Research Article

Evaluation of Parathyroid hormone serum level in HCV-related hepatocellular carcinoma.

Mahmoud Khattab *, Elham Ahmed*, Hend M. Moness, Arwa Mohamad***
* Department of internal medicine, Faculty of Medicine, Minia University, Minia, Egypt.
** Department of clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt.

Abstract
Background: The role displayed by parathyroid hormone (PTH) in HCV-related hepatocellular carcinoma (HCC) is not clearly defined. **Aim**: To investigate serum levels of parathyroid hormone, ionized Calcium (Ca) and corrected calcium in HCV-induced cirrhosis with HCC and to assess if their levels have relation to the child score and MELD score of HCC. **Patients and methods:** This study recruited patients with HCV-related HCC and HCV infected patients without HCC. A group of healthy subjects was also studied as controls. Liver and renal biochemical profiles, complete blood count and INR were determined. The serum levels of intact PTH (iPTH), ionized calcium and corrected calcium were measured in the three groups. **Results:** This cross-sectional observational study included fifty cirrhotic patients with HCV-related HCC, 70 HCV infected chronic liver disease patients without HCC and 30 healthy controls. iPTH serum level was significantly higher in the HCC patients compared to the other HCV infected chronic liver disease patients and to the healthy controls, ($p<0.001$). In addition, ionized calcium and corrected calcium serum levels were significantly raised in HCC than in HCV infected chronic liver disease patients without HCC, ($p<0.001$). There was no significant difference in iPTH levels between child B and child C HCC patients. Multiple regression analysis indicated the both iPTH and ionized calcium levels were independent predictors for HCC in HCV infected patients, (OR for PTH was 1.01; 95%CI: 1.004-1.02 with p= 0.002. and OR for corrected calcium was 3.18; 95%CI: 1.96-5.14 with p= <0.001). **Conclusion:** This study indicated the prevalent high levels of iPTH in HCC and associated hypercalcemia and this was not related to liver function. iPTH can be considered as a predictor for HCC occurrence in HCV infected chronic liver disease patients.
Keywords: parathyroid hormone, hepatocellular carcinoma

Introduction
HCC is one of the major health problems worldwide and 600,000 patients are dying from this disease annually (Forner et al., 2012).[1] Parathyroid hormone (PTH) secretion is controlled by vitamin D and calcium via the vitamin D receptor and calcium-sensing receptor, respectively. (Fisher and Fisher, 2007).[2] HCC associated hypercalcemia is probably due to either bone metastasis or secretion of hormones such as intact parathyroid hormone (PTH) or its related peptide (PTHrP) by the tumor (Abe et al., 2011).[3]

Patients and method
This cross sectional observational study recruited HCV-induced cirrhotic patients with HCC (group 1) and HCV infected cirrhotic patients without HCC (group 2) and healthy controls (group3); through the period from May 2017 up to April 2018.

Ethics related statement: The study protocol was approved by Institutional Research Board (IRB) of Minia School of Medicine, Egypt. Informed written consent was obtained from all patients who participated in this study. The study was conducted in accordance with the guidelines of 1975 declaration of Helsinki. The diagnosis of HCC was based on the characteristic criteria proposed by the national guidelines for diagnosis of HCC.[4] the diagnosis of liver cirrhosis was done relying upon the clinical, laboratory and ultrasonic criteria.[5] Criteria of exclusion included patients who were receiving vitamin D or calcium supplements for 6 months before the start of the study, patients with parathyroid adenoma as diagnosed by cervical ultrasound and CT when indicated.

Patients with liver cirrhosis due to any other cause than HCV and patients with chronic renal insufficiency were also excluded from the study. All patients were subjected to clinical assessment, complete blood count determined by automated cell counter, Sysmex KX-21N (TAO Medical Incorporation, Japan), liver and renal biochemical profiles were done by auto-analyzer Konelab i60 (Thermo-electro, Clinical chemistry automation systems, Finland), International Normalized Ratio (INR) done by using fully automated coagulometer STAGO (Diagnostic STAGO– France). viral markers (HCV-Ab, HBsAg, and HIV antibodies) were measured by fully automated ChemiLumine-scence technology (Cobas E 411-Roche-Roche Diagnostics GmbH Germany) and serum alpha fetoprotein were performed by ELISA. Serum PTH was assessed using immunoassay which is an adapted two-site sandwich ELISA. The kits were supplied by Calbiotech EIA kit (calbiotech Inc., 1935 Cordell Ct., El Cajon, CA). Serum calcium level determined by Sunostik semi-autobio-chemistry analyzer using Spinreact calcium kits (o-cresolphtalein v/v. Colorimetric Test). Corrected calcium was calculated as [0.8 x (4- serum albumin)] + serum calcium.

Statistical Analysis

SPSS program software version 24 was used for data analysis. Kolmogorov-Smirnov test was used to detect the normality of distribution of the quantitative data. Mean, standard deviation and minimum& maximum of the range were used for parametric quantitative data; while, for nonparametric quantitative data, the median was used. Multiple regression analysis and stepwise regression were done to determine the predictors of HCC.ROC curve was done to determine the cutoff point, AUC, sensitivity, specificity, PPV, NPV and accuracy of PTH, calcium. The level of significance was consid-ered when P value < 0.05.

Results

Our study showed that the age of HCC patients is significantly higher than the age of the control group but there was no significant difference in the age between HCC group and chronic liver disease group. There was statistically higher male distribution in group 1(HCC) than group 2 (chronic liver disease).

Using Fisher exact test for qualitative data between groups, the Child-Turcotte-Pugh score was significantly different between HCC and non HCC cirrhotic patients (<0.001). MELD score showed significant higher values in HCC group compared to chronic hepatitis patients with p= 0.009 (Table 1).

There was no statistical difference between the three groups on comparing both urea, creatinine. iPTH had significantly higher level in group 1(HCC) than group 2 (non-HCC cirrhotic patients) and group 3 (healthy controls); while the level in group 2 was still higher than group 3 (Mean±SD: 168.1±125 ng/L for HCC & 125±92ng/L for non HCC cirrhotics vs. 50.5±12.7ng/L for healthy controls; with p=0.013, p=< 0.001, and p=< 0.001 respectively). The corrected calcium had significantly higher level in group 1 than levels in groups 2 and 3 (Mean±SD: 10.8±1.2 mg/dl, 8.8±1.1mg/dl and 9.8±1.3 for groups 1, 2 and 3, respectively, with p=< 0.001). Also, the ionized calcium had significantly higher level in group 1 than levels in groups 2 and 3 (means±SD: 2.7±0.3 mg/dl, 2.2±0.3mg/dl and 2.4±0.3 mg/dl for groups 1, 2 and 3 respectively; with p=< 0.001 and p=0.002) (Table 2).

There was no significant difference in the serum levels of iPTH between child B and child C hepatocellular carcinoma patients (175.1±120 vs 165.8±133.5 ng/L, p 0.790). In addition there was no significant difference in its levels as regard to MELD score (Table 3).

Using multiple logistic regression analysis, corrected calcium and PTH were predictors for HCC with (p=<0.001 & p=0.003 respectively) (Table 4). Using multiple stepwise logistic regression analysis, the OR for corrected calcium was 3.18; 95%CI: 1.96-5.14 with p= <0.001. The OR for PTH was 1.01; 95%CI: 1.004-1.02 with p= 0.002. The OR for AST was 1.03; 95%CI: 1.01-1.05 with p=0.002. The OR for age was 1.11; 95%CI: 1.02-1.21 with p=0.014 (Table 5).

Table 5 shows the accuracy and sensitivity indices for some of the studied variables in the prediction of HCC in HCV infected patients. With optimal cut off points of >100 ng/L for

iPTH, >9.63 mg/dl for corrected calcium, >58 years for age and >50 U/L for AST; the corrected calcium had the highest value of AUC (0.80), while AUC for PTH was 0.790, AUC for AST was 0.739 and AUC for age was 0.686. P value was < 0.001 for all of them, (figure1).

Table 1: comparison of demographic and clinical data between the study subjects

	Group I HCC N=50	Group II Chronic liver disease N=70	Group III Healthy control (N=30)	P value		
				I vs II	I vs III	II vs III
Age (years)						
Range	(45-85)	(45-75)	(45-60)	0.001*	<0.001*	<0.001*
Mean ± SD	61.5±7.3	56.7±7	51.3±3.9			
Sex						
Male	34(68%)	34(48.6%)	18(60%)	0.034*	0.467	0.294
Female	16(32%)	36(51.4%)	12(40%)			
Child score				<0.001*	-	-
A	0(0%)	30(42.9%)				
B	34(68%)	27(38.6%)	-			
C	16(32%)	13(18.6%)				
Meld score %				0.009*	-	-
MR 1.9%	24(48%)	50(71.4%)				
MR 6%	26(52%)	20(28.6%)				

- 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

Table (2): Comparison of laboratory data between the study subjects.

	Group I HCC N=50	Group II Chronic liver disease N=70	Group III Healthy control (N=30)	P value		
				I vs II	I vs III	II vs III
Urea (mg/dl)						
Range	(16-75)	(20-73)	(33-58)	0.959	0.596	0.209
Mean ± SD	48.3±15.5	48.4±12	45.3±7			
Creatinine (mg/dl)						
Range	(0.3-1.5)	(0.6-1.5)	0.7-1.1	0.530	0.714	0.945
Mean ± SD	1.03±0.33	1.1±0.2	1-0.34			
AlT (U/ml)				0.030*	0.003*	0.351
Range	(12-641)	(15-111)	(15-30)			
Mean ± SD	83.2±104.1	43.9±18.9	40.3±11.4			
Median	56.5	41.5	40.5			
AST (U/ml)				<0.001*		
Range	(16-570)	(16-115)	(28-65)			
Mean ± SD	112.6±113.4	53.8±24	41.3±8.1	0.003*	<0.001*	0.033*
Median	69.5	53	42			
PTH (ng/l)				<0.001*		
Range	(41-833)	(17-381)	(28-70)			
Mean ± SD	168.1±125	93.1±78.9	50.5±12.7	0.013*	<0.001*	<0.001
Median	124	63.5	52			*
Corrected calcium (mg/dl)				<0.001*		
Range	(8.4-12.8)	(7-11.1)	(7.9-11.8)	<0.001*	0.002*	0.002*
Mean ± SD	10.8±1.2	8.8±1.1	9.8±1.3			

AST: aspartate transaminase, INR: International normalized ratio.

Table 3: comparison of iPTH serum levels according to Child-paugh classification and Meld score

Child- paugh classification	**Child B** N= 34(68%) 175.1±120	**Child C** N= 16(32%) 165.8±133.5	**P-Value** 0.790
Meld score	MR 1.9% N=24(48%) 162.3±80	MR 6% N= 26(52%) 168.3±70	0.778

Table (4): Multiple logistic regression analysis for prediction of HCC

	OR	**95% CI**	**P value**
Age	1.2	0.99-1.22	0.064
Sex **Male** **Female**	 Ref 1.99	 0.54-7.37	 0.302
ALT	1.004	0.97-1.04	0.786
AST	1.021	0.999-1.04	0.061
Corrected calcium	3.17	1.78-5.66	<0.001*
PTH	1.013	1.004-1.021	0.003*
MELD score **MR 1.9%** **MR 6%**	 Ref 2.1	 .43-10.37	 0.362

AOR: Adjusted Odds Ratio, CI: Confidence Interval. Ref.: Reference

Table (5) Multiple stepwise logistic regression analysis for prediction of HCC

	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

Table (6): ROC curve analysis for prediction of HCC

	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

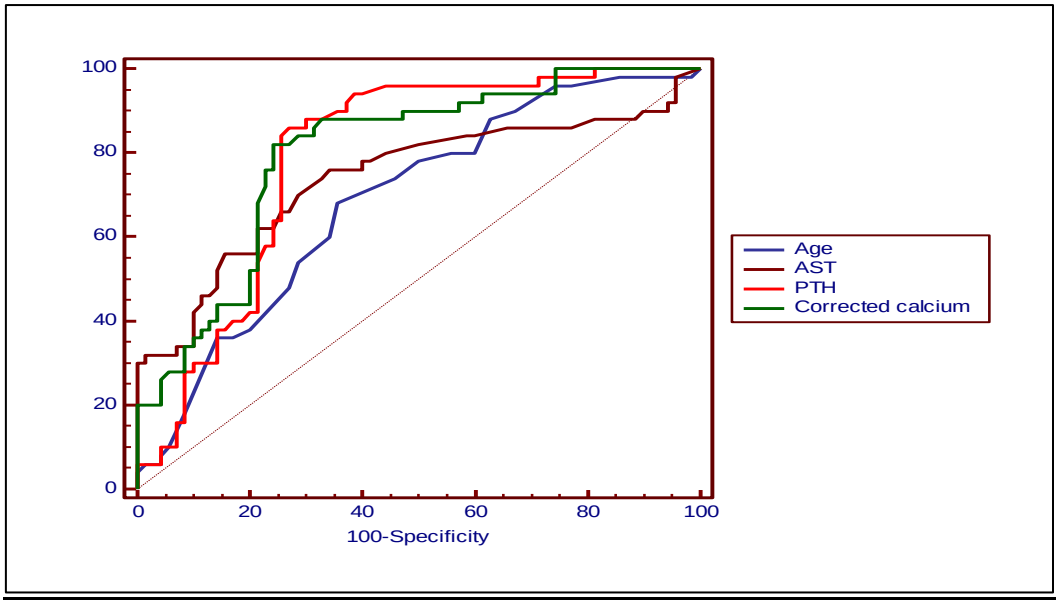

Figure (1) ROC curve presenting specificity and sensitivity for corrected calcium, PTH, AST and age.

Discussion

In our study, HCC was observed in males twice more frequent than in females with significantly higher male distribution in HCC patients. This was also reported by Hammad et al., 2013 who stated that HCC was significantly higher in males than females.[6] The reasons for this gender disparity could be explained by differences in exposure to risk factors (Lange et al., 2013).[7] Data related to age in our results showed that HCC was more common in old age. On multiple stepwise logistic regression analysis in relation to chronic liver disease, age was found to be a predictor to HCC (OR; 1.11; 95% CI; 1.02-1.2; P<0.014). El zayadi et al., 2005 reported that HCC in Egypt is significantly more prevalent among older age groups than younger age groups and it was suggested that HCV infection in old patients induces a rapid progression to HCC independent of HCV genotype.[8] Omata et al, 2010, reported that old age is a risk factor for HCC, especially in areas where HCV infection is endemic as Egypt.[9]

Our result also showed that there was statistical significant difference between the HCC group and HCV infected chronic liver disease as regard the liver transaminases AST and ALT. The raised AST level was significantly associated with HCC (OR; 1.03; 95% CI; 1.01-1.05); at cut off value >50 U/L with sensitivity 76% and specificity 65.71%. This finding was supported by large cohort study that was done on 1108 patients with HCC by Carr and Guerra, 2016, who found an association between increasing levels of liver enzymes (AST, ALKP and GGTP) and HCC aggressiveness.[10]

We investigated serum PTH level in the study groups, and we found a significant higher level of serum PTH in patients with HCC compared to the chronic liver disease and the healthy controls. At the same time, our results showed statistically significant higher levels of corrected calcium in patients with hepatocellular carcinoma compared to both chronic liver disease and controls.The corrected calcium was one of HCC predictive factors (OR; 4.0; 95%CI: 2.3-6.9). When multiple stepwise logistic regression analysis was done in relation to chronic liver disease, PTH, age, AST and corrected calcium levels were significantly associated with HCC. PTH was considered as a significant predictor for HCC (OR; 1.11; 95% CI; 1.004-1.02; P<0.014) at cut off value >100 ng/l with sensitivity 84% and specificity 74.29%. These findings come in contrary with Duarte et al., 2001 who found serum PTH was similar in patients with and without cirrhosis.[11] In addition our results were not consistent with findings of Miroliaee et al., 2010, who studied PTH in 40 healthy volunteers, 39 chronic hepatitis without cirrhosis and 51 cirrhotic

patients. They found no difference in serum PTH level between cirrhotic and non-cirrhotic patients.[12] Corrected calcium at a cut-off value >9.63, had sensitivity 82% and specificity 75.71% in the prediction of HCC in relation to chronic liver disease. Although many studies shown that hypercalcemia due to both bony metastatic lesions and the production of parathyroid hormone-related protein (PTHrP) from the malignant cells[13,3,14], yet three case reports by Koyama et al., 1999, Mahoney et al., 2006 and Abe et al., 2011 described cases with hypercalcemia that is caused by HCC secreting intact parathyroid hormone (iPTH).[15,16,3]. Our study has found no significant relation between the functional grading in HCC patients and the level of iPTH.

In conclusion,
our study indicated that the HCV-induced HCC is associated with significant high levels of IPTH and serum calcium which have a significant predictive value in the diagnosis of HCC.

Conflict of interest: None

Acknowledgment: The authors acknowledge Dr. Mostafa A. Asem for conducting the statistical analysis of the data.

References

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. The Lancet. 2012. 379 (9822): 1245–1255.
2. Fisher L, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. Clin Gastroenterol Hepatol; 2007.5: 513 –520.
3. Abe Y, Makiyama H, Fujita Y, Tachibana Y, Kamada G, Uebayashi M., Severe hypercalcemia associated with hepatocellular carcinoma secreting intact parathyroid hormone: a case report. Intern Med.; 2011. 50(4):329-33.
4. Attwa MH, El-Etreby SA. Guide for diagnosis and treatment of hepatocellular carcinoma.World journal of hepatology; 2015. 7(12):1632.
5. Johannes Wiegand, Thomas Berg. The Etiology, Diagnosis and Prevention of Liver Cirrhosis. Dtsch Arztebl Int 2013; 110(6): 85−91
6. Hammad LN, Abdelraouf SM, Hassanein FS, Mohamed WA, chaalan MF. Circulating IL-6, IL-17 and vitamin D in hepatocellular carcinoma: potential biomarkers for a more favorable prognosis? J Immunotoxicol, 2013. 10: 380-386.
7. Lange CM, Miki D, Ochi H, Nischalke H-D, Bojunga J. Genetic Analyses Reveal a Role for Vitamin D Insufficiency in HCV-Associated HepatocellularCarcinoma Development. PLoS ONE; 2013. 8(5): e64053.
8. El-Zayadi AR, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, Selim O, Saeid A., 2005. Hepatocellular carcinoma in Egypt: a single center study over a decade. World J Gastroenterol 11(33): 5193-5198.
9. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, Kudo M, Lee JM, Choi BI, Poon RT, Shiina S, Cheng AL, Jia JD, Obi S, Han KH, Jafri W, Chow P, Lim SG, Chawla YK, Budihusodo U, Gani RA, Lesmana CR, Putranto TA, Liaw YF, Sarin SK. Asian Pacific Association for the Study of the Liver consensus recommenddations on hepatocellular carcinoma. Hepatol Int2010.; 4(2):439-74.
10. Carr BI, Guerra V. A hepatocellular carcinoma aggressiveness index and its relationship to liver enzyme levels. Oncology; 2016.90: 215–220.
11. Duarte MP, Farias ML, Coelho HS, Mendonca LM, Stabnov LM, Do Carmo d Oliveira M, Lamy RA, Oliveira DS. Calcium–parathyroid hormone–vitamin D axis and metabolic bone disease in chronic viral liver disease. J Gastroenterol Hepatol: 2001. 16:1022–1027.
12. Miroliaee A, Nasiri-Toosi M, Khalilzadeh O, Esteghamati A, Abdollahi A, Mazloumi M., 2010. Disturbances of parathyroid hormone-vitamin D axis in non-cholestatic chronic liver disease: a cross-sectional study. Hepatol Int.; 4(3):634-40.
13. Oldenburg WA, van Heerden JA, Sizemore GW, Abboud CF, Sheedy PF. 2nd. Hypercalcemia and primary hepatic tumors. Arch Surg, 1982.117: 1363-1366.
14. Sultan-e-Rome1, Sharif S, Shah AA., Parathryoid hormone related peptide causing hypercalcaemia in a patient with hepatocellular carcinoma. J Pak Med Assoc.; 2013. 63(2):263-4.
15. Koyama Y, Ishijima H, Ishibashi A, Katsuya T, Ishizaka H, Aoki J, Endo K. Intact PTH-producing hepatocellular

carcinoma treated by transcatheter arterial embolization. Abdom Imaging, 1999. 24: 144-146.
16. Mahoney EJ, Monchik JM, Donatini G, De Lellis R., 2006. Life threatening hypercalcemia from a hepatocellular carcinoma secreting intact parathyroid hormone. Localization by sestamibi single photon emission computed tomographic imaging. Endocr Pract 12: 302-306.