D level is significantly low in Nigerian patients with HBV-related CLD compared with normal controls, and the degree of deficiency is directly related to the stage of disease progression.

Keywords: *Blood donors, chronic hepatitis B, hepatocellular carcinoma, liver cirrhosis, vitamin D*

Résumé
Contexte: La vitamine D fait référence à un groupe de seco-stéroïdes composés et synthétisés principalement dans le foie. La fonction hépatique est cruciale pour le métabolisme physiologique de la vitamine D. La vitamine D elle-même est considérée comme inactive et est hydroxylée en 25-hydroxy-vitamine D {25(OH)D_3} dans le foie et est la principale vitamine D circulante. De faibles niveaux de 25(OH)D_3 peuvent indiquer une fonction hépatique réduite et pourraient contribuer à des lésions hépatiques éventuellement entremis par une augmentation de l'inflammation et de la fibrose ainsi qu'une réponse antivirale réduite. Il a été démontré que la réplication du virus de l'hépatite B est élevée dans l'infection chronique par l'hépatite B lorsque le taux sérique de vit D est faible.
Objectif **:** Cette étude visait à évaluer le taux de vitamine D sérique chez des patients atteints d'une

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maladie hépatique chronique (MPC) liée au VHB par rapport à des témoins sains.
Méthodes: Il s'agissait d'une étude comparative transversale impliquant des patients consentants atteints de MPC liée au VHB et des témoins sains appariés selon l'âge et le sexe. $25(OH)D_3$ a été analysée dans les sérums des deux groupes à l'aide de méthode d'électrochimiolumine scentimmunométrique avec analyseur de chimie Roche Cobas e411 . CHB a été diagnostiquée avec un sérum positif à l'antigène de surface de l'hépatite B (HBsAg) d'une durée e" 6 mois ou la présence d'anticorps de base de l'immunoglobuline G (IgG) (HBcAb) et d'HBsAgséro-positivité en l'absence d'immunoglobuline M contre l'essence d'antigène Hépatite B (IgM-anti-HBc) et d'anticorps anti-HBsAg (anti-HBs). La cirrhose du foie a été diagnostiquée à l'aide d'une échographie abdominale, tandis que le diagnostic d'HCC a été fait à l'aide d'une échographie abdominale, de l'alpha-fœto-protéine (AFP), d'une tomodensitométrie abdominale tri-phasique (TDM) et d'une biopsie hépatique si nécessaire. Les données obtenues ont été saisies dans SPSS (version 20) et analysées à l'aide de statistiques d'inférences et descriptives.
Résultats: Les groupes étaient composés de 96 patients atteints de maladies hépatiques liées au HCB et de 96 témoins. Le groupe MPC lié à la CHB était composé de CHB non compliqué (30), de cirrhose du foie (30) et de carcinome hépatocellulaire (36). Les témoins étaient des donneurs de sang sains. Soixante-treize (76 %) des patients du groupe des maladies hépatiques liées à l'HCB présentaient de faibles taux sériques de $25(OH)D_3$ tandis que seulement 22 (22,9 %) des témoins présentaient de faibles taux sériques de vitamine D. Parmi le groupe MPC lié au VHB, 11 (36,7 %) des patients atteints de CHB, 30 (100 %) des patients atteints de cirrhose du foie (CF) et 32 (88,9 %) des patients atteints de carcinome hépatocellulaire (HCC) présentaient de faibles taux sériques de vitamine D . Le taux sérique moyen de $25(OH)D_3$ parmi le groupe témoin était de 25,77 ± 8,59 ng/ml, et celui parmi les groupes MPC de 13,11 ± 10,54 ng/ml (P<0,001) {IC à 95 % (9,919-15,393)}. Les valeurs moyennes de vitamine D sérique de : CHB, cirrhose et CHC étaient de 21,53 ± 4,88 ng/ml, 8,13 ± 3,16 ng/ml et 10,25 ± 13,53 ng/ml, respectivement. Cependant, aucune différence statistiquement significative n'a été trouvée entre les taux sériques moyens de vitamine D des patients CF et HCC (valeur P = 0,058).
Conclusion **:** Le taux sérique de vitamine D est significativement bas chez les patients nigérians atteints de MPC liée au VHB par rapport aux témoins normaux, et le degré de carence est directement lié au stade de progression de la maladie.

Mots clés : *Donneurs de sang, hépatite B chronique, carcinome hépatocellulaire, cirrhose du foie, vitamine D*

Introduction

Vitamin D refers to a group of seco-steroid compounds [1], synthesized predominantly in the liver. The hormone precursor is present in two forms [2], namely, ergocalciferol or vitamin D_2 which is present in plants and some fish, and cholecalciferol or vitamin D_3, which is synthesized in the skin by sunlight.

The liver is crucial for physiologic vitamin D metabolism [3, 4]. Vitamin D itself is considered inactive until it is hydroxylated to 25–hydroxyvitamin D, $\{25(OH)D_3\}$ in the liver and then the 1 hydroxylation in the kidney to 1,25 –dihydroxy vitamin D $[1,25(OH)_2D]$ by the enzyme 1-α-hydroxylase. The former is the main circulating vitamin D metabolite and is used for classification of the vitamin D status. The latter being the active metabolite has a higher affinity for the vitamin D receptors (VDR) compared to $25(OH)D_3$. Recently, local conversion of $25(OH)D_3$ to $1,25(OH)_2D$ has been observed in many extra-renal tissues including the liver [5-7].

Vitamin D status may be particularly relevant for patients with liver diseases. Low serum vitamin D levels may be indicative of reduced liver function and might also contribute to liver damage by leading to increased inflammation and fibrosis.

Vitamin D deficiency is very common (92%) among patients with chronic liver disease (CLD) and at least one-third of them suffer from severe vitamin D deficiency [8]. The importance of vitamin D deficiency is high-lighted by the increasingly recognized role vitamin D has in immunoregulation, cellular growth and differentiation, anti-oxidation, and regulation of fibroblast proliferation and collagen synthesis [9, 10].

Low serum $25(OH)D_3$ concentration has been associated with liver dysfunction and predicts hepatic decompensation and mortality in patients with CLD [1]. Also, deficiency of vitamin D has been associated with an increased risk of cancer [11]. Higher serum $25(OH)D_3$ concentrations has been associated with significantly lower risk of CLD deaths [12], a possible protective role of vitamin D in CLD.

In the setting of chronic hepatitis B (CHB) infection, low serum $25(OH)D_3$ has been associated with increased viral replication and reduced response to antiviral treatment [4]. The aetiology of low serum vitamin D in CHB infection has been attributed to a high necro-inflammatory activity and immune mediated body response by suppression of liver 25-hydroxylases [13].

The serum level of $25(OH)D_3$ is considered the best indicator of total body vitamin D stores. However, it is unclear if this reflects the actual

physiological state of vitamin D activity in the body. It is possible that there are effects of vitamin D that occur intracellularly independent of a measured serum level of vitamin D [14]. Interpretation of $25(OH)D_3$ can be challenging owing to a wide variability in patient's weight, ethnicity, assays, laboratory procedures and validation of references [15-17].

Studies on the relationship between CHB infection and serum vitamin D are hardly available in sub-Saharan Africa, though the main reason for the study was to determine if vitamin D deficiency contributed to the progression of chronic hepatitis B to end-stage liver disease in which case vitamin D supplementation may be useful in CHB to retard progression.

Methods

This was a cross-sectional comparative analytical study conducted at the University of Abuja Teaching Hospital, Gwagwalada, Abuja and Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun state, Nigeria, between July and December 2015.

The study population was comprised of 192 patients. Ninety-six of them (cases) had HBV-related CLD while the controls who were healthy blood donors were also 96 in number. The cases were recruited consecutively from the Gastroenterology Clinic and from patients on admission in the medical wards while the controls were similarly recruited from prospective blood donors at the Blood Bank after an informed consent was obtained from both groups. Patients with hepatitis C, HIV infection, significant history of alcohol ingestion or other causes of liver diseases other than hepatitis B infection were excluded from the study. Also, patients already on CHB anti-viral chemotherapy were excluded.

The study was approved by the Ethics and Research Committee of the University of Abuja Teaching Hospital, Abuja, with approval number: FCT/UATH/HREC/PR/392.

The extracted serum samples were used for the assay of $25(OH)D_3$, HBsAg, anti-HCV, HIV, alpha feto-protein.Serum $25(OH)D_3$ was assayed using Roche Cobas e411 chemistry analyzer by a one–step sandwich electrochemiluminis centimmunometric method. Serum $25(OH)D_3$ levels at <20ng/ml, 20-29ng/ml, and >30ng/ml were termed as deficient, insufficient, and normal respectively [18].

All serum samples belonging to both the cases and controls were tested for HBsAg, HIV and Anti-HCV. The HBsAg-CTK onsite rapid test kit manufactured by CTK Biotech Inc. USA was used for the HBsAg tests. It had a sensitivity and specificity of 99.5% and 99.7% respectively. The Determine HIV-1/2 kit manufactured by Alere Medical Co, Japan was used for the HIV tests. It was an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. It had a specificity and sensitivity of 97.96% and 100% respectively. The HCV-Ab CTK onsite rapid test kits manufactured by CTK Biotech Inc. USA was used to screen for anti-HCV. The test is a qualitative, membrane-based immunoassay for the detection of antibodies to HCV. It had a specificity and sensitivity of 99.6% and 98.9% respectively. All these tests were performed according to the information provided in the respective manufacturer's instruction manual.

The serum level of Alpha-Fetoprotein (AFP), a tumour marker for hepatocellular cancer (HCC), was assayed for in all the cases using the kit manufactured by Randox Laboratories Ltd, United Kingdom. AFP level of 0-20ng/ml was regarded as normal, 21 – 400ng/ml as elevated while > 400ng/ml was regarded as significantly elevated to suggest HCC in the presence of supportive clinical and imaging features [19]. Both the cases and the controls underwent abdominal ultrasonography (Abd USS) with emphasis on the liver size, parenchymal architecture and echotexture, liver margins, hepatobiliary and portal system.

Liver biopsies and triphasic abdominal computerized tomographic (Abd CT) scans were done to confirm the diagnosis of HCC in those with suspected HCC lesions on Abd USS. Liver biopsies were done in those with no contraindication to it (Prothrombin time >4second prolonged, International Normalized Ration (INR) >1.5, Bleeding time >10 minutes, Platelet count <80,000/cm^2, massive ascites, severe cardiopulmonary insufficiency, or where patient did not give consent) while triphasic Abd CT was done in those with contraindications to liver biopsy.

Clinical diagnoses were based on the history obtained, physical examination, laboratory investigations and abdominal ultrasound reports. The cases were grouped into

(a) **Uncomplicated CHB infection**: these were cases of asymptomatic CHB infection who did not have features suggestive of liver cirrhosis (LC), portal hypertension or HCC, and who had normal or insignificantly elevated serum AFP,

(b) **Complicated CHB infection**: these were patients diagnosed with LC and HCC.

LC: defined as patients with CHB infection who had symptoms, physical examination findings and abdominal ultrasound features of LC but not HCC.

The AFP levels of these patients were within normal limits or not significantly elevated (<200ng/ml).

HCC: defined as patients with CHB infection who had symptoms, physical examination findings and abdominal ultrasound features suggestive of HCC. Confirmation of the diagnosis was by liver biopsy histology, contrast-enhanced abdominal computerized tomography imaging or significantly elevated AFP (>400ng/ml) in the presence of a suggestive abdominal ultrasound feature.

The controls used were healthy, voluntary blood donors recruited from the blood bank. The controls tested negative to hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (anti-HCV) and HIV screening. Also, they had no significant history of alcohol use or other risk factors of liver disease and have never been on either hepatitis B or C antiviral agents.

Data obtained were analyzed using SPSS software version 20. Continuous variables were presented as mean ± standard deviations (SD), or as ranges while categorical variables were expressed as proportions and percentages. Differences in categorical variables were analyzed using Chi square or Fisher's exact test while differences in continuous variables were analyzed using Student's *t* test and Analysis of Variance (ANOVA) as appropriate. The comparison of serum 25(OH)D_3 level of CHB and healthy controls was done using Paired Sample t test with p value <0.05 regarded as significant, and the correlation between 2 continuous variables were analyzed using Spearman's bivariate correlation.

Results

A total of 192 patients comprising of 96 patients with CHB viral infection and 96 healthy controls completed the study. The mean ± SD age of all the participants was 36.2±14 years with a range of 15-80years. The modal age was 28 years with a median age of 33 years. Male sex (147, 76.6%) accounted for majority of the study population with a male to female ratio (M: F) of 3.3:1. Majority of them were employed (108, 56.3%) and married (114, 59.4%). Most of the participants had tertiary education (117, 60.9%) Table 1.

There were 30(31.3%) uncomplicated and LC while HCC were 36(37.7%). The mean serum vitamin D (25-OH) level was 19.44±11.50ng/ml among the total study population. Low serum vitamin D level was seen in 95 (49.5%) out of all the 192 study participants. Insufficient levels were detected in 58 (30.2%) participants while 39 (20.3%) of them had normal serum levels of the vitamin.

The mean level of 25(OH)D_3 in the CHB-group was 13.11±10.54ng/ml (Figure 1). Seventy-three out of the 96 (76%) CHB-related liver disease cases had low serum levels of vitamin D (25-OH), insufficient levels of serum vitamin D were seen among 17 patients, while 6 had normal levels of serum vitamin D accounting for 17.7% and 6.2%, respectively (Table 2).

On the other hand, the mean level of 25(OH) D3 in healthy controls was 25.77±8.59ng/ml (Figure 1). Twenty-two (22.9%) out of the control group had low levels of serum vitamin D. Forty- one of them (42.7%) had insufficient levels of serum vitamin D.

Table 1: Socio-demographic distribution of the CHB related liver disease population and Controls

	cases	controls	Total study population
AGE (mean age) mean±SD	38.6 ± 18.7	33.3 ± 11.3	36.2 ± 14
Sex n(%)			
Male	70(72.9%)	77(80.2%)	147(76.6%)
Female	26 (27.1%)	19 (19.8%)	45 (23.4%)
Marital status			
Single	32 (33.3%)	46 (47.9%)	78 (40.6%)
Married	64 (66.7%)	50 (52.1%)	114 (59.4%)
Level of education			
Tertiary	53 (55.2%)	64 (66.7%)	117 (60.9%)
Non-tertiary	43 (44.8%)	32 (33.3%)	75 (39.1%)
Occupation			
Employed	51 (53.1%)	57 (59.4%)	108 (56.3%)
Not employed	45 (46.9%)	39 (40.6%)	84 (43.7%)

CHB= Chronic hepatitis B

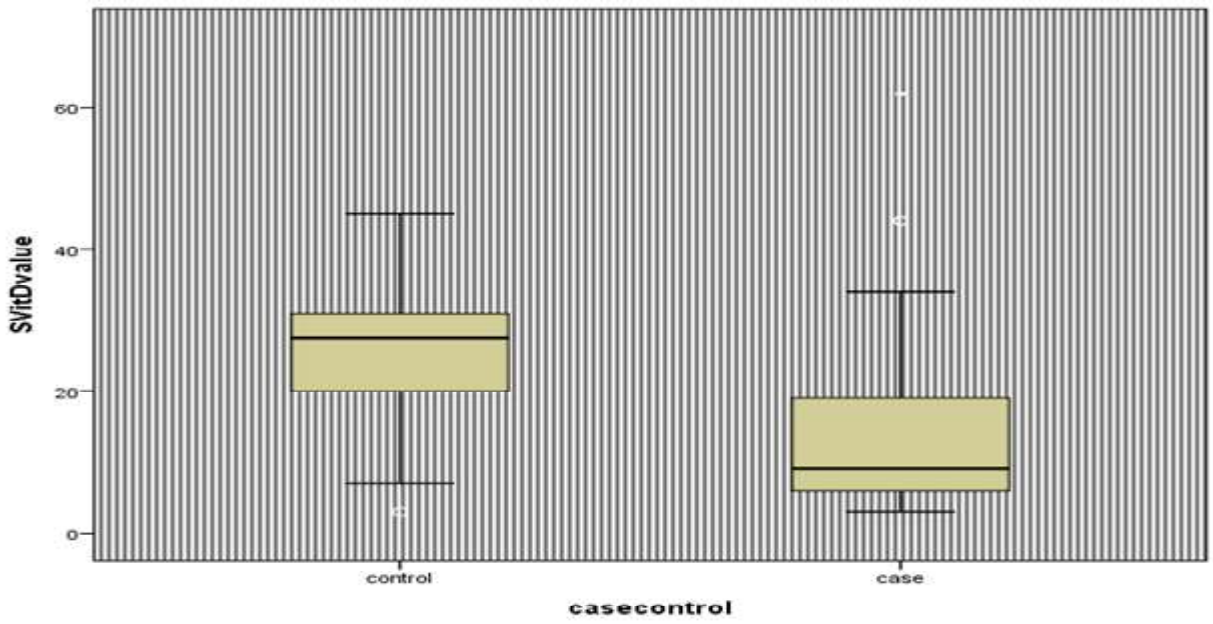

Fig. 1: Mean serum vitamin D levels of healthy controls and those with CHB-related liver diseases. (SVit D: serum vitamin D)

Normal serum levels of vitamin D was seen in 33 controls (33.4%) (Table 2).

Eleven (36.7%) of the uncomplicated CHB patients had low levels of serum 25(OH)D_3. All the LC patients (100%) had low levels of serum 25(OH)D_3. Thirty-two (88.9%) of patients with HCC also had low levels of serum 25(OH)D_3 (Table 2). Four (11.1%) of the patients with HCC had normal serum levels of 25(OH)D_3. There was an inverse relationship between severity of CHB infection (i.e., LC and HCC) and serum levels of vitamin D. There were significant differences in the mean serum level of 25(OH)D_3 in the control group and the different categories amongst the cases (Table 3). The analysis also revealed significant difference in the serum vitamin

Table 2: Frequency of the different serum levels of vitamin D among the cases (sub-categories) and the controls.

Study Population	Mean Serum Vitamin D level	Categories of serum Vitamin D level	Frequency
Uncomplicated-CHB cases	21.53 ± 4.9	Low	28 (93.3%)
		Normal	2 (6.7%)
LC cases	8.13 ± 3.6	Low	30 (100%)
		Normal	0 (0%)
HCC cases	10.25 ± 13.5	Low	32 (88.9%)
		Normal	4 (11.1%)
Controls	25.77 ± 8.59	Low	63 (65.6%)
		Normal	33 (34.4%)

CHB= Chronic hepatitis B
LC= Liver cirrhosis
HCC= Hepatocellular carcinoma

The mean serum levels of 25(OH) D3 in CHB patients was significantly lower than that of healthy controls (P- value = 0.000). The mean serum 25(OH)D_3 in the control and CHB-groups were 25±8.59ng/ml and 13.11±10.54ng/ml respectively. There was a statistically significant difference between the mean serum vitamin D levels of the healthy controls compared to patients with CHB-related liver disease (p-value <0.01).

D between the control and CHB groups (p-value < 0.05). The mean serum vitamin D levels of the control group when compared with the CHB-related liver disease cases, showed statistically significant mean differences (P-value <0.006). Similar findings were observed for uncomplicated CHB patients against CHB cirrhosis, and CHB-HCC (Table 3).

Table 3: Comparison of the mean serum levels of vitamin D in patients with CHB related liver diseases (Uncomplicated CHB, CHB-cirrhosis, and CHB-HCC) and controls.

Reference Category	Category	Mean difference	Standard error	p-value	Lower bound 95%	Upper bound CI
Controls	Uncomplicated CHB	4.237	1.250	0.006	0.87	7.60
	CHB-cirrhosis	17.638	1.049	0.000	14.83	20.44
	CHB-HCC	15.521	2.419	0.000	8.87	22.17
Uncomplicated CHB	Controls	-4.237	1.250	0.006	-7.60	0.87
	CHB-cirrhosis	13.400	1.062	0.000	10.49	16.31
	CHB-HCC	11.283	2.424	0.000	4.62	17.95

CI: confidence interval, CHB: chronic hepatitis B, HCC: hepatocellular carcinoma
LC: Liver cirrhosis

Discussion

The role of serum vitamin D in the inflammation seen in chronic hepatitis B viral infection and subsequent complications is still not well established, hence it's a growing area of research. This study was able to show a significantly lower level of serum vitamin D in CHB patients and those with chronic complications compared with healthy controls. It went further to also show that greater decline in serum vitamin D levels were seen in HBV associated liver cirrhosis and hepatocellular cancer cases compared to the decline seen in uncomplicated CHB cases. Though, there was a significantly lower level of vitamin D in the sera of uncomplicated CHB cases compared to the healthy controls, it was not as low as the levels seen amongst the LC and HCC cases in comparison to the healthy controls. All these clearly showed that the reduced level of vitamin D seen amongst the cases reflects the ongoing liver inflammation and possibly an index of worsening or progressive liver disease in such patients.

The significantly lower level of serum Vit D seen amongst the cases could be attributable to the reduction in the synthesis of 25-hydroxyvitamin D due to the progressive hepatocyte damage from inflammation and, later, fibrosis in these conditions. The slightly higher level of mean serum vitamin D seen in HCC compared to those with LC despite both conditions being advanced stage liver disease is probably due to paraneoplastic activities of the malignant liver cells.

The fact that some healthy controls had low serum levels of vitamin D in this study was an interesting but not a unique finding. En-Qiang *et* al [20], reported a 1.56% (2/128) prevalence of low serum vitamin D in his control subjects also. This was also reported by Hilger *et al* [21], in a review of vitamin D status among populations worldwide. They found that vitamin D status could be affected by factors like geographical location, age, and nutrition.

CHB infection and its liver-related chronic complications were the only form of CLD included in the study since CHB is the most common cause of CLD in our environment. However, whether this is peculiar to only CHB infection and CHB-related liver complications might need further studies. Some other studies which limited the aetiology of the liver disease to only CHB as well got similar results albeit a much lower prevalence of low mean serum Vit D levels amongst the study participants [20, 22]. However, Arteh *et al* [8] studied multiple aetiologies (i.e., HBV, HCV, Alcohol) and got similar results as in this study.

The study was limited in scope as the interplay between pro-fibrogenic and antifibrogenic factors at the molecular level was not studied. Vitamin D is said to have anti-fibrogenic properties and such molecular studies could have helped strengthen the association between low serum vitamin D and progressive liver damage.

Future studies may need to establish a direct causal role of vitamin D in the ongoing inflammation associated with HBV infection or as a risk factor for the development of complications. However, the findings in this study raise the possibility that vitamin

D supplementation in the diet of patients with uncomplicated CHB may retard or even abort the development of sequelae such as LC and HCC. This could be a subject for future longitudinal studies

Conclusion

There was a high prevalence of low serum vitamin D in patients with chronic hepatitis B-related liver disease compared to healthy controls and this level directly correlated with the severity of liver disease.

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