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A CROSS-SECTIONAL STUDY OF VITAMIN-D AND CALCIUM LEVELS IN PATIENTS LIVING WITH VIRAL HEPATIC INFECTIONS IN BENIN CITY, NIGERIA.

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ABSTRACT

The occurrence of vitamin D (25-(OH)D) insufficiency and hypocalcemia is associated with instances of chronic liver diseases. This investigation aimed to ascertain the prevalence of hypocalcemia and 25-(OH)D deficiency in people afflicted with viral liver infections and evaluate their serum calcium and 25-(OH)D concentrations to their respective characteristics. Forty-two individuals, with ages ranging from 20 to 70 years participated in the study. Within the sample population, a total of 29 individuals were diagnosed with hepatitis virus. The remaining thirteen participants consisted of healthy controls who were residents of Benin City. Blood samples were collected from the participants, followed by centrifugation to separate the serum. The resulting serum was then stored at a temperature of -20 degrees Celsius. The amounts of calcium and vitamin D were quantified using Calcium AMP/CPC tests and vitamin-D ELISA kits, respectively. The findings of the study indicated that 54.8% of the participants exhibited a deficiency in vitamin D, while 11.9% of the participants displayed hypocalcemia. A significant ($p < 0.001$) decrease in calcium and 25-(OH)D levels was also observed among patients diagnosed with hepatitis A, B, and C compared to the control group. People who took 25-(OH)D supplements, had significantly higher calcium levels ($p < 0.001$) than people who did not take supplements. Based on our research, it seems likely that giving 25-(OH)D supplements to people with viral liver infections could help them get better in future programs aimed at treating vitamin D deficiency and low calcium levels.

Keywords: *Vitamin-D Deficiency, Hypocalcemia, Liver Infection, Hepatitis, Lifestyle.*

INTRODUCTION

Viral hepatitis is a major global public health concern infecting millions of people annually (Jeffries *et al.*, 2018). It is estimated that about 2.3 billion people are infected worldwide with one or more of the hepatitis viruses and around 1.4 million deaths occur from viral hepatitis infection each year (WHO, 2010; Wiktor & Hutin, 2016). Viral hepatitis refers to a liver infection caused by five primary viruses, namely hepatitis A, B, C, D, and E. According to the Centres for Disease Control and Prevention (CDC, 2023), the prevailing forms of viral hepatitis include hepatitis A (HAV), hepatitis B (HBV), and hepatitis C (HCV). According to Wasley *et al.* (2006), all three of these viruses have the potential to cause acute illness characterized by symptoms such as nausea, stomach discomfort, lethargy, malaise, and jaundice. Moreover, the presence of acute infection caused by HBV and HCV has the potential to progress into chronic infections, which can result in severe complications that pose a threat to an individual's life. According to Wasley *et al.* (2006), those who are chronically infected may progress to the development of cirrhosis and hepatocellular carcinoma.

It is estimated that one billion people suffer from deficiency or insufficiency of vitamin D globally (Holick, 2007a; Gordon *et al.*, 2004). In patients with chronic liver diseases, the prevalence of vitamin D deficiency is much higher and practically universal (Fisher and Fisher, 2007). A high prevalence of vitamin D deficiency of serum levels below 20 mg/mL has also been found in patients with HBV and HCV infection worldwide (Hoan *et al.*, 2018; Kitson and Roberts, 2012). These reports therefore suggest that the pathogenesis of chronic liver diseases caused by hepatitis B and C viruses could be linked to Vitamin D deficiency (Hoan *et al.*, 2018). Vitamin D insufficiency has also been linked to complications from portal hypertension (Anty *et al.*, 2014) and fibrosis severity in patients with chronic liver disease of various etiologies (Targher *et al.*, 2007). Another study found that vitamin D deficiency in chronic liver illness may be caused by a hepatic synthesis defect (Fisher and Fisher, 2007). Experts describe vitamin D insufficiency as a lack of 25-hydroxyvitamin D (25(OH)D) levels less than 75 nmol/L (30 ng/mL). On the other hand, Vitamin D deficiency is defined by values less than 50 nmol/L (20 ng/mL).

Calcium is the most abundant mineral in the body and participates in many biological processes including the formation of bones and teeth and, the normal functioning of nerves and muscles and plays a role in blood coagulation and in several enzymatic processes (Espay, 2014; Akirov, *et al.*, 2017). Serum calcium levels are usually maintained within a normal range and ionized calcium is tightly regulated by the actions of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (1,25[OH]2D) on the kidney, bone, and gastrointestinal tract (Holick, 2007a; Body & Bouillon, 2003). Optimal vitamin D levels are therefore necessary to increase the efficiency of calcium absorption (Holick, 2007a). Without adequate vitamin D, the body absorbs no more than 10% to 15% of dietary calcium (Holick, 2007b). In the vitamin D –D-sufficient state, intestinal calcium absorption increases to 30% - 40% (Holick, 2007a). Hypocalcemia is defined as a total serum calcium concentration < 8.8 mg/dL (< 2.20 mmol/L) in the presence of normal plasma protein concentrations or as a serum ionized calcium concentration < 4.7 mg/dL (< 1.17 mmol/L) (Monegal *et al.*, 1997). The available evidence regarding the prevalence of hypocalcemia in hospitalized patients is minimal. Nevertheless, previous studies have indicated that the occurrence of hypocalcemia in hospitalized patients is approximately 18%, whereas in intensive care units, it can reach as high as 85% (Cooper & Gittoes, 2008).

According to a study conducted by Devaraj *et al.* (2021), there is an association between hypocalcemia and vitamin D-dependent metabolism in individuals diagnosed with chronic liver disease.

There is limited information on the vitamin D and calcium profiles of Nigerians with various types of hepatitis infections, even in light of the previously mentioned evidence suggesting a possible link between viral hepatic infections and cases of vitamin D deficiency and hypocalcemia. The important body minerals are not correlated with the demographic characteristics or lifestyle choices of the affected persons in the cases when research of this kind has been carried out. More research is therefore needed to determine the prevalence of vitamin D deficiency and hypocalcemia in Nigerians with viral hepatitis. Furthermore, it appears from the literature that nobody has previously compared the vitamin D levels of people with a diagnosis of hepatitis A virus infection with a control group of people in good health. Similarly, there hasn't been any previous research on how common vitamin D deficiency is in people who have hepatitis A. This study's goal is therefore, to evaluate the calcium and vitamin D levels, hence the prevalence of vitamin D deficiency and hypocalcemia, in residents of Benin City, Nigeria who have hepatitis A, B, or C.

MATERIALS AND METHODS

Study Design

This study involved a cohort of 42 individuals, consisting of 29 patients diagnosed with viral liver infections and 13 individuals who were deemed healthy controls. The age range of the participants ranged from 20 to 70 years. The study sample comprises a randomly selected cross-section of individuals residing in Benin City, Nigeria. The sample includes a total of 18 male participants and 24 female participants. The study participants consisted of individuals diagnosed with viral hepatitis who were selected from Central Hospital in Benin City, Nigeria. The sample included two groups: newly diagnosed patients (n = 16) who were identified using serological testing for hepatitis virus, and previously diagnosed patients (n = 13) those taking antivirals for at least six months. In the control group, healthy people without a history of viral hepatitis or liver disease, autoimmune disorders, cancer, or vitamin D3 or multi-vitamin use in the past three months were included. The hepatitis statuses of the control people were ascertained using serological testing for the hepatitis virus conducted before the initiation of the investigation. A structured health and lifestyle questionnaire was employed to ascertain the history of liver illnesses, autoimmune diseases, or cancer, and the utilisation of vitamin D3 or multi-vitamin supplementations. The exclusion criteria included kidney failure, bone diseases like osteopenia and osteoporosis, and current or past medical conditions or diagnoses of hepatocellular carcinoma or other malignancies, as well as those receiving therapy with supplements of calcium, magnesium, phosphorus, or vitamin D. Furthermore, those who willingly left the study for any number of reasons were eliminated as well. After providing a detailed explanation of the study's goals to each participant, the researchers got their agreement. To every individual involved in this study, a well-structured questionnaire was sent.

Questionnaire/ Ethical Approval

The questionnaire comprised inquiries specifically formulated to obtain information regarding various ages, genders, places of birth, career, marital status, frequency of smoking and alcohol intake, level of sunshine exposure, current

medication usage and duration, dietary supplement use, and past medical issues. The Edo State Ministry of Health Ethical Committee approved this study on January 17, 2022, with reference number 737/5/T1/009.

Laboratory Investigations

In adherence to aseptic protocols, sterile needles, and syringes were utilized to collect fasting blood samples measuring 5 millimetres from the ante-cubital vein of each participant. The sample was distributed into a sterile, moisture-free, unadorned container and left undisturbed for a few minutes to clot. The contents of the unadorned vessel underwent centrifugation at a speed of 4000 revolutions per minute for approximately 5 minutes to facilitate the separation of the serum from the coagulated substance. Before this, the specimen was allowed to coagulate undisturbed for a brief period. Subsequently, the serum was transferred to a separate container that had been sterilized. The serum samples were kept at a temperature of -20 degrees Celsius before analysis.

The sera samples were tested for total antibodies against HAV, HBV, and HCV using a competitive enzyme immunoassay (EIA) test. Global Company supplied the test kits for this study, according to Shah and Maghsoudlou (2016) and Khol and Ascoli (2017). The tests were done following the manufacturer's instructions.

An ELISA kit was used to quantify total 25-hydroxyvitamin D in human serum and plasma. The reagent kit with the number VDS6295 was supplied by Calbiotech. The tests were done following the manufacturer's instructions. Vitamin D deficiency is defined as a blood 25-(OH)D concentration below 20 ng/ml, according to Duarte *et al.* (2001).

This study used the Fortress Diagnostics Calcium AMP/CPC kit to estimate the serum calcium concentration according to the manufacturer's instructions. A blood-ionized calcium concentration below 4.7 mg/dL (or 1.17 mmol/L) constitutes hypocalcemia, according to Monegal *et al.* (1997).

Statistical Analysis

Mean, standard deviation and percentages were employed to describe continuous and categorical variables, respectively. The two study groups were compared using the independent sample t-test. A one-way analysis of variance and post hoc multiple comparison tests were used to determine the least significant difference in data from numerous normal distribution groups. Data with an abnormal distribution was tested with the Kruskal-Wallis test. This study measured normality with Shapiro-Wilk's normality test. We used a significance level of $P < 0.05$ for statistical analysis. Statistical analyses were performed using SPSS for Windows (25.0).

RESULTS

Table 1: Socio-Demographic Characteristics of the Study Population

Characteristics	**Control Group (n = 13)** **Mean ± SD or n (%)**	**Patients Living with Viral Hepatic Infections (n = 29)** **Mean ± SD or n (%)**	**X^2 or t- stat**	**P - Value**
Age (years)	32.69 ± 10.82	45.24 ± 12.63	-3.19	0.003
20-39 years	9 (69.2)	10 (34.5)	6.07	0.048
40-59 years	4 (30.8)	15 (51.7)		
≥60 years	0 (0)	4 (13.8)		
Sex				
Males	8 (61.5)	10 (34.5)	1.92	0.199
Females	5 (38.5)	19 (65.5)		
Marital Status				
Married	4 (30.8)	22 (75.9)	8.61	0.006
Single	9 (69.2)	7 (24.1)		
Occupation				
Employed	8 (61.5)	25 (86.2)	5.87	0.050
Unemployed	5 (38.5)	3 (10.3)		
Retired	0 (0)	1 (3.5)		
State of Origin				
Edo State	9 (69.2)	12 (41.4)	7.06	0.029
Other States	4 (30.8)	17 (58.6)		

Table 1 provides study population sociodemographics. 29 viral liver infection patients and 13 healthy controls were studied. According to an independent sample t-test, the mean age of the patients (45.24 years) was significantly higher ($p = 0.003$) than healthy controls (32.69 years). The control participants comprised a higher proportion of individuals aged 20-39 (69.2%), males (61.5%), single (69.2%), employed (61.5%), and residents of Edo state (69.2%). Patients with viral hepatic infections were more likely to be females (65.5%), in the age range of 40–59 years (51.7%), married (74.9%), employed (86.2%), and from Edo state (41.4%).

Table 2: Selected Lifestyle Profile of the Study Population

Lifestyle	**Control Group (n = 13)**	**Patients Living with Viral Hepatic Infections (n = 29)**
	N (%)	**N (%)**
Smoking Habit		
No	11 (84.6)	22 (69.0)
Yes	2 (15.4)	9 (31.0)
Extent of Smoking (n, 2 v.9)		
One stick/day	0 (0)	4 (44.4)
≥2 sticks/day	2 (100.0)	5 (55.6)
Alcohol Consumption		
No	6 (46.2)	5 (17.2)
Yes	7 (53.8)	24 (82.8)
Extent of Alcohol Consumption (n, 7 v. 24)		
1 bottle of alcoholic beverage	3 (42.8)	16 (66.7)
2 bottles	0 (0)	7 (29.1)
3 bottles	2 (28.6)	0 (0)
4 bottles	1 (14.3)	1 (4.2)
5 bottles	1 (14.3)	0 (0)
Use of Dietary Supplements		
No	12 (92.3)	27 (93.1)
Yes	1 (7.7)	2 (6.9)
Regular Exposure to Sunlight		
No	0 (0)	0 (0)
Yes	13 (100.0)	29 (100)

Table 2 displays a few chosen lifestyles of the research sample. The data indicates that a significant portion of the control group does not use dietary supplements (92.3%), does not smoke (84.6%), does not consume alcohol (82.8%), and is exposed to sunlight (100%). All the smokers in the control group (n = 2) indicated they smoke up to 2 or more sticks of cigarettes per day. The majority (42.8%) of the control group that drink alcohol reported they take 1 bottle of alcoholic beverage daily. A greater percentage of the patients living with viral hepatic infections, do not smoke (69%); drink alcohol (83.9%); are daily exposed to sunlight (100%); and do not use dietary supplements (93.1%). Of

the nine persons that smoke, 44.4% smoke 1 stick/day, while 55.6% smoke up to 2 sticks/day. A greater percentage (66.7%) of the patients who drink alcohol reported they take 1 bottle of alcoholic beverage daily.

Table 3: The Incidence of Patients' Hepatic Viral Infections, Ongoing Medication, Type of Medications, Duration of Treatment, and Experiences of Difficulty in Limb Movement

Characteristics	**Frequency**	**Percentage**
Type of Viral Hepatic Infections		
Hepatitis A	6	20.7
Hepatitis B	17	58.6
Hepatitis C	6	20.7
Ongoing Medications		
No	16	55.2
Yes	13	44.8
Type of Medication		
None	16	55.2
DDA Tablets	2	6.9
Entecavir	2	6.9
Lamivudine	3	10.3
Tenofuvin	6	20.7
Duration of Treatment		
None	16	55.2
< One Month	7	24.1
≥One Month	6	20.7
Difficulty in Limb Movement		
No	25	86.2
Yes	4	13.8

A few of the clinical features of individuals with viral liver infections are displayed in Table 3. The bulk of the patients (58.6%), according to the data, had hepatitis B. In each condition, the incidence of hepatitis A and C was 20.7%. Of the patients, 44.8% were taking medication at the time of the trial, and 55.2% were not. 6.9 percent of the patients were treated with DDA and entecavin respectively, 10.3% were treated with lamivudine, and 20.7% were treated with Tenofuvin. Twenty-four percent of those on medication had been on such medications for less than 1 month, while 20.7% had been on those drugs for one month or more. The majority (86.2%) of the patients reported they were not experiencing any difficulties in limb movement (a symptom of osteomalacia), while 13.8% complained of some difficulties in limb movement.

Table 4: The Serum Levels of Vitamin D in the Different Study Groups

Type of Viral Hepatic Disease	Number of Patients	Vitamin D Level (ng/ml)	Number of Patients with Vitamin D Deficiency
Control	13	62.41 ± 17.16	0
Hepatitis A	6	7.91 ± 5.77*	6
Hepatitis B	17	11.87 ± 9.18*	13
Hepatitis C	6	13.20 ± 8.28*	4
Statistics (Kruskal Wallis test)		*X^2 = 27.8; P = 0.000*	23

*Significantly lower compared with the control.

Table 4 shows the average vitamin D serum levels across research groups. The non-parametric Mann-Whitney U test compares two independent groups. The test results show significantly lower vitamin D levels ($p < 0.001$) in patients with hepatitis A, B, and C compared to the control group (62.41 ± 17.16 ng/ml). Mean vitamin D levels were not statistically different between hepatitis cohorts. This study found that 54.8% of participants had vitamin D deficiency. None of the healthy controls showed vitamin D deficiency. Vitamin D deficiency was found in all six (100%) hepatitis A patients, 13 (76.5%) of the HVB patients, and four (66.7%) of the HVC patients.

Table 5: The Serum Calcium Levels of the Different Study Groups

Type of Viral Hepatic Disease	Number of Patients	Calcium Level (mmol/L)	Number of Patients with Hypercalcemia
Control	13	2.35 ± 0.19	0
Hepatitis A	6	1.67 ± 0.30*	0
Hepatitis B	17	1.62 ± 0.54*	3
Hepatitis C	6	1.21 ± 0.46*	2
Statistics (ANOVA)		*F = 12.6; P = 0.000*	5

*Significantly lower compared with the control.

Table 5 displays the average serum calcium levels for each research group. The analysis of variance revealed significantly lower calcium levels in hepatitis A, B, and C patients (1.67 ± 0.30 mmol/L; p = 0.002, 1.62 ± 0.54

mmol/L; $p < 0.001$, and 1.21 ± 0.46 mmol/L compared with the control group (2.35 ± 0.19 mmol/L). Hepatitis B patients had increased mean calcium levels compared with hepatitis C patients ($p = 0.048$). However, mean calcium levels did not differ between hepatitis A and B ($p = 0.800$) or A and C ($p = 0.066$) respectively. Additionally, 11.9% ($n = 5$) of research participants exhibited hypocalcemia, while none of the control group did. No hypocalcemia was found in hepatitis A patients. Two (33.3%) and three (17.6%) of the hepatitis C and B groups had hypocalcemia.

Table 6: The Calcium and Vitamin D Levels of Patients Living with Liver Diseases Compared according to some of their Characteristics

Variables	**N**	**Calcium Level (Mean ± SD; *P – Value*)**	**Vitamin D Level (Mean ± SD; *P – Value*)**
Age			
20-39 years	10	1.57 ± 0.59	11.62 ± 8.62
40-59 years	15	1.59 ± 0.47	11.20 ± 9.0
≥60 years	4	1.31 ± 0.42	11.07 ± 7.07
		P = 0.616	*P = 0.991*
Sex			
Males	10	1.62 ± 0.61	12.42 ± 9.04
Females	19	1.51 ± 0.44	10.75 ± 8.17
		P = 0.562	*P = 0.619*
Smoking Habit			
No	20	1.52 ± 0.48	11.98 ± 8.70
Yes	9	1.61 ± 0.57	9.86 ± 7.82
		P = 0.656	*P = 0.538*
Alcohol Consumption			
No	5	1.54 ± 0.34	15.06 ± 8.70
Yes	24	1.54 ± 0.53	10.55 ± 8.25
		P = 0.976	*P = 0.280*
Difficulty in Limb Movement			
No	25	1.57 ± 0.52	11.46 ± 8.62
Yes	4	1.39 ± 0.37	10.47 ± 7.44
		P = 0.530	*P = 0.831*
Use of Supplements			
No	27	1.51 ± 0.50	11.38 ± 8.66
Yes	2	2.0 ± 0.03	10.50 ± 2.12
		P < 0.001	*P = 0.888*
Ongoing Medication			
No	16	1.48 ± 0.43	11.43 ± 8.20
Yes	13	1.62 ± 0.59	11.20 ± 8.88
		P = 0.473	*P = 0.943*
Duration of Treatment			
<1 month	7	1.63 ± 0.53	13.71 ± 10.84
≥1 Month	6	1.60 ± 0.70	8.26 ± 5.38
		P = 0.931	*P = 0.289*

Table 6 shows the mean calcium and vitamin D levels of patients living with viral hepatic infections compared according to some of their characteristics. Data indicated no significant differences in mean calcium and vitamin D levels of subjects when compared among age groups; between sexes; smokers and non-smokers; between those who drink alcohol and those who don't; between those who experience difficulty in movement or not; between those who are undergoing liver disease medications and those who are not; and between those who have been on medications for a period of <1 month and ≥1 month. In contrast, patients who take vitamin D supplements indicated significantly higher ($p < 0.001$) mean calcium levels compared with those who don't. Between groups, mean vitamin D levels were not significantly different.

DISCUSSION

The present study revealed the presence of three different viral liver infections (hepatitis A, B, and C) among the patient population. The majority (58.6%) of the patients suffering from liver infections presented with hepatitis B infection. 20.7 percent of the patients indicated hepatitis A and C infections respectively. The findings of this study are in agreement with previous reports which showed that the hepatitis B virus is the most common liver infection and a major cause of liver disease morbidity and mortality worldwide (Heaney, 2004; Medscape, 2022). It is estimated that two billion people (or 1 in 3) worldwide, have been infected and about 300 million people are living with a chronic hepatitis B infection (Heaney, 2004). Each year up to 1 million people die from hepatitis B even though the fact that it is preventable and treatable (Heaney, 2004). In Nigeria, HBV is reported to be the most common cause of liver disease (Hepatitis B Foundation, 2023). It has also been reported that up to 58.1% of patients with chronic liver disease in southern parts of the country were found to be HBsAg positive (WHO, 2013). The risk of contracting HBV in Nigeria has been attributed to inadequate funding, poor attention, and the political will to address Nigeria's HBV plight by the government on one hand, and the other hand, the high rate of exposure to the HBV infection, community misconceptions, and lack of awareness, resources and affordable diagnostics by the general population (Hepatitis B Foundation, 2023; Musa *et al.*, 2015).

This study found that people with liver illnesses such as hepatitis A, B, and C had significantly lower vitamin D levels than healthy people. Vitamin D deficiency was found in 54.8% of participants. Additionally, 100% of hepatitis A patients, 76.5% of B patients, and 66.7% of C patients had vitamin D deficiency. There were no studies comparing vitamin D levels in hepatitis A patients and healthy controls. Based on current information, vitamin D inadequacy in hepatitis A patients is understudied. The novelty of this study may be attributed to this particular aspect. On the other hand, previous studies (Lesi *et al.*, 2004; Ajuwon *et al.*, 2021; Chen *et al.*, 2015; Hoan *et al.*, 2016) have shown that average serum 25OHD levels were significantly lower in chronic hepatitis B patients compared with controls. Another study has also shown significantly low vitamin D levels in hepatitis C virus patients as compared to healthy subjects (Gao *et al.*, 2016). Furthermore, this study showed the prevalence of 54.8% of vitamin D deficiency (referring to serum 25(OH)D concentrations < 20 ng/ml), which is slightly lower compared to the global prevalence of vitamin D deficiency among chronic liver disease populations ranging from 64 to 92% (Zhao *et al.*, 2016, Falak *et al.*, 2020). Vitamin D deficiency is very common and frequently observed in HBV- and HCV-associated chronic liver diseases and sufficient levels have been shown to play an important role during antiviral treatment of HBV and HCV infections

(Arteh *et al.*, 2010). It is not well understood, the mechanisms behind the vitamin D deficiency observed in liver infections in this study. Nevertheless, it is worth noting that the deficit of vitamin D in abnormal liver states can be attributed to several factors such as inadequate food intake, impaired absorption, reduced generation of vitamin D binding protein by the liver, and the presence of fibrotic, inflammatory, and immunomodulatory diseases commonly associated with viral hepatitis infections (Arteh *et al.*, 2010). Moreover, the occurrence of vitamin D deficiency among African populations has been linked to factors such as skin pigmentation, traditional attire that covers the entire body, and the prevalence of infectious diseases like tuberculosis, HIV/AIDS, and malaria (Fisher and Fisher, 2007; Hoan *et al.*, 2018; Prentice *et al.*, 2009; Cusick *et al.*, 2014).

The study found that hepatitis patients had lower calcium levels than healthy controls, which is consistent with their greater vitamin D levels. Calcium is absorbed more readily in the intestines, released more easily from bones, and reabsorbed more readily in the kidneys' distal tubules when vitamin D is present. Additionally, it has the potential to induce secondary hyperparathyroidism (Kibirige *et al.*, 2013). The liver assumes a significant part in the metabolic processes associated with vitamin D. In the liver, vitamin D derived from the skin and diet is hydroxylated into 25-hydroxy vitamin D [25(OH)D], which represents the major circulating form of vitamin D, and which is used to determine a patient's vitamin D status (Mehta *et al.*, 2010). Findings from this study agree with previous studies which showed a strong correlation between low levels of calcium deficiency in patients with chronic liver diseases (Aykara *et al.*, 2020; DeLuca, 2004). Interestingly, hypocalcemia was observed in 11.9% of the study population, with patients living with HBV and HCV infections indicating 33.3% and 17.6% incidences of hypocalcemia respectively The incidence of hypocalcemia in people infected with the hepatitis virus is not well understood. Jagdish *et al.* (2021) conducted a prior investigation that revealed hypocalcemia in patients suffering from decompensated cirrhosis caused by chronic viral hepatitis (hepatitis B cirrhosis, 54.5%, and hepatitis C cirrhosis, 95.1%). In a different Chinese study, 87.7% of patients with decompensated cirrhosis had hypocalcemia (Ionele *et al.*, 2022).

This study examined the relationship between mean calcium and vitamin D levels and demographic and lifestyle variables like age, gender, smoking and alcohol consumption, osteomalacia symptoms, vitamin D supplement use, liver disease medications, and treatment length. Patients who received vitamin D treatment had considerably higher calcium levels ($p < 0.001$) compared to those who did not. Statistics showed that age, gender, smoking, alcohol, osteomalacia symptoms, liver disease medication use, and treatment length did not alter mean calcium and vitamin D levels. It is noteworthy that despite the lack of significant differences, slightly lower values of vitamin D were seen in patients who were females, smokers, alcohol drinkers, and in those aged ≥60 years and have difficulty in limb movement, but higher values were seen in those with <1-month duration of treatment. Calcium level was also slightly lower in those aged ≥60 years, in females, and in those with difficulty in limb movement, but higher in those with < 1-month duration of treatment. The aforementioned findings indicate that the inclusion of vitamin D supplementation may hold significance in future therapy approaches for hypocalcemia and its related complications. Furthermore, in the current study, it was found that factors such as age, gender, smoking behaviour, alcohol consumption, presence of osteomalacia symptoms, use of liver disease medication, and duration of treatment did not demonstrate a substantial impact on the calcium and vitamin D levels of individuals with hepatitis infections.

LIMITATIONS OF STUDY

The small sample size that was utilized in this investigation is one of its main shortcomings. This occurred because volunteers declined to participate in the hepatitis virus serological screening tests. The absence of statistical significance in vitamin D and calcium levels with respect to age, gender, smoking and alcohol consumption habits, osteomalacia symptoms, usage of liver disease medication, and length of treatment may have been explained by this. The population of the patients and the control group could not be matched, in a similar manner.

CONCLUSION

We found that hepatitis A, B, and C virus patients had lower serum 25-(OH)D and calcium levels than controls. The study population had 54.8% vitamin D deficiency and 11.9% hypocalcemia. Statistical analysis showed no correlation between calcium and vitamin D levels and age, gender, smoking, drinking, osteomalacia symptoms, liver disease medication use, or treatment length. The study's small patient sample may have caused this. Supplementing vitamin D may alleviate hypocalcemia and related issues in viral liver infection patients.

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AUTHOR CONTRIBUTIONS

The study was conceived and designed by EEB and BIGA. The project's execution and manuscript revision were aided by MI, EOA, SU, FOO, UD, ONA, and UIA. The manuscript was written and revised by all of the authors. The authors committed to taking responsibility for every part of the work, having reviewed and approved the completed document.

CONFLICT OF INTEREST

No conflicts of interest were reported.

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