Serum levels of Vitamin D3, Zinc, and Parathyroid Hormone in HCV-induced Hepatocellular Carcinoma.

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Abstract

Background and Aim

The role of the metabolic syndrome in hepatocellular carcinoma (HCC) has been previously reported. This study aims to investigate the possible role of vitamin D3, Zinc, Parathyroid hormone (PTH), calcium and phosphorus serum levels as non-traditional metabolic risk factors in HCV-related HCC.

Method

This cross-sectional observational study recruited HCV infected patients with and without HCC. All patients were subjected to demographic, biochemical, and hematological assessment. Serum levels of vitamin D3, Zinc, PTH, calcium, and phosphorus were determined in all the study participants.

Results

This study includes 50 patients with HCV-related HCC compared to 40 patients with HCV-related liver cirrhosis and 30 patients with HCV chronic hepatitis C (CHC) without HCC. Our results show significantly higher age, male sex, aspartate transaminase (AST), PTH and corrected serum calcium levels in the HCC patients compared to values in the other two groups, ($p < 0.001$); while significant lower vitamin D3 and zinc levels were detected among the HCC patients compared to patients with non-HCC liver cirrhosis and CHC, ($p < 0.001$).Vitamin D3 deficiency was detected in 96% of the HCC patients, while it was detected in only 22.5% of the cirrhotic patients and in none of the CHC patients, ($p < 0.001$). However, on multiple stepwise regression analysis, only the age, AST, PTH, and corrected calcium levels were the independent predictors for HCC when studied in relation to chronic liver disease.

Conclusion

This study indicates the prevalent deficient levels of vitamin D3 and zinc in HCC patients; however, a causal relationship is not established in this study.

Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer and third cause of death due to cancer universal.[1,2] A variety of risk factors for the development of HCC have been clearly identified. Hepatitis B and C viral infections are the main HCC risk factors.[3] In addition, dietary aflatoxin exposure, alcoholic and non-alcoholic cirrhosis are considered risk factors of HCC.[4] Metabolic syndrome (MS) associated with insulin resistance is recognized as a potential risk factor for the development of hepatocellular carcinoma.[5]

The liver plays an important role in the metabolism of vitamin D which is hydroxylated in the liver into 25-hydroxyvitamin D (Vitamin D3) and then transported to the kidney to undergo a second hydroxylation, to form 1, 25(OH) Vitamin D.[6] Vitamin D3 is particularly known for its function in calcium homeostasis and

bone mineralization.[7] A lot of evidence proposes that vitamin D3 has many other functions as anti-proliferative, pro-apoptotic, differentiating, anti-angiogenic and anti-invasive roles in malignancy.[8]

The liver plays a major role in maintaining systemic Zinc homeostasis. Zinc metabolism occurs mainly in the liver; hence, Zinc is affected by liver diseases. Furthermore, Zinc has a role in cellular metabolism as cell division, growth and differentiation.[9] Zinc deficiency was reported to affect hepatocyte functions and immune responses in inflammatory liver diseases.[10]

PTH secretion is regulated by means of vitamin D and calcium through the vitamin D receptor and calcium-sensing receptor, respectively. The negative association between serum 25(OH) D and serum PTH is a documented physiologic phenomenon.[11]

This study aims to investigate the status of vitamin D, Zinc, PTH, calcium and phosphorus serum levels as possible non-traditional metabolic risk factors in HCV-induced HCC.

Patients And Methods

Patients: This cross-sectional observational study recruited HCV-induced cirrhotic patients with and without evident HCC through the period from May 2019 to April 2020. At the same time, a group of chronic hepatitis C (CHC) patients without cirrhosis were included in the study. Diagnosis of HCC was based on the current diagnostic guidelines for HCC.[12] Cirrhosis and chronic hepatitis were diagnosed by clinical examination, laboratory tests, ultrasonography, and Fib-4 test (F0-F3 ≤3.25 and F4> 3.25).[13,14] Exclusion criteria includes patients with HCC and/or cirrhosis with etiology other than HCV infection, patients who were receiving vitamin D or calcium supplements for 6 months before the start of the study, patients with cholestasis, and patients with chronic renal insufficiency.

Ethics Related Statement: The study protocol was approved by the Institutional Research Board at the Faculty of Medicine, Minia University, Egypt. Informed consent was achieved from all subjects contributed in our study. This research was performed in agreement with the guidelines of 1975 Declaration of Helsinki.

Clinical and Laboratory Assessment:

All patients were subjected to physical examination and laboratory investigations including complete blood count (CBC) which was determined by automated cell counter, in addition to Sysmex KX-21N (TAO Medical Incorporation, Japan), prothrombin time concentration and International Normalized Ratio for prothrombin activity (INR) which were done using fully automated coulometer STAGO (Diagnostic STAGO– France). Viral markers (HCV Abs, HBs Ag, HIV Abs) were tested by fully automated ChemiLuminescence technology (Cobas E 411-Roche-Roche Diagnostics GmbH Germany). Liver and renal biochemical profiles and serum calcium levels were done by auto-analyser: Konelab i60 (Thermo-electro, Clinical chemistry automation systems, Finland). Serum alfa-fetoprotein levels were done by auto-analyser: Konelab i60 (Thermo-electro, Clinical chemistry automation systems, Finland). Serum zinc level

was measured using kits supplied by Abcam (ab102507, USA). Serum phosphorus level was measured by kit supplied by Abcam (ab65622, USA). The patients were classified according to serum Zinc levels in patients with zinc deficiency (< 70 µg/dl), patients with normal zinc levels ≥ 70 µg/dl).[15] Finally both Parathyroid hormone (PTH) and Serum 25-OH vitamin D3 levels were measured by mini Vida an automation using the ELFA (Enzyme Linked Fluorescent Assay) technique (Mini Vidas, bioMerieux, France). According to serum 25-OH vitamin D3 levels, all patients were classified into patients with vitamin D3 deficiency (< 20 ng/dl), patients with vitamin D3 insufficiency (20-30 ng/dl), and patients with vitamin D3 sufficiency (> 30 ng/ml).[16]

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using SPSS program software version 24. Test of normality (Kolmogorov-Smirnov) was done to determine the distribution of the quantitative data. Descriptive statistics were done for parametric quantitative data by mean, standard deviation, and minimum & maximum of the range; while the nonparametric quantitative data was presented by the range and median. The categorical data was presented as number and percentage. An independent sample T test was used for analyzing the parametric quantitative data of the two groups, and Mann Whitney test was used for the non-parametric quantitative data. Chi-Square test was used for analyzing the qualitative data. Simple regression analysis was done to determine the possible predictive factors for HCC. Multiple logistic and stepwise regressions were done to detect the independent predictors for HCC. ROC curve study was done to determine the cutoff point, AUC, sensitivity, specificity, PPV, NPV and accuracy for the significant independent predictors for HCC. The level of significance was considered when P value was < 0.05.

Results

This study includes 50 HCV-related cirrhotic patients with HCC (Group 1), 40 HCV cirrhotic patients without HCC (Group 2) and 30 HCV chronic hepatitis (CHC) patients without cirrhosis (Group 3). Table (1) presents the statistical significance of the baseline demographic and clinical characteristics of the studied groups. The data indicates that the cirrhotic groups with and without HCC have significantly higher age compared to the CHC patients, ($p < 0.001$), while there is no significant difference in age between the cirrhotic and HCC groups, ($p = 0.729$). There is statistically higher male distribution in the HCC group (68%) compared to the non-HCC cirrhotic group (45%; p value = 0.028). However, both the CTP and MELD scores are not significantly different between the HCC and the non-HCC cirrhotic patients.

Regarding the laboratory data, the results show that the total leucocytes count (TLC), serum albumin, ALT and AST have higher significant values in the HCC group compared to the cirrhotic group without HCC, Table (2).

Table (3) shows that vitamin D3 has significantly lower level in the HCC group than in the non-HCC cirrhotic and CHC groups (values of: 14.7 ± 8.4ng/ml, 28.5 ± 11.3 ng/ml, and 29.3 ± 6.2 ng/ml for the three

groups, respectively, with p = < 0.001). However, there is no significant difference in vitamin D levels between the non-HCC cirrhotic and CHC groups, (p = < 0.605). According to the guideline classification for the cutoff values for deficient and insufficient levels of vitamin D3[16], vitamin D3 deficiency was detected in 96% of our HCC patients while it was detected in only 22.5% of the cirrhotic patients and in none of the CHC patients, p < 0.001, Table (4). At the same time, zinc level in the HCC group has significantly lower level than in the non-HCC cirrhotic (p < 0.02) and in the CHC patients (p < 0.001), Table (3). Zinc levels are stratified according to the recommended cutoff value (patients with normal zinc levels ≥ 70 µg/dl).[19] Zinc is found to be deficient in 96% of the cases with HCC while this is observed in 75% of the cirrhotic patients without HCC, and in only 16.7% of the CHC patients, p < 0.001, Table (5). PTH has significantly higher levels in the HCC group than in the non-HCC cirrhotic group (p < 0.013), and the levels in the non-HCC cirrhotic group are significantly higher than in the CHC group (p < 0.001), Table (3). The corrected calcium has significantly higher level in the HCC group than the levels in the non-HCC cirrhotic and CHC groups; (p < 0.001), Table (3). The ionized calcium levels present the same statistical significance in the studied groups. There is no statistically significant difference between the three groups as regards serum phosphorus level, Table (3).

Utilizing multiple stepwise logistic regression analysis for the prediction of HCC when chronic liver disease (CLD) (the cirrhotic and CHC patients) is the reference, the corrected calcium, the PTH, the AST and the age are the independent predictors for HCC. The OR for corrected calcium is 3.18; 95%CI: 1.96–5.14 with p = < 0.001; the OR for PTH is 1.01; 95%CI: 1.004–1.02 with p = 0.002; the OR for AST is 1.03; 95%CI: 1.01–1.05 with p = 0.002 and the OR for age is 1.11; 95%CI: 1.02–1.21 with p = 0.014 in the prediction of HCC, Table (6).

Table (7) and Figure (1) show ROC curve analysis and accuracy indices for the prediction of HCC when chronic liver disease is the reference group. With optimal cut off points of > 100 ng/L for PTH; >9.63 mg/dl for corrected calcium, > 58 years for age, and > 50 U/L for AST; these variables have AUC values of 0.800, 0.790, 0.739 and 0.686, respectively, with P values of < 0.001 for all of them. Corrected calcium has the highest total accuracy of 85.6 %.in the prediction of HCC development on top of chronic liver disease.

Discussion

Our study aims to investigate the status of some non-traditional metabolic variables in relation to HCC in HCV-induced cirrhotic patients. We estimated the serum levels of vitamin D3, Zinc, parathyroid hormone (PTH), calcium, and phosphate in HCV patients with and without HCC in a trial to study their possible predictive or risk abilities in the development of HCC.

In our study, in the HCC patients, the male distribution is twice more frequent than in females (68% vs 32%); with significantly higher male distribution in the HCC group than in the cirrhotic one (68% vs 45%, p < 0.028). This reflects the male predominance in HCC as has been reported by Hammad et al.[17], who stated that HCC was significantly more frequent in males than in females (77.7% and 22.3% respectively).

However, in our study, on multiple logistic regression analysis, the sex distribution is not a significant predictor for the occurrence of HCC.

The reasons for this gender disparity could be explained by differences in exposure to risk factors. Furthermore, estrogen is believed to have a protective role against the development of HCC as differences in subtypes of estrogen receptors expressed in males vs. females have been shown to contribute to the progression of HCV related HCC.[18]

Our data also show that HCC is more common in older age patients; albeit with no significant difference of age between the HCC and non-HCC cirrhotic patients. The age is significantly higher in the HCC and the cirrhotic than in the CHC patients, ($p < 0.001$). On multiple stepwise logistic regression analysis in relation to chronic liver disease rather than to cirrhosis, older age is found to be significantly associated with HCC (OR: 1.11; 95% CI; 1.02–1.2; $P < 0.014$). El Zayadi et al.[19] have reported that HCC in Egypt is significantly more prevalent among older age groups than younger age groups and they have suggested that HCV infection in old patients induces a rapid progression to HCC independent of HCV genotype. Omata et al.[20] have reported that older age is a risk factor for HCC, especially in areas where HCV infection is endemic as in Egypt.

Furthermore, our results show that both the AST and ALT levels are significantly higher in the HCC patients when compared to the non- HCC cirrhotic and CHC patients. The raised AST is significantly associated with HCC (OR: 1.03; 95% CI; 1.01–1.05). At a cut off value of > 50 U/L, AST has sensitivity 76% and specificity 65.71%. This finding is supported by large cohort study that has been done on 1108 patients with HCC by Carr and Guerra[21], who have found an association between increasing levels of liver enzymes (AST, ALP and GGT) and HCC aggressiveness.

Our data present significantly lower levels of vitamin D3 in the HCC group compared to the cirrhotic and the CHC groups. Furthermore, vitamin D3 deficiency is significantly more frequent in patients with HCC (96%) compared to the cirrhotic and chronic hepatitis patients, ($p < 0.001$). These results are similar to those of a study done by Finkelmeier et al.[22], who have measured vitamin D3 in 200 patients with HCC and cirrhosis and have compared its level to the stages of both HCC and stages of cirrhosis based on MELD, CTP, BCLC, and The Cancer of the Liver Italian Program (CLIP) scores. In their study, vitamin D3 levels are negatively correlated with the stages of cirrhosis as well as stages of HCC. Furthermore, their patients with severe vitamin D3 deficiency had the highest mortality risk. Our study indicates that the mean vitamin D3 levels are not significantly different between the cirrhotic and the CHC patients. However, vitamin D deficiency is more frequent in the cirrhotic than in the CHC groups (22.5% vs zero%, respectively; $p = 0.004$). This later finding is in agreement with Duarte et al.[23], who have studied vitamin D3 level in 100 patients with chronic viral hepatitis, (49 noncirrhotic and 51 with cirrhosis), where they have found that vitamin D3 was low in only 3 cirrhotic patients without significant difference between the cirrhotic and the non-cirrhotic groups. However, Miroliaee et al.[24] have reported that vitamin D deficiency is significantly more frequent in cirrhotic patients compared to non-cirrhotic patients (76.5 vs.17.9%; $p = 0.001$).

Vitamin D3 and its derivatives have immune, neuroendocrine activities, anti-carcinogenic properties.[25,26] Recently, Diaz et al.[27] have reported the ability of vitamin D3 to enhance the anti-tumor activity of chemotherapeutic drugs by activating apoptosis. However, a causal relationship has remained mostly unclear because most of the studies were small or concentrated on the assessment of vitamin D3 serum levels at the date of HCC occurrence which may result in false statistical associations due to the influence of impaired liver function on circulating vitamin D3. Another study done by Caputo et al.[28] has reported an inhibitory effect of vitamin D3 on the growth of the human liver cancer cell lines which express functional receptors able to specifically bind vitamin D3.

Although our results indicate significant very low levels of vitamin D3 in HCC patients with more significant frequency of its deficiency, yet on multiple stepwise logistic regression analysis, vitamin D3 is not a significant independent predictor for HCC. It is not clear whether this reflects a type two statistical error due to the small sample size or this deficiency may reflect a functional synthetic error for vitamin D3 by the HCC diseased liver rather than a risk factor for HCC development.

We also investigated serum Zinc levels in our study groups. Our results show that Zinc level is significantly lower in patients with hepatocellular carcinoma compared to levels in liver cirrhosis and CHC patients This finding is in agreement with results reported by other studies.[29,30] HCC malignancy is ZIP14-deficient tumor, so with Zinc deficiency, the abolished cytotoxic effects of Zinc on malignant cells cannot be ruled out and low Zinc levels may predispose patients to HCC development. At the same time, the malignant cells derive adaptation mechanisms through which they lower down the concentration of zinc to avoid its cytotoxic effects on them at normal zinc levels[30]. A recent study has reported data about the protective role of long-term Zinc supplementation against development of HCC.[31] However, again, in spite of the significant low levels of zinc in our HCC patients, yet on multiple stepwise regression analysis, zinc is not a significant predictor for HCC.

Our results indicate a significant higher level of serum PTH in patients with HCC compared to its levels in the cirrhotic and the CHC patients. In addition, the serum PTH significantly increases in patients with liver cirrhosis in comparison to CHC patients. When multiple stepwise logistic regression analysis was done in relation to CLD rather than cirrhosis, PTH, age, AST and corrected calcium levels were significantly associated with HCC, Table (6). PTH is considered a predictor for HCC (OR: 1.11; 95% CI; 1.004–1.02; $P < 0.014$) at cut off value of > 100 ng/l with sensitivity 84% and specificity 74.29%.

On the other hand, our study shows statistically significant higher level of corrected calcium in patients with hepatocellular carcinoma compared to levels in liver cirrhosis and CHC patients. Our data show also that the corrected calcium is one of HCC independent predictive factors (OR: 4.0; 95%CI: 2.3–6.9; $p < 0.001$). Corrected calcium at a cut-off value of (> 9.63) had sensitivity 82% and specificity 75.71% in the prediction of HCC in relation to chronic liver disease. Hypercalcemia associated with malignant disease is not uncommon. This is ascribed to both bony metastatic lesions and the production of parathyroid hormone-related protein (PTHrP) from the malignant cells.[32] Two case reports have described cases with hypercalcemia that is caused by HCC secreting intact parathyroid hormone (iPTH).[33,34] When effective,

Trans Arterial Chemoembolization (TACE) against the HCC results in stepping down the serum iPTH level and calcium to within the normal range, suggesting a correlation between the carcinoma and the iPTH.[35]

Conclusion

This study indicates the prevalent deficient levels of vitamin D3 and Zinc in HCV-induced HCC; however, a causal relationship is not established. This is associated with significantly higher levels of corrected calcium and PTH serum levels. It is not clear whether these changes reflect a mere significant association or otherwise these metabolic parameters may have a risk for development of HCC.

Limitations Of The Study

The small sample size of HCC group of patients and the cross sectional nature of the study are limitations that do not enable us to conclude if vitamin D3 and Zinc are hazard risk factors for development of HCC in HCV infected patients or their deficient levels are just an association.

Abbreviations

HCC: hepatocellular carcinoma**, PTH:** Parathyroid hormone, **HCV:** hepatitis c virus**, CHC:** chronic hepatitis c, **AST:** aspartate transaminase**, MS:** Metabolic syndrome**, 1, 25(OH) Vitamin D:** 1, 25-hydroxyvitamin D**, CBC:** complete blood count**, INR:** prothrombin activity, **HCV Abs:** hepatitis c virus antibodies, **HBs Ag:** hepatitis B virus antigen, **HIV Abs:** human immunodeficiency virus antibodies, **AUC**: area under the curve, **PPV**: positive predictive value, **NPV:** negative predictive value**, CTP:** Child-Turcotte-Pugh**, MELD**: Model for End-Stage Liver Disease, **TLC:** total leucocytes count, **CLD:** chronic liver disease, **ROC curve**: A receiver operating characteristic curve, **ALP**: Alkaline Phosphatase, **GGT**: Gamma Glutamyltransferase, **BCLC:** The Barcelona-Clınic Liver Cancer , **CLIP:** The Cancer of the Liver Italian Program, **ZIP14:** Zinc-regulated protein14, **PTHrP**: parathyroid hormone-related protein, **iPTH**: intact parathyroid hormone: TACE: transarterial chemoembolization

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Research Board at the Faculty of Medicine, Minia University, Egypt. Informed consent was achieved from all subjects contributed in our study. This research was performed in agreement with the guidelines of 1975 Declaration of Helsinki.Consent for publication

Consent for publication “Not applicable”.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest: All authors declare that they have no competing interest**.**

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Authors' contributions: Mahmoud Khattab gave the concept of the study and the study design, Elham Ahmed shared in study design, analyzed the data and submitted of the manuscript, Magdy Fouad shared analyzed the data and interpretation of the data, Mohammad Omar Abdelaziz, Arwa Mohamad, Ragaa Abd- Elshaheed Matta and Muhammed Khattab shared in recruitment of the patients, Hend M. Moness performed the laboratory investigations of the study the study; Nashwa Mohamed Adel performed the laboratory investigations of the study.

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Tables

Table (1): Comparison of demographic and clinical data between the study subjects

	Group I HCC N = 50	Group II LC N = 40	Group III Chronic hepatits N = 30	P value		
Age (years) Range Mean ± SD	(45–85) 61.5 ± 7.3	(47–75) 60.8 ± 6	(45–60) 51.3 ± 3.9	< 0.001*		
				I vs II	I vs III	II vs III
				0.729	< 0.001*	< 0.001*
Sex Male Female	34(68%) 16(32%)	18(45%) 22(55%)	16(53.3%) 14(46.7%)	0.083		
				I vs II	I vs III	II vs III
				0.028*	0.190	0.490
Varices No Yes	6(12%) 44(88%)	0(0%) 40(100%)	0(0%) 30(100%)	0.009*		
				I vs II	I vs III	II vs III
				0.032*	0.079	----
Ascites No Mild Moderate Marked	12(24%) 11(22%) 20(40%) 7(14%)	4(10%) 4(10%) 22(55%) 10(25%)	30(100%) 0(0%) 0(0%) 0(0%)	< 0.001*		
				I vs II	I vs III	II vs III
				0.079	< 0.001*	< 0.001*
Splenomegally Mild Moderate Huge	10(20%) 29(58%) 11(22%)	2(5%) 30(75%) 8(20%)	28(93.3%) 2(6.7%) 0(0%)	< 0.001*		
				I vs II	I vs III	II vs III
				0.095	< 0.001*	< 0.001*
Child score A B C	0(0%) 34(68%) 16(32%)	0(0%) 27(67.5%) 13(32.5%)	30(100%) 0(0%) 0(0%)	< 0.001*		
				I vs II	I vs III	II vs III
				1	< 0.001*	< 0.001*
Meld score % MR 1.9% MR 6%	24(48%) 26(52%)	20(50%) 20(50%)	30(100%) 0(0%)	< 0.001*		
				I vs II	I vs III	II vs III
				1	< 0.001*	0.605

- HCC: hepatocellular carcinoma, LC: liver cirrhosis.

- MELD: Model for End-Stage Liver Disease

- MR: Mortality rate.

- Kruskal Wallis test for non-parametric data (expressed by median) between the three groups followed by Mann Whitney test between each two groups.

- Chi square test and Fisher exact test for qualitative data between groups.

*- *: Significant difference at P value < 0.05.*

Table (2): laboratory data of the study subjects

	Group I HCC N = 50	Group II LC N = 40	Group III Chronic hepatits N = 30	P value		
Urea(mg/dl) Range Mean ± SD	(16–75) 48.3 ± 15.5	(20–73) 50.8 ± 14.3	(33–58) 45.3 ± 7	0.240		
				I vs II	I vs III	II vs III
				0.565	0.596	0.209
Creatinine(mg/dl) Range Mean ± SD	(0.3–1.5) 1.03 ± 0.33	(0.6–1.5) 1.05 ± 0.23	(0.6–1.3) 1.08 ± 0.17	0.763		
				I vs II	I vs III	II vs III
				0.886	0.714	0.945
HB(gm/dl) Range Mean ± SD	(5.7–16.5) 10 ± 2.3	(4.5–13.5) 9.1 ± 2.2	(8.5–12.5) 10.4 ± 0.9	0.025*		
				I vs II	I vs III	II vs III
				0.099	0.691	0.027*
TLC ($x10^3$) Range Mean ± SD Median	(2.6–16.2) 8.3 ± 3.9 7.7	(1.6–13.4) 5.9 ± 2.8 5.3	(4.3–11.2) 7.9 ± 2 7.8	< 0.001*		
				I vs II	I vs III	II vs III
				< 0.001*	0.831	< 0.001*
Platelets (cells/cmm3) ($x10^3$) Range Mean ± SD Median	(37–442) 140.6 ± 76.2 128.5	(36–274) 122.6 ± 63.4 108	(96–312) 156.1 ± 60.3 125.5	0.028*		
				I vs II	I vs III	II vs III
				0.183	0.283	0.003*
INR Range Mean ± SD Median	(1–2) 1.4 ± 0.2 1.4	(1-2.4) 1.5 ± 0.3 1.4	(1-1.6) 1.1 ± 0.2 1			
				I vs II	I vs III	II vs III
				0.034*	< 0.001*	< 0.001*
Total Bilirubin(mg/dl) Range Mean ± SD Median	(0.3–2.9) 1.5 ± 0.7 1.4	(0.2–2.1) 1.4 ± 0.6 1.5	(0.5–1.2) 0.8 ± 0.1 0.7	< 0.001*		

HB: hemoglobin, TLC: total leucocytic count, ALT: Alanine transaminase, AST: aspartate transaminase, INR: International normalized ratio. HCC: Hepatocellular carcinoma, LC: liver cirrhosis.

- Kruskal Wallis test for non-parametric data (expressed by median) between the three groups followed by Mann Whitney test between each two groups

- (¶) One-way ANOVA test for parametric quantitative data between the three groups followed by post hoc Tukey analysis between each two groups

- *: Significant difference at P value < 0.05

	Group I HCC N = 50	Group II LC N = 40	Group III Chronic hepatits N = 30	P value		
				I vs II	I vs III	II vs III
				0.062	< 0.001*	< 0.001*
Direct Bilirubin(mg/dl) Range Mean ± SD Median	(0.2–1.8) 0.9 ± 0.4 0.9	(0.1–1.8) 0.9 ± 0.4 1	(0.2–0.8) 0.4 ± 0.1 0.4	< 0.001*		
				I vs II	I vs III	II vs III
				0.347	< 0.001*	< 0.001*
Albumin(gm/dl) Range Mean ± SD Median	(1.2–4.3) 3 ± 0.7 2.9	(1.6–3.9) 2.6 ± 0.5 2.5	(3.4–4.5) 3.7 ± 0.3 3.6	< 0.001*		
				I vs II	I vs III	II vs III
				0.005*	< 0.001*	< 0.001*
ALT(U/ml) Range Mean ± SD Median	(12–641) 83.2 ± 104.1 56.5	(15–111) 46.6 ± 22.8 44	(15–73) 40.3 ± 11.4 40.5	0.008*		
				I vs II	I vs III	II vs III
				0.030*	0.003*	0.351
AST(U/ml) Range Mean ± SD Median	(16–570) 112.6 ± 113.4 69.5	(16–115) 53.8 ± 24 53	(28–65) 41.3 ± 8.1 42	< 0.001*		
				I vs II	I vs III	II vs III
				0.003*	< 0.001*	0.033*
HB: hemoglobin, TLC: total leucocytic count, ALT: Alanine transaminase, AST: aspartate transaminase, INR: International normalized ratio. HCC: Hepatocellular carcinoma, LC: liver cirrhosis.						
- Kruskal Wallis test for non-parametric data (expressed by median) between the three groups followed by Mann Whitney test between each two groups						
- (¶) One-way ANOVA test for parametric quantitative data between the three groups followed by post hoc Tukey analysis between each two groups						
- *: Significant difference at P value < 0.05						

Table (3) Level of PTH, vitamin D_3***, corrected calcium, ionized calcium, zinc & phosphorus between the study subjects.***

	Group I HCC (N = 50)	Group II LC (N = 40)	Group III Chronic hepatits (N = 30)	P value		
vitamin D_3 (ng/ml) Range Mean ± SD Median	(6–69) 14.7 ± 8.4 14	(2–58) 28.5 ± 11.3 29	(20–40) 29.3 ± 6.2 29	< 0.001*		
				I vs II	I vs III	II vs III
				< 0.001*	< 0.001*	0.605
PTH (ng/l) Range Mean ± SD Median	(41–833) 168.1 ± 125 124	(17–381) 125 ± 92 91	(28–70) 50.5 ± 12.7 52	< 0.001*		
				I vs II	I vs III	II vs III
				0.013*	< 0.001*	< 0.001*
Corrected calcium(mg/dl) Range Mean ± SD	(8.4–12.8) 10.8 ± 1.2	(7-11.1) 8.8 ± 1.1	(7.9–11.8) 9.8 ± 1.3	< 0.001*		
				I vs II	I vs III	II vs III
				< 0.001*	< 0.001*	0.004*
Ionized calcium (mg/dl) Range Mean ± SD	(2.1–3.2) 2.7 ± 0.3	(1.5–2.8) 2.2 ± 0.3	(2–3) 2.4 ± 0.3	< 0.001*		
				I vs II	I vs III	II vs III
				< 0.001*	0.002*	0.003*
Zinc (mcg/mL) Range Mean ± SD Median	(13–75) 36 ± 17.4 34.5	(16–118) 49.5 ± 27.6 43	(4-114) 82.4 ± 24.9 88	< 0.001*		
				I vs II	I vs III	II vs III
				0.023*	< 0.001*	< 0.001*
Phosphorus(mg/dl) Range Mean ± SD	(1.5-7) 3.1 ± 1.2	(2-4.4) 3.2 ± 0.5	(2-4.4) 3.2 ± 0.5	0.770		
				I vs II	I vs III	II vs III
				0.924	0.912	0.750

- Kruskal Wallis test for non-parametric data (expressed by median) between the three groups followed by Mann Whitney test between each two groups

- (¶) One-way ANOVA test for parametric quantitative data between the three groups followed by post hoc Tukey analysis between each two groups

*- *: Significant difference at P value < 0.05*

Table (4): **Statistical significance of the frequency of vitamin D status between groups**

	Group I HCC N = 50	Group II LC N = 40	Group III Chronic hepatitis N = 30	P value		
Vitamin D3 deficiency (< 20 ng/dl)	48 (96%)	9(22.5%)	0	< 0.0001		
				I vs II	I vs III	II vs III
				< 0.0001	< 0.0001	0.004
Vitamin D3 insufficiency (20–30 ng/dl)	1 (2%)	15 (37.5%)	19(63.3%)	< 0.0001		
				I vs II	I vs III	II vsIII
				< 0.0001	< 0.0001	0.032
Vitamin D3 normal (> 30ng/dl)	1 (2%)	16(40%)	11(36.7%)	< 0.0001		
				I vs II	I vs III	II vsIII
				< 0.0001	< 0.0001	0.777

Table (5): Statistical significance for the frequency of zinc deficiency between the study groups

	Group I HCC N = 50	Group II LC N = 40	Group III Chronic hepatitis N = 30	P value
zinc deficiency (< 70 µg/dl)	48 (96%)	30 (75%)	5(16.67%)	< 0.001 I vs II II vs III I vs III 0.03 < 0.001 < 0.001
normal zinc (≧ 70 µg/dl)	2 (4%)	10 (25%)	25(83.33%)	

Table (6): Multiple stepwise logistic regression analysis for prediction of HCC (Ch. Liver disease is reference group)

	OR	95% CI	P value
Age	1.11	1.02–1.21	0.014*
AST	1.03	1.01–1.05	0.002*
PTH	1.01	1.004–1.02	0.002*
Corrected calcium	3.18	1.96–5.14	< 0.001*

- AST: aspartate transaminase

- *PTH:* parathyroid hormone

- *AOR:* Adjusted Odds Ratio

- *CI:* Confidence Interval

- *Ref.:* Reference

- *: Significant level at P value < 0.05

Table (7): Accuracy indices for the prediction of HCC; (Ch. Liver disease is reference group)

	Age	AST	PTH	Corrected calcium
Optimal cutoff	> 58	> 50	> 100	> 9.63
AUC	0.686	0.739	0.790	0.800
95% CI	0.595–0.768	0.651–0.815	0.707–0.859	0.717–0.867
P value	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Sensitivity %	68	76	84	82
Specificity %	64.29	65.71	74.29	75.71
PPV%	57.6	61.3	70	70.7
NPV%	73.8	79.3	86.7	85.5
Accuracy %	62.5	66.7	78.3	78.3
AUC; area under the curve				
PPV; positive predictive value				
NPV; negative predictive value				

Figures

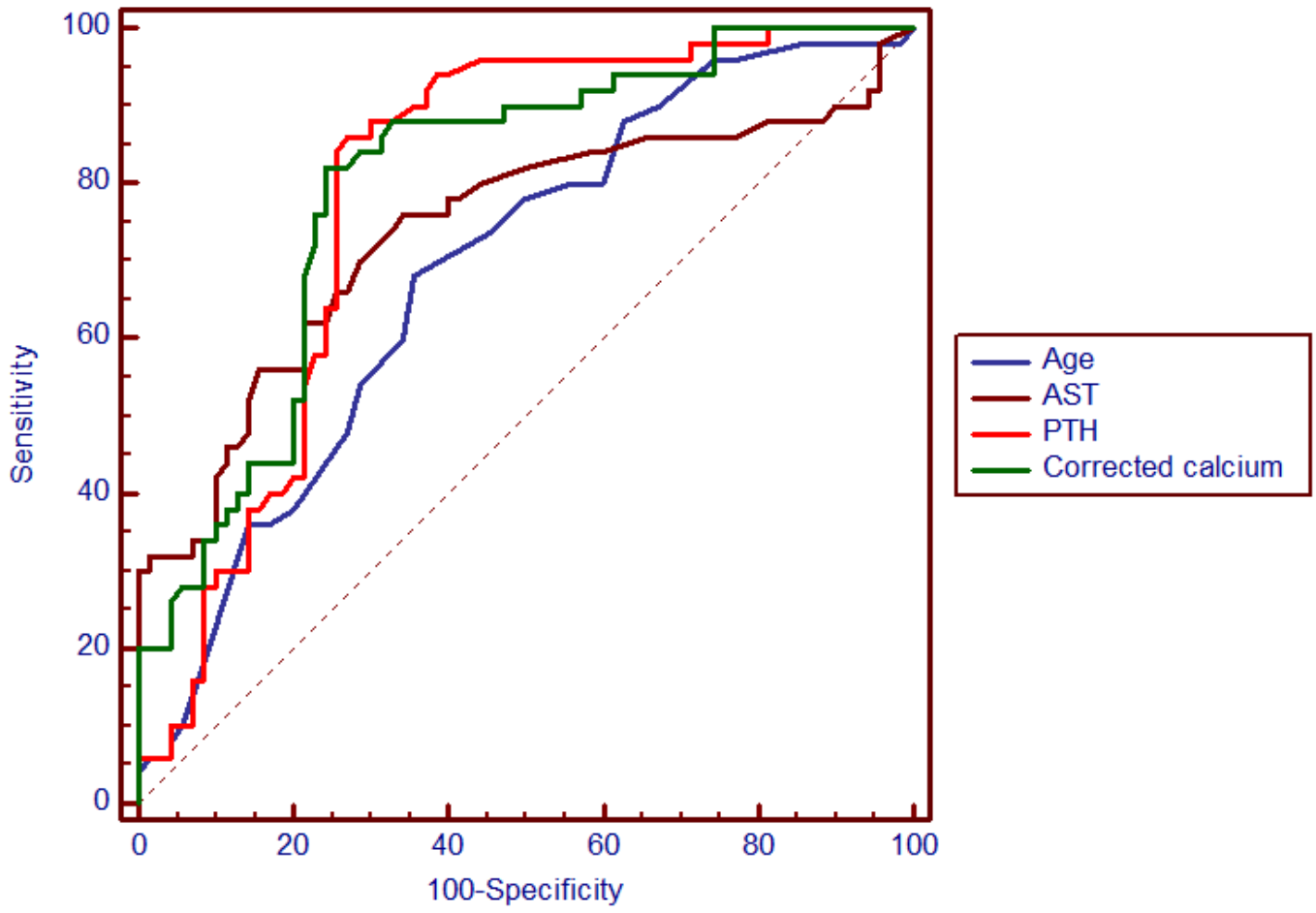

Figure 1

ROC curve presenting specificity and sensitivity for corrected calcium, PTH, AST and age