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Hypovitaminosis D and Hepatocellular Carcinoma in Patients with Liver Cirrhosis

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ABSTRACT

Background: The most frequent primary liver cancer in those with cirrhosis and chronic liver disease is hepatocellular carcinoma (HCC). Anti-infective and immune-modulating capabilities have been discovered for the multifunctional steroid hormone known as vitamin D. Vitamin D deficiency was found to be linked with advancement of Chronic liver disease (CLD) as non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), as well as the hepatitis C virus (HCV). **Objective:** The aim of the current work was to investigate the association between vitamin D deficiency and HCC in patients with liver cirrhosis. **Patients and Methods:** Ninety individuals with liver cirrhosis (LC) participated in this trial. The included subjects were divided into two groups; **Group A** consisted of 45 cirrhotic patients without HCC, and **Group B** consisted of 45 cirrhotic patients with HCC. **Results:** Liver function tests: INR, ALT, AST, total bilirubin were all statistically substantially higher in group B than in group A (P=0.001, 0.001, 0.001, and 0.011, respectively). However, group B's serum albumin and platelet count were considerably lower than group A's (P=0.003 and 0.001, respectively) compared to each other. In comparison to group A's alpha-fetoprotein (AFP) of 8.69±1.84, group B's AFP of 254.33±32.69 was statistically substantially higher (P = 0.001).Vitamin D levels in group B were substantially lower (19.33±4.68) than in group A (26.31±4.95) (P= 0.00). With an area under the curve (AUC) of 0.802, vitamin D was significant at a cutoff level of ≤ 20.5 ng/ml with a sensitivity of 80.3% and a specificity of 75% for increasing the risk of HCC. **Conclusion:** It could be concluded that it is crucial to maintain an optimum blood level of vitamin D in cirrhotic individuals since our findings indicate a substantial correlation between vitamin D levels and HCC risk.

Keywords: Cirrhosis; Hepatocellular Carcinoma; Vitamin D.

INTRODUCTION

People all over the world are affected by hepatocellular carcinoma (HCC). It comes in 2nd place in prevalence among mortality causes of cancer related diseases while it comes in 6th place as a factor predisposing to malignancy globally. This tumor is aggressive and has a bad prognosis, as seen by the discrepancy between its incidence and mortality rates. Development of HCC could be attributed to various predisposing factors including chronic viral hepatitis, non-alcoholic, and alcoholic fatty liver diseases, as well as other chronic inflammatory liver diseases [1].

Calcium homeostasis and bone metabolism, among other biological processes, are regulated by vitamin D receptors (VDR), that can be found in more than 30 tissues involving pituitary, brain, prostate, kidneys, intestine, parathyroid gland, skeletal and cardiac muscle, mammary glands, non-parenchymal liver endothelial cells, as well as the immune system[2].

Vitamin D is mostly obtained either from sun exposure or food sources. In the first step of activating vitamin D, the liver converts cholecalciferol to 25-hydroxyvitamin D (25-OHD). With a half-life of 15 to 21 days, the primary vitamin D circulating metabolite is related to the carrier protein; vitamin D binding protein (DBP) [3].

The kidney is where 25-OHD undergoes its second activation to 1,25-dihydroxyvitamin D, with the bone, parathyroid gland, monocytes, placenta, breast, as well as brain, being less involved. This metabolically active compound has a half-life of just ten to twenty hours. Consequently, evaluating 25-OHD levels in the blood is a common method of assessing vitamin D status[4].

Vitamin D deficiency was found to be linked with advancement of chronic liver diseases as non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), as well as the hepatitis C virus (HCV). New evidence suggests vitamin D has antimicrobial and immune-boosting properties [5].

As liver function declines, levels of 25-OHD, which is created as the first phase of vitamin D hydroxylation, decrease with time. At least one-third of those with chronic liver disease have a significant lack of vitamin D (25-OHD insufficiency), and studies suggest that up to 92% of those with the condition are vitamin D deficient [6].

Wu *et al.* [7] revealed that DBP as well as VDR play important functions in the development of HCC tumors and that people with chronic liver disorders who have polymorphisms in these genes have an increased chance of developing HCC in patients with CLDs. They have partially demonstrated vitamin D's anti-tumor properties. Thus, vitamin D and its analogues may provide new treatment targets and HCC prognostic biomarkers, which may be crucial for primary and secondary HCC prevention and the tracking of its development.

This research aims to investigate the association between vitamin D deficiency and HCC in liver cirrhosis.

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PATIENTS AND METHODS

This study included a total of 90 patients with liver cirrhosis, attending at Department of Internal Medicine, Benha University Hospital.

The included subjects were divided into two groups; **Group A** consisted of 45 cirrhotic patients without HCC, and **Group B** consisted of 45 cirrhotic patients with HCC. The American Association for the Study of Liver Diseases' recommendations served as the foundation for the HCC diagnosis [8].

Inclusion criteria: Patients ≥ 18 years with established liver cirrhosis as determined clinically, and by laboratory and ultrasonographic studies.

Exclusion criteria:

Patients with malignancies other than HCC, those with HCC receiving any type of treatment, those who had taken vitamin D supplements in the past two months, those who had a history of taking a wide range of medications that may affect vitamin D levels, such as antifungal, anticonvulsant, or glucocorticoid medications, and those who were vitamin D deficient due to other conditions like inflammatory bowel disease (IBD), chronic kidney disease (CKD), or malabsorptive disorders were excluded from this study.

Each patient underwent thorough history review, thorough clinical examination for signs of LC and biochemical tests, such as CBC, ALT, AST, serum albumin, PT, INR, AFP. Enzyme-linked immunosorbent test ((ELISA) used to evaluate serum 25-hydroxyvitamin D levels. The 25-hydroxyvitamin D levels were measured and categorized as sufficient (> 30 ng/ml), insufficient (20-30 ng/ml), or deficient (<20 ng/ml).

Abdominal ultrasonography and triphasic CT abdomen were done to detect radiological features of LC and diagnosis of HCC.

Using the Child Pugh score, the severity of the illness was evaluated [9]. **(Table 1)**

Table (1): Child-Pugh classification of severity of cirrhosis [9].

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/L)	2 to 3 mg/dL (34.2 to 51.3 micromol/L)	>3 mg/dL (>51.3 micromol/L)
Albumin	>3.5 g/dL (35 g/L)	2.8 to 3.5 g/dL (28 to 35 g/L)	<2.8 g/dL (<28 g/L)
Prothrombin time (seconds over control) or	<4	4 to 6	>6
INR	<1.7	1.7 to 2.3	>2.3
Encephalopathy	None	Grade 1 to 2	Grade 3 to 4

Total Child-Turcotte-Pugh scores of 5 to 6 indicate a state of well-compensated illness (Child-Pugh class A), whereas scores of 7 to 9 indicate substantial functional compromise (Child-Pugh class B), and scores of 10 to 15 indicate decompensated disease (Child-Pugh class C)[9].

Method and sampling: Blood was withdrawn, serum was separated and divided in two aliquots, one was stored at -20°C until vitamin D determination, and the other was used for other laboratory investigations.

***Ethical Consideration*:**

This study was ethically approved by Institutional Review Board of the Faculty of Medicine, Benha University. Written informed consent of all the participants was obtained. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Statistical Analysis

Using SPSS 22 for Windows (SPSS Inc., Chicago, Illinois, USA), all data were gathered, tabulated, and statistically evaluated. For communicating quantitative data, the mean and standard deviation were utilised (SD). To express qualitative data, frequency and percentage were utilised. The data were also summarised using the Chi-square test, linear correlation coefficient, and standard student "t" test. In order to evaluate different testing procedures and set threshold levels for test findings, the receiver operating characteristic (ROC) curve was created.

The Cantor technique was used to calculate the areas under ROC curves and their standard errors, compare them using the normal distribution, and account for correlation of data collected from the same instances. According to the area under a ROC curve (AUC) value, outstanding is defined as 0.90 to 1; good as 0.80-0.90; fair as 0.70-0.80; bad as 0.60-0.70; and failing as 0.50-0.6. The greatest accuracy point served as the ideal cutoff point. Every statistical comparison used a two-tailed significance test. P value < 0.05 was considered significant.

RESULTS

Age, sex, smoking habits, and body mass index (BMI) did not show statistically significant variations between the studied groups' demographic data **(Table 2).**

Table (2): Demographic data of studied patients

			Group A	Group B	t/ X^2	P
Age (years)	**Range**		**25 – 82**	**34 – 96**	**0.321**	**0.749**
	Mean ± SD		**60.42 ± 10.90**	**59.69 ± 10.77**		
BMI (kg/m^2)	**Range**		**23.8 – 32.4**	**23.1 – 33.2**	**1.218**	**0.226**
	Mean ± SD		**27.43 ± 2.04**	**26.80 ± 2.79**		
Gender	**Female**	**N**	**20**	**18**	**0.182**	**0.670**
		%	**44.4%**	**40.0%**		
	Male	**N**	**25**	**27**		
		%	**55.6%**	**60.0%**		
Smoking	**Not**	**N**	**31**	**22**		
		%	**68.9%**	**48.9%**		
	Smoker	**N**	**14**	**23**	**3.717**	**0.054**
		%	**31.1%**	**51.1%**		
Total		**N**	**45**	**45**		
		%	**100.0%**	**100.0%**		

Child-Pugh classification showed that 71.1% of group B patients were in class C compared with 31.1 % of group A patients in same class. **(Table 3)**

Table (3): Child-Pugh classification of the studied patients

			Group		X^2	P
			Group A	Group B		
Child-Pugh classification	**A**	**N**	**11**	**3**		
		%	**24.4%**	**6.7%**		
	B	**N**	**20**	**10**	**14.948**	**0.001****
		%	**44.4%**	**22.2%**		
	C	**N**	**14**	**32**		
		%	**31.1%**	**71.1%**		
Total		**N**	**45**	**45**		
		%	**100.0%**	**100.0%**		

INR, ALT, AST, PT, as well as Total bilirubin, were all higher in group B than in group A in this study, with statistically significant differences (P=0.001, 0.001, 0.001, 0.001 and 0.011, respectively). However, serum albumin and platelet count showed statistically significant differences (P=0.003 and P=0.001, respectively). **(Table 4)**

Table (4): Laboratory investigations of the studied patients

		Group A	Group B	T	P
Alb (g/dl)	**Mean ± SD**	**3.13 ± 0.78**	**2.68 ± 0.64**	**3.040**	**0.003***
Total Bilirubin (mg/dl)	**Mean ± SD**	**4.33 ± 1.01**	**5.52 ± 1.21**	**2.604**	**0.011***
ALT (U/L)	**Mean ± SD**	**46.67 ± 8.34**	**62.24 ± 8.79**	**8.619**	**0.001***
AST (U/L)	**Mean ± SD**	**76.00 ± 10.01**	**100.94 ± 8.55**	**12.707**	**0.001***
WBCs ($X10^9$/L)	**Mean ± SD**	**7.66 ± 1.86**	**7.95 ± 1.92**	**0.683**	**0.496**
Hb (g/dl)	**Mean ± SD**	**9.81 ± 1.41**	**10.06 ± 1.22**	**0.911**	**0.365**
PLT ($X10^9$/L)	**Mean ± SD**	**99.04 ± 10.41**	**70.58 ± 9.99**	**13.233**	**0.001***
PT (sec.)	**Mean ± SD**	**17.71 ± 2.33**	**22.36 ± 3.79**	**7.013**	**0.001***
INR	**Mean ± SD**	**1.48 ± 0.19**	**1.86 ± 0.32**	**7.013**	**0.001***

Comparison of serum AFP between the two studied groups: it was statistically higher in group B (254.33± 32.69) than in group A (8.69± 1.84); P-value was 0.001. **(Table 5)**

Table (5): Alpha-fetoprotein (AFP) distribution between studied patients

		Group A	Group B	t	P
AFP (ng/mL)	**Range**	**6 – 13**	**181 – 321**	**50.327**	**0.001***
	Mean ± SD	**8.69 ± 1.84**	**254.33 ± 32.69**		

Group B had a much significant lower vitamin D level (19.33± 4.68) than in group A (26.31±4.95); P-value was 0.00. Also, 29 patients of group B (64.6 %) were deficient in vitamin D versus 5 patients of group A (11.1 %); P=0.00. **(Table 6)**

Table (6): Vitamin D distribution between studied patients

			Group A	Group B	t/ X^2	P
VIT D (ng/ml)	**Range**		**17.8 – 41.3**	**10.5 – 33.3**	**5.615**	**0.00****
	Mean ± SD		**26.31 ± 4.95**	**19.35 ± 4.68**		
VIT D	**Normal**	**N**	**13**	**7**		**0.00****
		%	**28.9%**	**15.6%**		
	Insufficiency	**N**	**27**	**9**	**27.741**	
		%	**60.0%**	**20.0%**		
	Deficiency	**N**	**5**	**29**		
		%	**11.1%**	**64.4%**		
Total		**N**	**45**	**45**		
		%	**100.0%**	**100.0%**		

Correlation between vitamin D and serum AFP showed that vitamin D was negatively correlated with AFP (r = -0.876 & P-value =0.001). **(Table 7)**, **(Figure 1)**

Table (7): Correlation between Vit D level and AFP

	VIT D Level L	
	r.	**P**
AFP	**- 0.876**	**0.001***

Spearman's correlation was used, r: correlation coefficient.

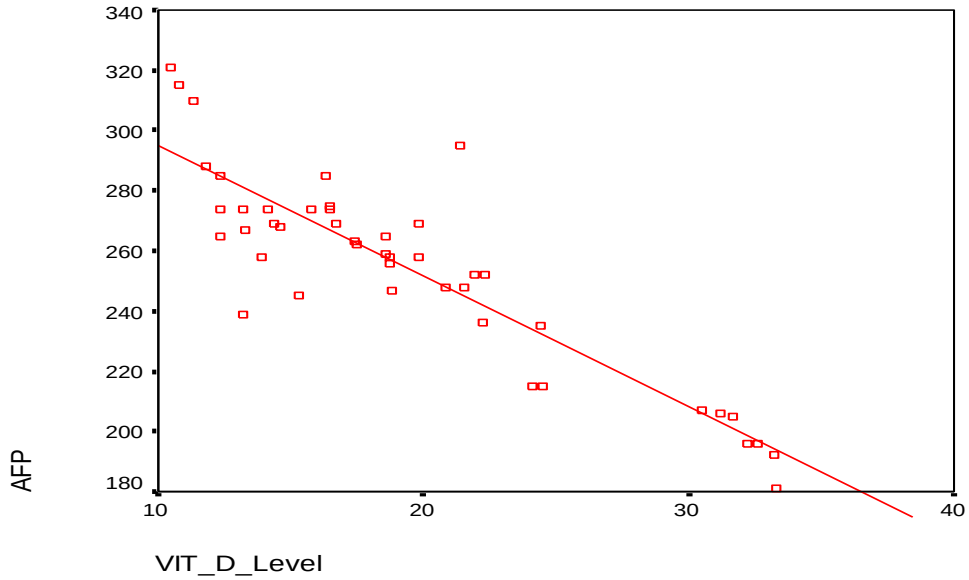

Figure (1): Negative significant correlation between Vit D level and AFP

On analyzing vitamin D and risk of HCC, it was significant at a cutoff level of ≤ 20.5 ng/ml with a sensitivity of 80.3% and 75% specificity for increasing the risk of HCC with an area under the curve (AUC) = 0.802). **(Table 8)**, **(Figure 2)**

Table (8): Vitamin D and the risk of HCC

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.802	≤20.5 ng/ml	0.00**	0.701	0.903	80.3%	75.0%

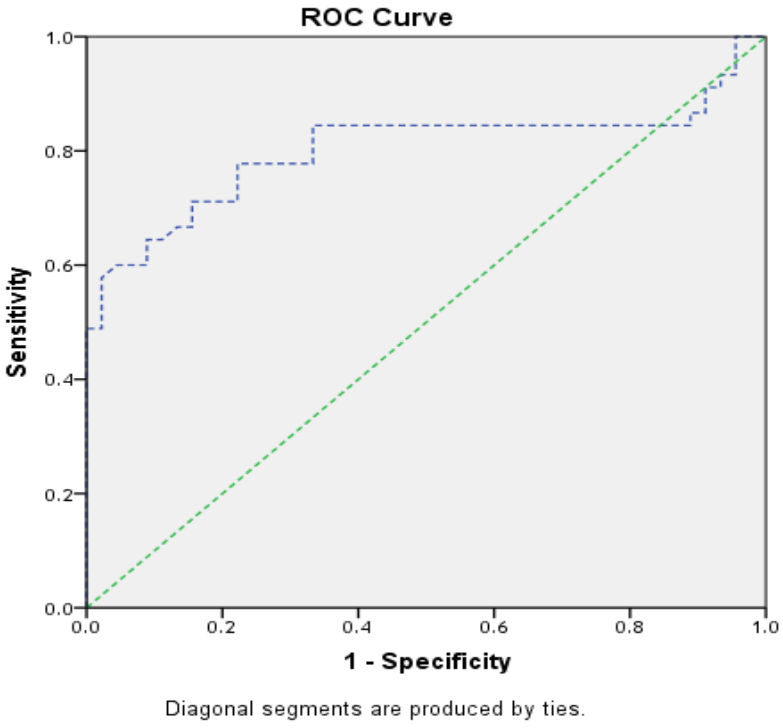

Figure (2): ROC analysis of 25-OH Vitamin D and risk of HCC

DISCUSSION

Hepatocellular carcinoma is a devastating consequence of liver cirrhosis (HCC). The annual chance of getting liver cancer for those with LC is between 1% to 8%, making it the sixth most prevalent malignancy diagnosed worldwide. Additionally, HCC is frequently detected at an advanced stage, which limits the range of available treatments [10].

The risk of hepatocellular carcinoma (HCC) in cirrhotic individuals is increased by a number of factors, including but not limited to co-infection with the human immunodeficiency virus (HIV), the hepatitis C virus (HCV), also the hepatitis B virus (HBV), as well as alcohol abuse, diabetes mellitus, steatosis, and obesity [11].

Interest in potential new factors that may be involved in the development of HCC has grown in response to evidence that some patients with chronic HCV infection may experience a de novo occurrence of HCC during or after treatment with new directly acting antiviral agents, despite these drugs' high efficacy in treating viral infection. Modern antiviral medications may efficiently remove the virus at extremely high rates with little side effects, in contrast to older antiviral therapy for HCV (interferon era)[12].

Vitamin D, a fat-soluble steroid hormone helps to maintain healthy calcium homeostasis and bone mineralization. Additionally, vitamin D modulates immunological response [13].

As liver damage progresses, levels of 25-OHD decline because the liver is where vitamin D is initially hydroxylated. Up to 92 percent of individuals with chronic liver illness have been shown to be vitamin D deficient, and at least one-third of them had severe 25-OHD deficiency. Patients with cirrhosis are more likely to have 25-OHD deficit than patients without cirrhosis, and it is more frequently connected with rising Child-Pugh classification than disease cause. Higher mortality in individuals with cirrhosis is directly linked to 25-OHD insufficiency and increased all-cause mortality [14].

Data on the connection between hypovitaminosis D and the incidence of HCC are currently few. This study's primary goal was to analyse the association between hypovitaminosis D assessed by 25-OHD measurement and HCC in patients with LC.

The results of our study showed that: regarding Child-Pugh classification, 31.1% of group A patients were Child-Pugh class C, however, 71.1% of group B patients were child-Pugh class C. **The study by Buonomo *et al.*[15]** investigated the mortality rates between those with and without hepatocellular carcinoma in a group of patients with liver cirrhosis to see whether there was a correlation between vitamin D insufficiency and the risk of dying from the HCC disease. Patients with Child-Pugh cirrhosis classes B or C were at a higher risk of developing HCC than those with cirrhosis of Child-Pugh class A. The total bilirubin, ALT, AST, PT, and INR values were all significantly higher in group B compared to group A (P 0.011, 0.001, 0.001, 0.001 and 0.001, respectively). However, there was a statistically significant difference between group B and group A in terms of serum albumin and platelet count (P = 0.003, 0.001, respectively). These results were consistent with those of **Elnakeeb *et al.*** [16] who reported that there were highly significant difference in serum levels of AST, ALT, Albumin, Bilirubin, PT and Platelet count with increased severity of liver function tests in HCC group more than other groups of the study (HCV cirrhotic patients without HCC and control group of apparently healthy subjects with no past history of HCC or HCV cirrhosis).

In this study, AFP was significantly higher in group B (254.33± 32.69) than in group A (8.69± 1.84) (P= 0.001), this was in agreement with **Liu *et al.*** [17] who stated that AFP levels significantly differed in patients with HCC (mean 250.65 ng/ml) than patients with liver cirrhosis (mean 2.32ng/ml) and p value <0.001. Regarding vitamin D levels between the two studied groups, it was significantly lower in group B (19.33± 4.68) than in group A (26.31±4.95) (P= 0.00). Also, 29 patients of group B (64.6 %) were deficient in vitamin D versus 5 patients of group A (11.1 %) (P=0.00). This was in line with the findings of **Buonomo *et al.*[15],** who discovered that individuals with HCC had considerably lower median blood vitamin D concentrations than those without the disease. The median 25-hydroxyvitamin D level at the start of that study was 15 ng/ml.

The research found that just 52 out of 345 people (15%) had adequate levels of vitamin D. Independent of the existence of HCC, they showed that low vitamin D levels were related with poor survival in patients with liver cirrhosis. **Wu *et al.*** [18] found that there were numerous strategies to address the likely factors for the current incidence of 25-OHD deficit in HCC patients. Firstly off, less outside activities, limited sun exposure, and decreased oral intake all contribute to insufficient vitamin D production in vivo. Secondly, because most HCC patients have substantially impaired liver function, there may be issues with vitamin D metabolism and absorption.

On the other hand, if the liver's 25-hydroxylase activity is reduced, vitamin D conversion to 25-OHD after binding to alpha globulin in the blood is disturbed. The current study's ROC analysis reveals that a higher risk of HCC may be associated with a lower serum 25-OHD level at a cutoff of 20.5, with a sensitivity of 80.3% and a specificity of 75% and a substantial AUC.

Our results were supported by study of **Yi *et al.*** [19] as they supported the idea that maintaining adequate blood vitamin D levels might help prevent liver cancer since vitamin D deficiency is thought to

increase the risk of liver cancer. **Kubesch *et al.* [20]** revealed that, the best threshold for predicting the likelihood of hepatic decompensation in patients with liver cirrhosis is 8.5 ng/mL of 25-OHD blood concentration, according to a study examining the correlation between vitamin D serum levels, systemic inflammation, and hepatic decompensation, they discovered a potentially functionally relevant connection between severe vitamin D deficiency, systemic inflammation, and the likelihood of hepatic decompensation in individuals with advanced liver cirrhosis. Finally, to evaluate the association between circulating vitamin D levels in the blood and the risk of liver cancer, additional international, population-based research is needed to address the heterogenicity problem.

Limitation of the study: The number of patients was small.

CONCLUSION

It could be concluded that vitamin D levels and HCC risk are significantly correlated, it is crucial for cirrhotic patients to maintain a healthy level of vitamin D in their serum.

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Author contribution: Authors contributed equally in the study.

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