Albumin-corrected serum calcium and serum parathyroid hormone-related polypeptide determined by enzyme-linked immunosorbent assay in cirrhotic patients with hepatocellular carcinoma – a pilot study

Ragai M.F.R. Fouda[a], Rania E. Sheir[c], Nora M. Selim[b], Ahmed A. Hammad[d]

Departments of [a]Internal Medicine, [b]Clinical Pathology, Faculty of Medicine, Kasr AlAiny Hospital, Cairo University, Cairo, [c]Department of Internal Medicine, Faculty of Medicine, Beni-Suef University, Beni-Suef, [d]Department of Internal Medicine, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Correspondence to Ragai M.F.R. Fouda, MD, MRCP, George Eliot Hospital, College Street-Nuneaton, Warwickshire CV107DJ, UK.
Tel: +44 24 7635 1351; fax: 024 76865175;
e-mail: drragaifouda@gmail.com

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Context
Parathyroid hormone-related peptide (PTHrP) is produced by many malignant tumors. It is responsible for most cases of hypercalcemia in patients with malignancy. Few published studies shed light on the relation between serum calcium levels and serum PTHrP levels in cirrhotic patients with hepatocellular carcinoma (HCC).
Aim
The aim of the current work was to evaluate serum PTHrP in cirrhotic patients with HCC and a possible correlation between serum PTHrP levels and albumin-corrected serum calcium levels in these patients.
Patients and methods
This is a cross-sectional study. The study included 35 cirrhotic patients with HCC (diagnosed depending upon α-fetoprotein and abdominal imaging studies). Data about their serum albumin and albumin-corrected serum calcium levels were collected. Sera of the studied patients were collected for determination of PTHrP levels by enzyme-linked immunosorbent assay (ELISA). Numerical data were summarized in the form of mean±SD. Strength of association between variables was tested using Pearson correlation coefficient.
Results
Approximately 8.6% of studied patients were hypercalcemic, and no statistically significant positive correlation was detected between serum PTHrP determined by ELISA and albumin-corrected serum calcium in these patients.
Conclusion
Approximately 8.6% of studied cirrhotic patients with HCC were hypercalcemic. NO statistically significant positive correlation was detected between serum PTHrP determined by ELISA and albumin-corrected serum calcium in these patients. Studies involving a larger number of patients could clarify the exact role of PTHrP in the development of hypercalcemia in cirrhotic patients with HCC.

Keywords:
calcium, hepatocellular carcinoma, parathyroid hormone related polypeptide

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Introduction

Nearly 90% of hepatocellular carcinomas (HCC) are due to underlying liver cirrhosis caused by chronic viral hepatitis B or C, alcoholism, α-one antitrypsin deficiency, and nonalcoholic steatosis [1,2]. Less frequently HCC can occur in a non-cirrhotic liver. A large multicenter Italian study demonstrated that out of 3000 cases of HCC, merely 52 (2%) of these cases were noncirrhotic HCC cases [3].

Paraneoplastic syndromes such as hypercholesterolemia, hypoglycemia, erythrocytosis and hypercalcemia may be encountered in some patients with HCC, either at presentation or later during the clinical course.

The incidence of HCC-associated hypercalcemia reported in the literature ranges between 1 and 50%. This wide range may be attributed to the inclusion of patients with bony metastases in some studies [4–7].

HCC-associated hypercalcemia, without bony metastasis, is uncommon. It is associated with more advanced HCC, larger tumor burden, and shortened survival [8].

Hypercalcemia among patients with HCC in the absence of bony metastases was attributed to the tumor-secreted hormones such as parathyroid hormone-related peptide (PTHrP). The humoral hypercalcemia of malignancy (HHM) was first

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proposed by Fuller Albright, and it refers specifically to PTHrP-mediated hypercalcemia [9]. This theory was later supported by the observation that hypercalcemia can be successfully treated with either tumor resection or embolization [6].

The aim of the current pilot study was to evaluate a possible correlation between serum PTHrP level and albumin-adjusted serum calcium in cirrhotic patients with HCC.

Patients and methods

Setting

The current pilot cross-sectional study was conducted in Kasr-AlAini University Hospital, Cairo, Egypt, which is one of the biggest tertiary centers in Egypt. Adult patients older than 18 years admitted to Internal medicine units of the hospital between June 2017 and January 2018 were included in this study. Informed consents were obtained from the study participants, and ethical approval was obtained from the Ethical Committee of Kasr-AlAini University Hospital.

Study population

The study involved 35 hospitalized cirrhotic HCC patients; the diagnosis of HCC was made based on one of the following criteria: histology; elevated serum α-fetoprotein (AFP) greater than 400 μg/l and radiological features compatible with HCC (on abdominal ultrasonography or triphasic computed tomography); or, radiological features compatible with HCC by two or more imaging modalities if AFP less than 400 μg/l [10].

Exclusion criteria

Patients with primary extrahepatic malignancy or liver metastases due to extrahepatic malignancy were excluded. Those with conditions known to be associated with elevated plasma PTHrP levels including pregnancy, lactation, systemic lupus erythematosus, HIV-associated lymphadenopathy, lymphedema of chest or pleural cavities, and benign tumors of the ovary, kidney, and the neuroendocrine system were also excluded from the current study. Those with bone metastases, receiving calcium or vitamin D supplements, and experiencing advanced stages of renal dysfunction (estimated creatinine clearance <30 ml/min) were also excluded [11].

Data collection

Data about demographic characteristics, clinical data (ascites and hepatic encephalopathy), and blood tests (liver function tests, serum creatinine, prothrombin time, and albumin-corrected serum calcium) of patients with HCC were recorded. Data about ascites, encephalopathy, serum albumin, serum bilirubin, and prothrombin time were used to calculate Child–Pugh score for patients with HCC.

All patients with HCC were cirrhotic, and they were classified according to their Child score into three classes: a score of 5–6 points for Child–Pugh class A score, a score of 7–9 points for Child–Pugh class B score, and a score of 10–15 points for Child–Pugh class C score [12].

Samples collection

Blood samples obtained from the study participants and that of 10 healthy controls were collected in EDTA tubes. Samples were then centrifuged, and their sera were stored in a freezer at −80°C till the time of serum assay testing.

Parathyroid hormone-related peptide serum assay

Sera of the study participants were collected for determination of PTHrP by a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) using the kits of Bionevan Co. (Beijing, China). According to the standard concentration and the corresponding optical density, the concentration of PTHrP in the samples was calculated from a standard curve using a linear regression equation.

Statistical methods

Categorical data were summarized in the form of frequencies and percentages. Numerical data were summarized in the form of mean±SD. Strength of association between variables was tested using Pearson's correlation coefficient. All *P* values were two sided, and a *P* value less than 0.05 was considered significant. Data management and analysis were performed using Statistical Analysis Systems (Microsoft Excel 2017; Microsoft Corporation, New York, USA) and Statistical Package for the Social Sciences version 13 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Results

The demographics and clinical and biochemical data of patients with HCC are outlined in Table 1. Thirty-five patients with HCC participated in the current study, and ∼60% of them were men, with a mean age of around 60.5 years (range: 52–80 years).

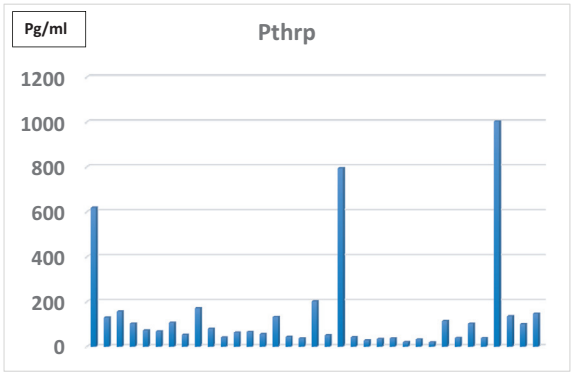
Approximately 51% of the patients with HCC belonged to Child–Pugh class C and ∼43% of them

Table 1 Demographic, clinical, and biochemical parameters of studied patients with hepatocellular carcinoma

Parameters	*n*/*n*(%) or mean±SD
Participants number	35
Age (years)	60.54±5.39
Males	20/35 (57.1)
Females	15/35 (42.9)
Ascites	30/35 (85.7)
Encephalopathy	4/35 (11.4)
Serum bilirubin (mg/dl)	3.36±2.66
Serum albumin (g/dl)	2.46±0.56
Prothrombin time more the control time (s)	5.76±4.98
Corrected serum calcium (mg/dl)	9.73±1.07
Serum PTHrP (pg/ml)	170.86±201.23
Child–Pugh score	
Mean score	10±1.6
Stage A	2/35(5.7)
Stage B	15/35(42.8)
Stage C	18/35(51.4)

HCC, hepatocellular carcinoma; PTHrP, parathyroid hormone-related protein.

Figure 1



PThrP =Parathyroid hormone related polypeptide.Pg/ml=picrogram/millilitre.

Serum PThrP of studied HCC patients.

belonged to Child–Pugh class B. Approximately 85% of the patients suffered from ascites, and ~11% of them had hepatic encephalopathy. Further data about HCC patient's liver functions tests and their biochemical parameters were also included in the aforementioned table.

Serum parathyroid hormone-related peptide level and albumin-corrected serum calcium in patients with hepatocellular carcinoma

The mean±SD serum level of PTHrP in patients with HCC was 140.55±40.07 pg/ml (range: 19–1002.5 pg/ml and confidence interval 95%: 63.28–220.36 pg/ml) (Fig. 1).

The mean±standard deviation of albumin-corrected serum calcium concentration was 9.8±1.07 mg/dl (range: 8.8–14.6 mg/dl). Thirty-two (91.4%) patients were normocalemic and three (8.6%) patients were hypercalcemic (Fig. 2).

There was no statistically significant correlation between serum PTHrP levels and albumin-corrected calcium levels (linear regression, r=–0.91; P=0.64) (Fig. 3).

Discussion

In the current study, 3 of the 35 studied cirrhotic patients with HCC (8.6%) were hypercalcemic, and there was no significant correlation between serum PTHrP detected by ELISA and albumin-corrected serum calcium level in these patients.

Three of the 35 studied patients with HCC (8.6%) were hypercalcemic. Prior studies reported that the prevalence of hypercalcemia in patients with HCC varied from 1.5 to 40% [5,6]. This big variation was attributed as previously mentioned to the possible inclusion of patients with HCC having bone metastases in some of these studies.

More recently, Chang *et al.* [8] in 2013 reported that 5.3% of patients with HCC had hypercalcemia, and Qu *et al.* [13] in 2014 reported that 8% of 175 patients with HCC included in their study had hypercalcemia. The results of the current study are extremely close to that reported by Qu *et al.* [13].

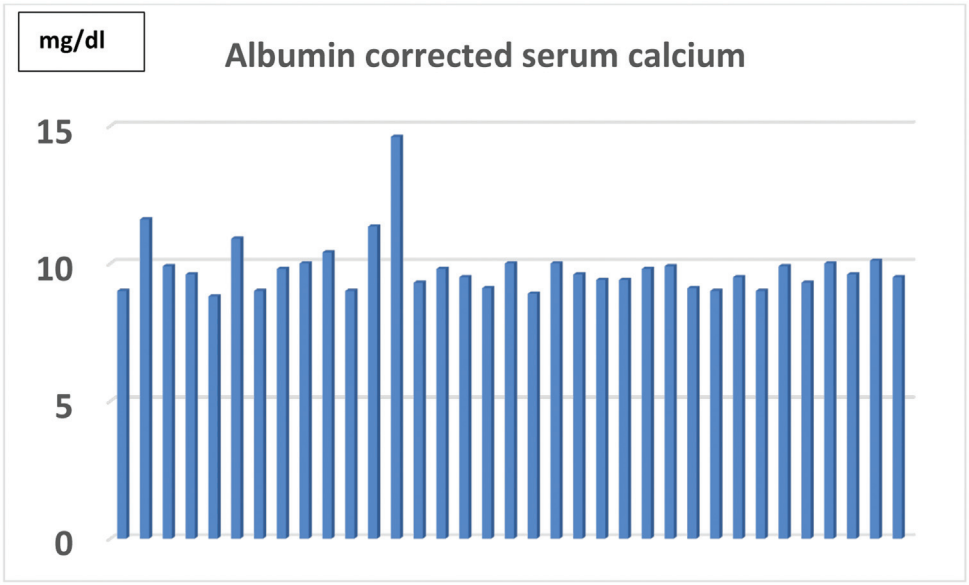
No significant correlation between serum PTHrP levels detected via ELISA and albumin-corrected serum calcium levels was detected in the current study. To the best of our knowledge, there are no reported studies in the English medical literature that evaluated the aforementioned correlation in cirrhotic patients with HCC.

Upon reviewing the studies that evaluated the correlation between albumin-corrected serum calcium and serum PTHrP in other solid malignancies, the results appeared to be conflicting.

Papworth *et al.* [14] in 2005 reported that in 243 patients with renal carcinoma, there was a significant positive correlation between PTHrP and serum calcium. Interestingly, Albright's postulation about the role of PTH-like factor in the development of hypercalcemia of malignancy was reported in a patient with renal carcinoma [9].

On the contrary, Deans *et al.* [15] reported that in 151 patients with gastroesophageal carcinoma, 26 (17.2%)

Figure 2



Albumin-corrected serum calcium of studied patients.

of them had elevated levels of C-terminal fraction of PTHrP, but similar to the results of this study, they were not able to detect a significant correlation between serum level of C-terminal fraction of PTHrP and that of serum calcium concentration.

The above conflicting results could be attributed to the use of different serum PTHrP assay methods in the previously mentioned studies, or complexity of the mechanism underlying hypercalcemia in malignancy.

PTHrP mechanism of action on bones is complex. It enhances the synthesis of osteoblast receptor activator for nuclear factor Kappa-B ligand (RANKL) [16], which results in increased interaction between osteoblast RANKL and receptor activator for nuclear factor Kappa-B (RANK) receptors on the surface of osteoclasts leading to enhanced osteoclastic activity and more bone resorption and consequent hypercalcemia [17].

Serum calcium levels in patients with solid malignancy is probably not regulated by the solo action of PTHrP, but it is probably regulated by multiple humoral factors released by solid tumors other than PTHrP.

Prior studies showed that hypercalcemia in solid malignancy patients could be attributed to various humoral factors, including interleukin 1 (IL-1), IL-3, IL-6, tumor necrosis factor-α, transforming growth factor a and b, lymphotoxin, and E series prostaglandins [17–19].

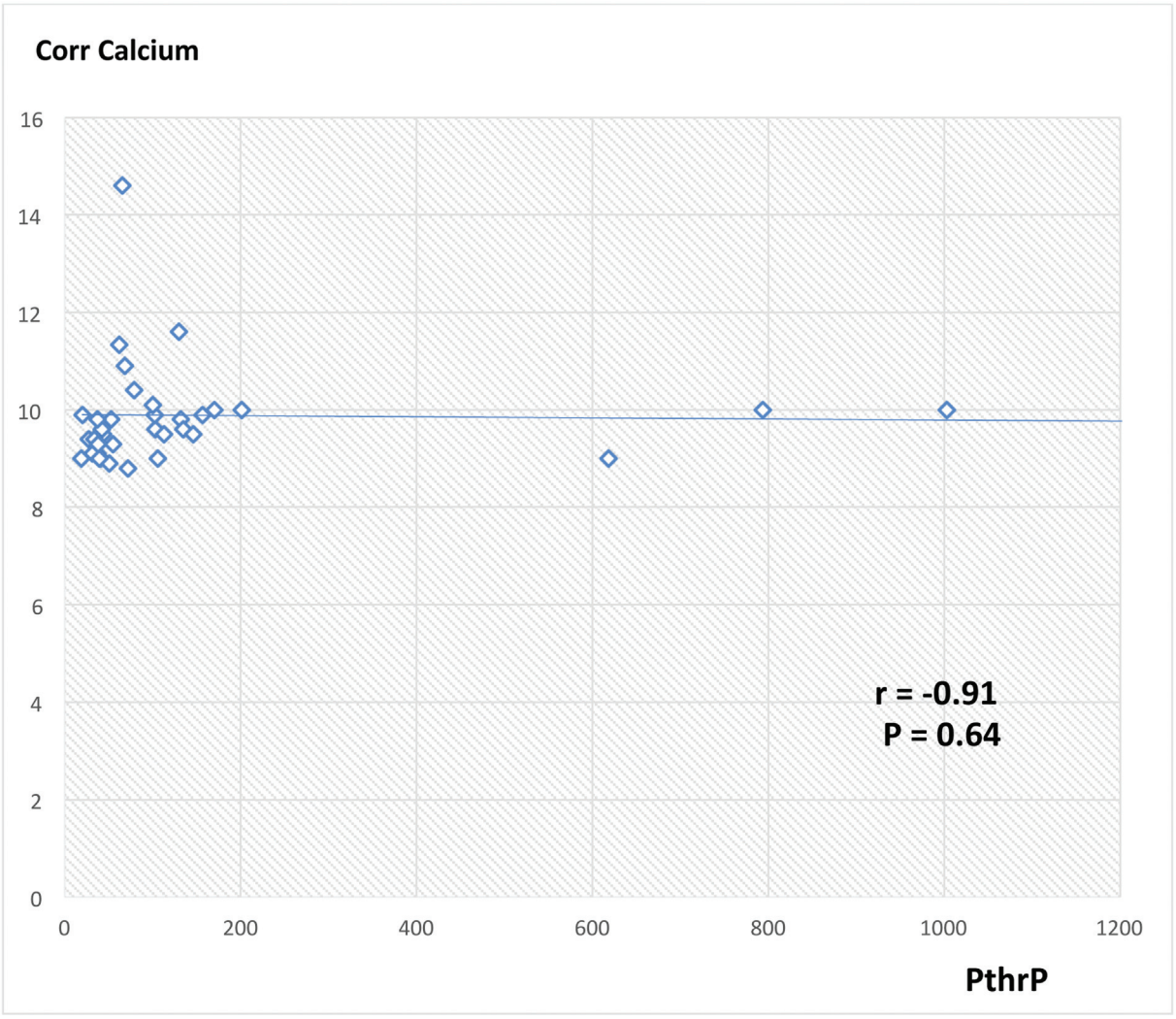
The current study sheds light on the interesting topic of hypercalcemia in cirrhotic patients with HCC, and to the best of our knowledge, no prior published studies have evaluated the important correlation between serum PTHrP and albumin-corrected serum calcium in these patients.

Despite being the first study to evaluate the above correlation, it has the following limitations, which might had affected the accuracy of its results: first, the small number of participants of the current study might had limited the accuracy of its results, but this is just a pilot study that aimed to shed light on a previously unexplored topic, and larger studies are needed to confirm or contradict its results; second, bone scans to exclude the presence of bony metastases were not performed as a routine part of the current study, so it is possible that patients with HCC having occult bone metastases were included in the current study; and third, diagnosis of HCC in all study participants depended upon evaluation of AFP, abdominal ultrasound, or computerized tomography in the absence of histological confirmation.

Conclusion

In conclusion, ~8.6% of studied cirrhotic patients with HCC were hypercalcemic. No statistically significant positive correlation was detected between serum PTHrP determined by ELISA and albumin-corrected serum calcium in these patients.

Figure 3



Corr Calcium=albumin corrected serum calcium and PThrP =Parathyroid hormone related polypeptide.

Correlation between albumin-corrected serum calcium and parathyroid hormone-related peptide.

The above results suggest that serum calcium levels in cirrhotic HCC patients are probably not regulated just by the solo action of PTHrP, but it is probably regulated by multiple humoral factors released by the tumor other than PTHrP.

Future studies regarding a possible correlation between various tumour related factors (other than PTHrP) and albumin-corrected serum calcium in cirrhotic patients with HCC could clarify humoral factor(s), which play the major role in regulating serum calcium level in these patients.

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Conflicts of interest
There are no conflicts of interest.

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