Received: 26 October 2022 | Revised: 4 January 2023 | Accepted: 28 January 2023

DOI: 10.1002/hsr2.1101

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Serum 25-hydroxyvitamin D and hyaluronic acid levels as markers of fibrosis in patients with chronic liver disease at the main tertiary referral hospital in Ghana: A case-control study design

Bartholomew Dzudzor[1] | Harris Hammond[1] | Kenneth Tachi[2] | Anna Alisi[3] | Sandro Vento[4] | Richard Kwasi Gyasi[5] | Justice Moses K Aheto[6]

[1]Department of Medical Biochemistry, University of Ghana Medical School, Korle-Bu, College of Health Sciences, University of Ghana, Accra, Ghana

[2]Department of Medicine and Therapeutics, University of Ghana Medical School, Korle-Bu, College of Health Sciences, University of Ghana, Accra, Ghana

[3]Research Unit of Genetics of Complex Phenotypes, Area of Translational Pediatrics and Cinical Genetics, "Bambino Gesù" Children's Hospital IRCCS, Rome, Italy

[4]Faculty of Medicine, University of Puthisastra, Phnom Penh, Cambodia

[5]Department of Pathology, University of Ghana Medical School, Korle-Bu, College of Health Sciences, University of Ghana, Accra, Ghana

[6]Department of Biostatistics, School of Public Health, College of Health Sciences, University of Ghana, Accra, Ghana

Correspondence
Bartholomew Dzudzor, Department of Medical Biochemistry, University of Ghana Medical School, College of Health Sciences, University of Ghana, P. O. Box 4236, Korle Bu, Accra, Ghana.
Email: bdzudzor@ug.edu.gh and bartdzudzor7@gmail.com

Funding information
University of Ghana Research Fund, Grant/Award Number: URF/7/032/2013-2014

Abstract

Background and Aims: Liver fibrosis leading to chronic liver disease (CLD) is a major cause of morbidity, mortality and health-care expenditure worldwide. The "gold standard" for diagnosis and staging of liver fibrosis is histological analysis of liver tissue obtained by liver biopsy, an invasive procedure. Therefore, there is the need to identify noninvasive and inexpensive markers for diagnosis and staging of liver fibrosis. This study aimed at evaluating the correlation of hyaluronic acid (HA) and 25-hydroxyvitamin D (25-OH vitamin D) serum levels as markers of fibrosis with histologically staged and graded liver biopsies obtained from CLD patients.

Methods: This was a case-control study involving 40 CLD patients requiring liver biopsies and 40 controls. Liver biopsies were staged to determine the degree of fibrosis. Serum levels of 25-OH vitamin D and HA were determined using ELISA. Statistical analyses were performed to determine differences in HA and 25-OH vitamin D levels between controls and patients as well as to correlate the biomarkers with the stages of fibrosis.

Results: CLD patients showed significant ($p < 0.001$) increase in the levels of AST, ALT, GGT, compared to the controls. Patients also had significantly ($p < 0.001$) lower serum 25-OH vitamin D and higher HA ($p < 0.001$) levels compared to the controls. Additionally, 25-OH vitamin D levels of the CLD patients were significantly different across the stages of liver fibrosis likewise serum HA levels. Furthermore, 25-OH vitamin D levels inversely correlated with the severity of liver fibrosis. A significant negative correlation ($r = -0.33$, $p < 0.05$) between CLD patients' HA and 25-OH vitamin D were found.

Conclusion: CLD patients had significantly reduced serum 25-OH vitamin D and higher HA. Both markers correlated with the degree of liver fibrosis. These findings

Abbreviations: 25-OH vitamin D, 25- hydroxyvitamin D; CLD, chronic liver disease; HA, hyaluronic acid.

have major clinical translatable implication in the use of vitamin D supplementation in the management of CLD in Ghana.

KEYWORDS
25-OH vitamin D, CLD, fibrosis, HA, inflammation, markers

1 | BACKGROUND

Chronic liver diseases (CLD) cause significant impairment in patients' quality of life and are responsible for significant morbidity and economic burden.[1,2] Liver fibrosis occurs as a result of the formation of fibrous scars that results from the accumulation of extracellular matrix (ECM) proteins, largely type I and type III collagens, which replace damaged normal tissue.[3]

Histological examination of liver biopsies is the "gold standard" for assessing and staging liver fibrosis. However, the use of liver biopsies to monitor disease progression is limited by the fact that it is an invasive procedure, associated with potential for sampling error, high inter-observer variability and high costs. Therefore, a search for markers suitable for early diagnosis of fibrosis has been ongoing for decades.

The complex process of ECM remodeling during fibrogenesis involves different molecules, such as a polysaccharide known as hyaluronic acid (HA).[4] HA, which is synthesized mainly by the liver and plays an important role in the formation of the ECM, is considered a valid marker of fibrosis because its production and release levels increase during fibrogenesis.[5–7] Indeed, HA serum levels increase in correlation with fibrosis progression in alcoholic liver disease, peaking in patients with alcoholic cirrhosis.[8] Moreover, patients with nonalcoholic fatty liver disease have also significantly greater HA levels than normal individuals, and in these patients HA help to differentiate patients with fibrosis from those without fibrosis.[9,10]

Apart from its critical role in the control of calcium homeostasis and bone health, vitamin D is a steroid hormone with numerous additional functions, and it is involved in the modulation of the immune system, insulin secretion, cell proliferation and cell differentiation through the inhibition of matrix metalloproteinases.[11–13] Therefore, vitamin D has been evaluated for its possible role in CLD. Also, the pathway to synthesis of the active form of vitamin D (1, 25 $(OH)_2$ vitamin D) involves hydroxylation of vitamin D2 and D3 to 25-hydroxyvitamin D (25-OH vitamin D) in the liver. Even though the cut off level for serum 25-OH vitamin D deficiency is controversial, levels below 30 ng/mL are generally considered deficient or insufficient.[14] Vitamin D deficiency or insufficiency is found in patients with liver diseases, and nearly in all kinds of CLD.[15–17] Unsurprisingly, low serum levels of 25-OH vitamin D have been associated with the severity of liver fibrosis in chronic hepatitis C patients.[18–20]

Furthermore, a large number of studies showed that low circulating vitamin D levels were associated with the development of hepatic fibrosis in patients with various CLD, thus suggesting the use of vitamin D levels to follow the progression of liver fibrosis.[21]

The aim of this study was to investigate if serum levels of HA and 25-OH vitamin D could be good markers of liver fibrosis in patients with CLD at the Korle Bu Teaching Hospital (KBTH).

2 | METHODS

2.1 | Study site

The study site was the KBTH, located in the Accra Metropolitan Area in the Greater Accra Region of Ghana. It is a tertiary healthcare facility and a major referral hospital, serving patients from both Ghana and the West African Sub-Region. KBTH has a bed capacity of about 2000 and 17 clinical and diagnostic departments.

2.2 | CLD patients and controls characteristics

Forty patients with CLD were recruited from the Gastrointestinal Unit of KBTH between June 2014 and August 2015. Age- and gender-matched control subjects with normal alanine amino-transferase (ALT) and aspartate aminotransferase (AST) levels and no history, symptoms or signs of CLD were recruited from healthy blood donors. The diagnosis of CLD was based on standard clinical, ultrasound and laboratory features. Only patients who consented and required liver tissue as part of their evaluation within the period were recruited into the study. Patients with history of active drug addiction, Wilson's disease, hemochromatosis, alpha-1 anti-trypsin deficiency or treated with drugs known to affect serum 25-OH vitamin D metabolism, including vitamin/mineral supplements, were excluded from the study.

Body mass index (BMI) was calculated for each patient using the formula BMI = weight (kg)/height $(m)^2$. ALT, AST, and γ glutamyl-transferase (GGT) levels were measured by standard laboratory methods. Reference liver function enzymes were obtained from MDS-Lancet Laboratories Ltd (GH) Korle Bu.

2.3 | Sample collection, processing, and sample size

About 5 mL of whole blood samples were collected from the patients and matched controls by phlebotomist into clot activator and gel separating tubes, covered immediately with aluminum foil to avoid direct exposure to sunlight and placed on ice. Samples were allowed to clot for 30 min followed by centrifugation at 1500 rpm for 10 min.

The serum was pipetted into Eppendorf tubes in aliquots of 1 mL and stored at −80°C while recruitment went on.

Liver tissue was obtained by standard blind liver biopsy procedure on the same day the clinical and anthropometric data were obtained from the patients. Initial liver ultrasound, blood count and clotting profiles were carried out to exclude contraindications. The position of the liver was then located by percussion and the proposed biopsy site anesthetized with 10 mL of 5% xylocaine. A G18 semi-automatic biopsy needle was inserted through the site and the tissue obtained. The patients were monitored for 4 h after the procedure for complications.

In arriving at the sample size of 40 participants each for the CLD patients and healthy controls for the study, patients were recruited over the period June 2014 through to August 2015 from the Gastrointestinal Unit of the KBTH. The patients on treatment within the period who required liver biopsies and consented to participate in the study were consecutively recruited. The study employed one case to one control in the participants' recruitment process. Since there were no literature on similar study conducted in Ghana and sub-Saharan Africa before this study, appropriate parameters needed to conduct sample size estimation were not available. Thus, the study relied on the duration of 15 months (June 2014 to August 2015) to recruit available study participants who met the inclusion criteria.

2.4 | Measurement of HA serum levels

The serum HA was measured at the Research Unit of Molecular Genetics of Complex Phenotypes, "Bambino Gesù" Children's Hospital IRCCS, Rome, Italy and determined using an ELISA kit according to the manufacturer protocol (Hyaluronan; R&D Systems).

2.5 | Measurement of 25-OH vitamin D serum levels

The serum 25-OH vitamin D levels were also measured at the Research Unit of Molecular Genetics of Complex Phenotypes, "Bambino Gesù" Children's Hospital IRCCS, Rome, Italy, using Quidel MicroVue (San Diego, USA) serum 25-OH vitamin D enzyme immunosorbent assay (EIA) as described by the manufacturer's protocol. Briefly, 50 µL of serum samples were pipetted into the wells, 15 µL of assay buffer added followed by incubation for 2 h at 25°C while shaking at 300 rpm. Plates were washed three times (3x) with wash solution (TRIS-HCL with deionized water) and 200 µL of horse radish peroxidase conjugate solution added followed by incubation for 30 min while shaking at 300 rpm at 25°C. Plate was again washed 3x with wash solution. Hundred microliters (100 µL) of chromogenic solution (tetramethylbenzidene-TMB substrate) was added to the wells and incubated for 15 min at 25°C while shaking at 300 rpm in a hood. A total of 100 µL of stop solution (1 M HCl) was then added and absorbance read within an hour at a wavelength of 450 nm using a spectrophotometer.

A calibration curve was plotted and the total serum 25-OH vitamin D (D2 and D3) concentrations of the samples were determined by dose interpolation from the calibration curve.

2.6 | Histopathology

The histology slides were reported by a single pathologist (RKG), who was unaware of the patient's identity and demographic details. Biopsies were considered adequate for evaluation if the specimen had a minimum length of 15 mm or the presence of at least 10 complete portal tracts. Biopsies were reported according to the Batt-Ludwig scoring system[22]; the grade of inflammation was done using a 4-point scale; G0 to G3–4 (where G0 = portal inflammation only, no activity; G1 = minimal, G2 = mild, G3 = moderate and G4 = severe inflammation) and the stage of fibrosis, S0 to S3–4 (where S0 = no fibrosis; S1 = portal fibrosis; S2 = periportal fibrosis; S3 = Septal fibrosis and S4 = cirrhosis).[22]

2.7 | Ethics and confidentiality

The principles of the Declaration of Helsinki and its appendices, as well as national legislation, were followed during the conduct of the study. The University of Ghana College of Health Sciences, Ethical and Protocol Review Committee granted ethical approval, and all patients and control subjects provided written informed permission. All demographic patient data were given unique identifiers to protect confidentiality. These data sheets were coded and only the principal investigator knew the database containing the unique identifiers.

2.8 | Statistical analysis

We summarized the data by using mean, median and interquartile range for continuous variables while frequencies and their associated percentages were used to summarize categorical variables as appropriate. To assess the relationship of inflammation to the histological characteristics of patients, we used a 4-point scale G0 to G3–4 as the grade of inflammation and S0 to S3–4 as the stage of fibrosis from the mildest to severest, respectively. The study examined differences in HA and serum 25-OH vitamin D levels between cases and controls using the Wilcoxon rank-sum test because both the HA and serum vitamin D levels were not normally distributed. The significant differences and/or associations between cases and controls were compared using Kruskal–Wallis equality-of-populations rank test. This is appropriate because the data were not normally distributed, and we had four levels of stages to be compared. A Spearman's correlation analysis was used to determine correlation between CLD patients' serum 25-OH vitamin D and HA levels with the stage of fibrosis. All analyses were done using Stata version 16.1. A p value below 0.05 was used to declare statistical significance under a two-tailed test.

3 | RESULTS

3.1 | Characteristics of CLD patients and healthy controls

A total of 40 CLD patients and 40 healthy control participants were recruited for this study (Table 1). The mean age of the CLD patients was 43.5 ± 10.7 and that of the healthy controls was 43.5 ± 10.3; there was no significant difference in the ages between the two groups. The majority of the participants were male. We found significant differences in both HA ($p < 0.001$) and 25-OH vitamin D ($p < 0.001$) between CLD patients and healthy controls. There were significant differences in the anthropometric measurements (weight and BMI) between CLD patients and healthy controls. CLD patients showed a significant increase in the levels of AST ($p < 0.001$), ALT ($p < 0.001$), and GGT ($p < 0.001$) compared to the healthy control participants (Table 1).

3.2 | Histological characteristics of CLD patients

The etiology of CLDs and histological severity are shown in Table 2. The hepatitis B virus (HBV) infection was the most common cause of liver disease (29, 72.5%) among the patients studied. With regards to the grade of inflammation, the majority of the patients presented with G1 (16, 40%), followed by G0 (12, 30%), G2 (7, 17.5%), and G3–4 (5, 12.5%). The majority of the CLD patients fall within stage S0 of fibrosis (23, 57.5%) indicative of no observable fibrosis, followed by stage S1 (9, 22.5%) indicating portal fibrosis.

3.3 | HA and vitamin D levels between CLD patients and healthy controls

Next, serum levels of HA and 25-OH vitamin D were analysed in CLD and healthy controls. As shown in Table 3, HA and 25-OH D levels between CLD patients and healthy controls were significantly different. The median HA levels were significantly higher in CLD patients (363.8 ng/mL) than in control subjects (178.5 ng/mL) ($z = -4.446$, $p < 0.001$), with effect size $r = 0.50$. The median 25-OH vitamin D levels was also significantly lower in the CLD patients (62.4 ng/mL) than in the control subjects (96.6 ng/mL) ($z = 6.899$, $p < 0.001$) with effect size $r = 0.77$.

3.4 | HA and vitamin D levels across stages of fibrosis and grades of inflammation

Furthermore, to determine the differences between serum levels of HA and 25-OH vitamin D across the stages of fibrosis and grades of inflammation among CLD patients, a Kruskal–Walli's test was performed (Table 4). The result showed that the HA levels of the CLD patients were significantly different across the grade of

TABLE 1 Characteristics of chronic liver disease patients and healthy control participants.

Characteristic	CLD patients ($n = 40$)	Healthy controls ($n = 40$)	p value
Age (years)	43.5 ± 10.7	43.5 ± 10.3	0.96
Sex (M/F)	22, 18	22, 18	–
Weight (kg)	77.6 ± 12.2	69.3 ± 7.7	0.002
BMI (kg/m^2)	27.5 ± 4.5	24.8 + 2.9	0.004
AST (0–40 IU/L)*	49.7 ± 31.5	26.9 ± 5.2	<0.001
ALT (0–41 IU/L)*	47.4 ± 29.8	25.7 ± 4.2	<0.001
GGT (<55 IU/L)*	124.3 ± 207	37.7 ± 8.7	<0.001
HA (ng/mL)	363.8 (280.4)	178.5 (131.9)	<0.001
25-OH Vitamin D (ng/mL)	62.4 (13.4)	96.6 (29.9)	<0.001

Note: Data are expressed as frequencies, mean ± *SD*, median (interquartile range) as appropriate. All p values were calculated by Wilcoxon rank-sum test. Asterisk (*) in first column indicate standard reference values were obtained from MDS-Lancet Laboratories Ltd, Ghana.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CLD, chronic liver disease; GGT, γ glutamyl-transferase; HA, hyaluronic acid.

TABLE 2 Etiology, grade, and stage of liver disease.

Characteristic	Frequency (%)
Cause of fibrosis	
Autoimmune hepatitis	2 (5.0)
Drug induced hepatitis	1 (2.5)
HBV	29 (72.5)
Multiple liver masses	1 (2.5)
HCV	3 (7.5)
Lymphoproliferative disorder	1 (2.5)
Nonalcoholic steatohepatitis	1 (2.5)
Idiopathic-Unknown?	2 (5.0)
Grade of inflammation	
G0	12 (30)
G1	16 (40)
G2	7 (17.5)
G3–4	5 (12.5)
Stage of fibrosis	
S0	23 (57.5)
S1	9 (22.5)
S2	4 (10.0)
S3–4	4 (10.0)

Note: Data are expressed in numbers and percentages.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

TABLE 3 Differences between CLD patients and healthy controls for HA and 25-OH vitamin D serum levels ($n = 80$).

Variables	n	Rank sum	Expected	Variance	p value
HA					
Cases	40	2082	1620		
Controls	40	1158	1620	10,800	<0.001
25-OH vitamin D					
Cases	40	903	1620		
Controls	40	2337	1620	10,800	<0.001

Note: Wilcoxon rank-sum test was used to examine the significant differences between groups.
Abbreviation: HA, hyaluronic acid.

inflammation $\chi^2(df = 3,\ n = 40) = 18.005$, $p < 0.001$ and stage of fibrosis $\chi^2(df = 3,\ n = 40) = 8.92$, $p = 0.006$. However, the differences were not always consistent with the severity of fibrosis. Only the stage S2 to S3–4 of fibrosis corresponded to an average increase in the level of HA in agreement with what has been reported in literature. Additionally, 25-OH vitamin D levels were also significantly different across the grade of inflammation $\chi^2(df = 3,\ n = 40) = 9.989$, $(p = 0.002)$ and stage of fibrosis $\chi^2(df = 3,\ n = 40) = 17.461$, $(p < 0.001)$. Taken together, the data revealed a step-wise decrease in the level of 25-OH vitamin D values in patients from liver fibrosis stage S0 to S3–4 (619 ng/mL for stage S0, 127 ng/mL for S1, 48 ng/mL for S2 and 26 ng/mL for S3–4). A similar trend was observed for the grades of inflammation (Table 4). Patients with higher stages of fibrosis had lower serum 25-OH vitamin D. Collectively, the data on serum 25-OH vitamin D levels demonstrated that stages of fibrosis correlated consistently with the severity of liver fibrosis and inflammation.

A Spearman's correlation analysis indicated that there was a negative significant correlation between CLD patients' HA and 25-OH vitamin D levels, $r = -0.33$, $n = 40$, $p < 0.05$ (Table 5).

TABLE 4 Differences between HA and 25-OH vitamin D serum levels with regards to stage of fibrosis and grade of inflammation among CLD patients only ($n = 40$).

Variables	n	Rank sum	χ^2 value	p value
HA				
Grade of inflammation			18.005	0.001
G0	12	136		
G1	16	385		
G2	7	120		
G3–4	5	179		
25-OH vitamin D				
Grade of inflammation			9.989	0.019
G0	12	341		
G1	16	299		
G2	7	129		
G3–4	5	51		
HA				
Stage of fibrosis			8.92	0.006
S0	23	376		
S1	9	224		
S2	4	72		
S3–4	4	148		
25-OH vitamin D				
Stage of fibrosis			17.461	0.006
S0	23.	619		
S1	9	127		
S2	4	48		
S3–4	4	26		

Note: Wilcoxon rank-sum test was used to examine the significant differences between groups.
Abbreviation: HA, hyaluronic acid.

TABLE 5 Correlation between HA and 25-OH vitamin D serum levels of CLD patients.

Serum measure	Correlation		
	Median	HA	25-OH vitamin D
CLD patients			
HA	363.8	1	
25-OH vitamin D	62.4	−0.3278*	1

Note: Spearman's correlation.
Abbreviation: HA, hyaluronic acid.
*$p < 0.05$.

4 | DISCUSSION

4.1 | Principal findings

In the present study, we have reported the first evidence that correlates serum levels of 25-OH vitamin D and HA with liver fibrosis among CLD patients in Ghana and tropical Africa. The study sought to evaluate the levels of HA and 25-OH vitamin D in the serum of CLD patients to determine the possibility of using them as reliable, consistent, inexpensive and noninvasive markers for diagnosis and staging of liver fibrosis. The study also aimed at correlating the levels of these two markers to the severity of liver fibrosis among the CLD patients. This is very important because taking of histological biopsies for staging requires certain expertize, it's invasive and may lead to certain complication and its cost is not affordable to many Ghanaians. The major findings in this study revealed that CLD patients had a significantly lower serum 25-OH vitamin D and significantly higher HA levels compared to the healthy controls. Furthermore, 25-OH vitamin D levels of CLD patients were significantly different across

the stages of liver fibrosis and grades of inflammation, similar to the trend observed for HA levels.

4.2 | Interpretation

CLDs are disorders that manifest progressively and the histological staging of liver biopsies to determine the degree of fibrosis is the main method of assessing the prognosis of the disease. First, we determined the serum levels of ALT, AST, and GGT in the patients' samples and compared to those of the healthy controls. The patients had much greater average activity of these enzymes than the controls, as predicted. This indicates that the patient's liver was not functioning properly. Persistently elevated serum levels of ALT have been linked to stages of fibrosis and its pathways of progression.[23] This agreed with our results where the average ALT detected is much higher in the cases compared to the healthy controls. Our analysis found the predominant cause of liver abnormalities in our patients to be HBV infection, followed by hepatitis C virus infection. These findings are not surprising, given that HBV infection is common in Ghana, with a prevalence of 12.3%.[24]

In the present study, we observed significantly higher levels of HA in the patients compared to the healthy controls. This finding agrees with what has been extensively reported in the literature.[7–10,25] Furthermore, we observed an increased level of HA in CLD patients from stage S2 to S3–4, which is consistent with previous studies.[5] However, the level of HA decreased from grade S0 to S1, which is contrary to most previous data.[5–8] The inconsistency in the HA results presented in this study might be due to different methods employed in histological staging of the liver biopsies in the various studies. In the method by Batts and Ludwig, used in this study, S0 is an indication of normal tissue or no fibrosis and S1 denotes portal fibrosis only. Probably this staging is able to clearly distinguish fibrosis from periportal fibrosis (S2) to septal fibrosis (S3) and cirrhosis (S4). The disparity observed in this study may also be due to the different etiology of CLD in this study compared to previous ones.[26] The correlation of HA presented in this study is however not very different from what was found in a study in Pakistan carried out by,[27] where the authors observed that HA did not consistently differentiate minimal and significant liver disease in chronic hepatitis C patients.

The pathways to the synthesis of active vitamin D (1,25 $(OH)_2$ D) require hepatic hydroxylation of vitamin D2 and D3 to 25-OH vitamin D, therefore impaired liver function might result in low 25-OH vitamin D levels in the serum. Low level of vitamin D in patients with CLD could be caused by defective or decreased hydroxylation in the liver, impaired absorption from the intestine and decreased intake from the diet.[28] In the present study, we found that serum levels of 25-OH vitamin D are significantly lower in patients with CLDs compared to the healthy control subjects. Furthermore, we have observed an inverse relationship between 25-OH vitamin D levels and the stage of liver fibrosis. Low serum levels of 25-OH vitamin D could be due to limited exposure to the sun because of long hours spent in air-conditioned vehicles and offices. This could be very important in causing vitamin D deficiency among patients with end stage liver diseases, who might spend most of their time hospitalized. In our study, low serum levels of 25-OH vitamin D correlated with the severity of the histologically staged level of fibrosis and necroinflammation. As liver fibrosis becomes severe from stage S1 to S3–4 indicating portal fibrosis to septal fibrosis and cirrhosis, 25-OH vitamin D levels drastically reduces (Table 4). The inflammation data of the biopsies compared with the 25-OH vitamin D levels also showed a similar inverse relationship. Our findings are in agreement with several others reported in the literature from other geographical regions.[11–18,21] Literature search indicated a paucity of information with regards to serum 25-OH vitamin D levels and liver fibrosis in tropical Africa, probably because of the assumption that people are adequately exposed to sunlight and therefore, there would not be a deficiency of vitamin D. Furthermore, this study also showed that 25-OH vitamin D serum levels were consistently associated with the severity of liver fibrosis and inflammation compared to HA serum levels. Additionally, serum 25-OH vitamin D seems to be a more reliable and consistent marker of liver fibrosis compared to HA in our study. The averaged vitamin D level observed among patients with septal fibrosis and cirrhosis was 26 ng/mL, which fall within deficient or insufficient levels. In fact, deficient levels of 25-OH vitamin D have been linked to many CLD of different etiology.[15–20]

4.3 | Strengths of the study

Liver biopsies for histological staging were collected from clinically diagnosed CLD patients by a single gastroenterologist throughout the study. A single pathologist, who was unaware of the clinical history of the CLD patients, consistently performed the histological staging during the study using the same scoring system. Collectively, these measures reduces the prospect of staging bias and strengthen the objectivity and validity of the study. Furthermore, this study have a good potential for translational application in liver disease diagnosis, staging of liver biopsies noninvasively and inexpensively. The results of this study could also influence the management of CLD patients in Ghana by encouraging in this group of patients', supplementation with vitamin D.

4.4 | Limitation of the data

Some challenges were encountered in the design and implementation of the study because it was the first to be conducted in Ghana. Sample size could be a limitation that could be bypassed by performing the next study on a larger population. We did not pre-determine the nutritional parameters or used fasting blood of the participants before evaluating the 25-OH vitamin D levels. This could influence the level of serum vitamin D at the time of taking the samples but the healthy controls blood samples were also obtained similar to that of the CLD patients. Furthermore, samples were taken early in the morning to reduce this possible confounding challenge.

5 | CONCLUSION

The findings reported in our study have potential effects on the management of CLD patients in Ghana and Africa. CLD patients have significantly reduced level of 25-OH vitamin D compared to the control subjects. Also, severity of liver fibrosis inversely correlates with the level of 25-OH vitamin D. Large scale clinical trials can be potentially organized in Ghana or sub-Saharan Africa to determine the dose-dependent requirement of vitamin D supplementation among CLD. However, to actualize the clinical translatable application of our findings, additional studies are warranted in Africa using larger sample sizes. These further studies will help to support more evidence-based decisions that will be critical to direct healthcare policy formulation in diagnosis, staging of liver fibrosis and management of CLD.

AUTHOR CONTRIBUTIONS

Bartholomew Dzudzor: conceptualization; data curation; funding acquisition; investigation; methodology; project administration; supervision; visualization; writing—original draft; writing—review & editing. **Harris Hammond**: data curation; investigation; methodology; writing—original draft; writing—review & editing. **Kenneth Tachi**: conceptualization; data curation; funding acquisition; investigation; project administration; supervision; visualization; writing—review & editing. **Anna Alisi**: data curation; investigation; methodology; resources; writing—review & editing. **Sandro Vento**: conceptualization; formal analysis; methodology; validation; writing—review & editing. **Richard Kwasi Gyasi**: data curation; investigation; visualization; writing—review & editing. **Justice Moses K Aheto**: data curation; formal analysis; methodology; software; validation; visualization; writing—original draft; writing—review & editing. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Kenneth Hayibor for re-formatting the statistical analysis and all the participants for taking part in the study and making it a success. This study was partially supported by URF/7/032/2013-2014 University of Ghana Research Fund awarded to Bartholomew Dzudzor.

CONFLICT OF INTEREST STATEMENT

Justice Moses K. Aheto is an Editorial Board member of Health Science Reports and co-author of this article. He was excluded from editorial decision-making related to the acceptance of this article for publication in the journal. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available at https://figshare.com/articles/dataset/Fibrosis_data/21350088. Bartholomew Dzudzor had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author Bartholomew Dzudzor affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID

Bartholomew Dzudzor http://orcid.org/0000-0003-2325-7063
Justice Moses K Aheto http://orcid.org/0000-0003-1384-2461

REFERENCES

1. Ritchie H, Spooner F, Roser M. Causes of death. *Our World in Data*. 2018. Retrieved at https://ourworldindata.org/causes-of-death
2. Younossi ZM, Golabi P, Henry L. A comprehensive review of patient-reported outcomes in patients with chronic liver diseases. *J Clin Gastroenterol*. 2019;53:331-341.
3. Friedman SL. Liver fibrosis—from bench to bedside. *J Hepatol*. 2003;38:38-53.
4. Gressner OA. In the search of the magic bullet. *Gastroenterology*. 2010;139(5):1453-1457.
5. Chwist A, Hartleb M, Lekstan A, Kukla M, Gutkowski K, Kajor M. A composite model including visfatin, tissue polypeptide-specific antigen, hyaluronic acid, and hematological variables for the diagnosis of moderate-to-severe fibrosis in nonalcoholic fatty liver disease: a preliminary study. *Pol Arch Med Wewn*. 2014;124(12):704-712.
6. Dvorak K, Stritesky J, Petrtyl J, et al. Use of non-invasive parameters of non-alcoholic steatohepatitis and liver fibrosis in daily practice--an exploratory case-control study. *PLoS One*. 2014;9(10):e111551.
7. Guéchot J, Serfaty L, Bonnand AM, Chazouillères O, Poupon RE, Poupon R. Prognostic value of serum hyaluronan in patients with compensated HCV cirrhosis. *J Hepatol*. 2000;32(3):447-452.
8. Stickel F, Poeschl G, Schuppan D, et al. Serum hyaluronate correlates with histological progression in alcoholic liver disease. *Eur J Gastroenterol Hepatol*. 2003;15(9):945-950.
9. Fitzpatrick E, Mitry RR, Quaglia A, Hussain MJ, DeBruyne R, Dhawan A. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *J Pediatr Gastroenterol Nutr*. 2010;51(4):500-506.
10. Lebensztejn DM, Wierzbicka A, Socha P, et al. Cytokeratin-18 and hyaluronic acid levels predict liver fibrosis in children with non-alcoholic fatty liver disease. *Acta Biochim Pol*. 2011;58(4):563-566.
11. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol*. 2008;8(9):685-698.
12. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med*. 2004;229(11):1136-1142.
13. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev*. 2005;26(5):662-687.
14. Rosen CJ. Vitamin D insufficiency. *N Engl J Med*. 2011;364(3):248-254.
15. Fisher L, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. *Clin Gastroenterol Hepatol*. 2007;5(4):513-520.
16. Miroliaee A, Nasiri-Toosi M, Khalilzadeh O, Esteghamati A, Abdollahi A, Mazloumi M. Disturbances of parathyroid hormone-vitamin D axis in non-cholestatic chronic liver disease: a cross-sectional study. *Hepatol Int*. 2010;4(3):634-640.
17. Lim LY, Chalasani N. Vitamin D deficiency in patients with chronic liver disease and cirrhosis. *Curr Gastroenterol Rep*. 2012;4(1):67-73.

18. Petta S, Marchesini G, Caracausi L, et al. Industrial, not fruit fructose intake is associated with the severity of liver fibrosis in genotype 1 chronic hepatitis C patients. *J Hepatol*. 2013;59(6):1169-1176.
19. Luo Y, Wu X, Ling Z, Cheng Y, Yuan L, Xiang C. Association between serum vitamin D and severity of liver fibrosis in chronic hepatitis C patients: a systematic meta-analysis. *J Zhejiang Univ Sci B*. 2014; 15(10):900-906.
20. Gabr SA, Alghadir AH. Handgrip strength and vitamin D as predictors of liver fibrosis and malnutrition in chronic hepatitis C patients. *Dis Markers*. 2021;2021:1-13.
21. Udomsinprasert W, Jittikoon J. Vitamin D and liver fibrosis: molecular mechanisms and clinical studies. *Biomed Pharmacother*. 2019;109:1351-1360. doi:10.1016/j.biopha.2018.10.140
22. Batts KP, Ludwig J. An update on terminology and reporting. *Am J Surg Pathol*. 1995;19:1409-1417.
23. Hamid S, Umar M, Alam A, et al. Pakistan Society of Gastroenterology. PSG consensus statement on management of hepatitis C virus infection--2003. *J Pak Med Assoc*. 2004;54:146-150.
24. Ofori-Asenso R, Agyeman AA. Hepatitis B in Ghana: a systematic review & meta-analysis of prevalence studies (1995-2015). *BMC Infect Dis*. 2016;16:130. doi:10.1186/s12879-016-1467-5
25. Orasan OH, Ciulei G, Cozma A, Sava M, Dumitrascu DL. Hyaluronic acid as a biomarker of fibrosis in chronic liver diseases of different etiologies. *Medicine and Pharmacy Reports*. 2016; 89(1):24-31.
26. Younossi ZM, Stepanova M, Younossi Y, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. *Gut*. 2020;69(3):564-568. doi:10.1136/gutjnl-2019-318813
27. Arain SA, Meo SA, Jamal Q. Serum hyaluronic acid level does not reliably differentiate minimal and significant liver disease in chronic hepatitis C. *Saudi Med J*. 2011;32(12):1241-1245.
28. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3): 266-281.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dzudzor B, Hammond H, Tachi K, et al. Serum 25-hydroxyvitamin D and hyaluronic acid levels as markers of fibrosis in patients with chronic liver disease at the main tertiary referral hospital in Ghana: a case-control study design. *Health Sci Rep*. 2023;6:e1101. doi:10.1002/hsr2.1101