Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Serum Calcium is a New Indicator to Evaluate Metabolic Syndrome in Hepatocellular Cancer

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SUMMARY

Background: **The role of serum calcium in hepatocellular cancer (HCC) patients with features of metabolic syndrome (MetS) is largely unknown. This study aimed to determine the association of serum calcium with clinical features, and the correlation between serum calcium and metabolic parameters, including serum fasting plasma glucose (FPG) and lipids, in HCC patients.**
Methods: **The study included 180 HCC patients. Unpaired *t*-test and covariance analysis were performed to evaluate the distribution of serum calcium among different categorical variables. Simple correlation analyses and partial correlation analyses were conducted to assess the correlations between serum calcium and metabolic parameters.**
Results: **HCC patients with cirrhosis had significantly lower total serum calcium than those without cirrhosis, and patients with distant metastasis had significantly higher corrected calcium than their counterparts after adjusting for confounders. Significant correlations between total calcium and metabolic parameters were observed in HCC patients, and these correlations were still significant after adjusting for cirrhosis and distant metastasis. However, the corrected serum calcium showed no significant correlation with metabolic parameters.**
Conclusions: **Serum calcium, especially total serum calcium, might be a more sensitive indicator for metabolic syndrome in HCC patients than FPG and lipids.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190943)

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Manuscript accepted November 3, 2019

KEY WORDS

hepatocellular cancer, calcium, metabolic syndrome, glucose, lipids

INTRODUCTION

Hepatocellular cancer (HCC) is a severe threat to human health worldwide. It was predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death [1]. It has been clear that hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, nonalcoholic fatty liver disease (NAFLD), and cirrhosis were etiological risk factors and strongly associated with development of HCC [2]. In addition, metabolic syndrome (MetS) was also considered a well-established risk factor for HCC. Dysglycemia and dyslipidemia, two important components of MetS, have been reported to be associated with hepatocellular cancer [3]. It

has been concluded by the American Diabetes Association and the American Cancer Society that type 2 diabetes mellitus (T2DM) was closely associated with an increased risk of liver cancer [4], while hyperlipidemia was protective against HCC and related to lower future HCC mortality [5].

Ionized calcium (Ca^{2+}) is a critical ion and participates in a variety of intracellular processes, including membrane excitability, activation of enzymes, and regulation of gene transcription [6]. It has been shown that the functional T-type Ca^{2+} channels probably participate in modulating the proliferation of hepatocellular carcinoma cells [7], and a monoclonal antibody 1B50-1 has a therapeutic effect on HCC by targeting the Ca^{2+} channel α2δ1 subunit [8]. Total serum calcium is a critical source of intracellular Ca^{2+} [9] and has been described as correlated with several different malignancies. Female patients with malignant pelvic masses possessed higher albumin-corrected serum calcium than those with benign pelvic masses [10], and albumin-corrected serum calcium was also positively associated with disease stage of cutaneous melanoma [11]. However, the relationship between serum calcium and HCC is not yet clear.

It has been demonstrated that serum calcium was tightly correlated with metabolic profiles in serum. There were significant positive correlations of serum fasting plasma glucose (FPG) with calcium in healthy subjects and T2DM patients [12,13], and albumin-adjusted serum calcium was also strongly correlated with total cholesterol (TC) and high-density lipoprotein (HDL) in women [14]. However, it was reported in another study that serum calcium was not significantly associated with FPG, low-density lipoprotein (LDL), HDL, and TC in overweight and obese vitamin D deficient women with polycystic ovary syndrome [15]. Therefore, the relationship between serum calcium and metabolic profiles remains controversial.

In this study, we aimed to determine the distribution of total serum calcium and albumin-corrected serum calcium among HCC patients and evaluate the association of serum calcium with FPG and serum lipids. Total serum calcium, FPG, and serum lipid profiles were detected, and the relationships between serum calcium and clinical features of HCC patients were assessed. In addition, we performed correlation analyses between serum calcium and metabolic parameters. It is hypothesized that serum calcium was correlated with FPG and serum lipids in HCC patients.

MATERIALS AND METHODS

Study population

A total of 235 patients initially diagnosed with HCC in Peking University People's Hospital from October 2015 to January 2019 were included in this study. The clinicopathological features, including age, gender, BMI, history of diabetes and hypertension, and some other aspects, were obtained. The research was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Peking University People's Hospital. Informed consent was obtained from all patients before their participation.

The inclusion criterium for this study was newly diagnosed and pathologically confirmed HCC in patients in our hospital from October 2015 to January 2019. The exclusion criteria included: 1) HCC patients with parathyroid disease or chronic kidney disease; 2) HCC patients with other malignancies; 3) missing pathological report or data; and 4) missing data for serum calcium, albumin, or metabolic parameters, including FPG, TG (triglycerides), HDL, LDL, and TC. Corrected calcium, the biologically active fraction of serum calcium, was estimated by a standard formula [16]:

Corrected serum calcium (mmol/L) = total serum calcium (mmol/L) + 0.02 x [40 - albumin (g/L)]

First, 235 patients were identified with confirmed HCC in our hospital from October 2015 to January 2019. Patients with CKD (chronic kidney disease) (n = 6), with a history of primary colorectal cancer, breast cancer or lung cancer (n = 14), missing pathological report (n = 21), or missing serum calcium, albumin, or metabolic parameters (n = 14) were excluded. Finally, a total of 180 patients were included in this study (Figure 1).

Next, the clinicopathological features and metabolic parameters of 180 patients with histologically confirmed HCC were summarized in Table 1. Newly diagnosed HCC patients, including 144 men and 36 women, had a mean ± SD age of 58.1 ± 11.5. Of all the patients, 31.7% were overweight with a BMI (body mass index) > 25 kg/m^2, 16.7% had a history of diabetes, and 27.2% had a history of hypertension. Of the patients, 79.4% were HBsAg-positive, 76.1% had cirrhosis, 18.9% had NAFLD, and 13.3% had a history of long-term alcohol abuse. For the pathologic characteristics of these patients, 63.9% had a tumor ≤ 5 cm^3, 65.6% had low/moderate-differentiated cancer, 39.4% had vessel invasion, and 15.6% had distant metastasis. Serum calcium and metabolic parameters, including FPG, TG, HDL, LDL, and TC, are displayed as mean ± SD in Table 1 as well.

Biochemical analysis

PB (Peripheral blood) samples were collected separately, and serum was separated by centrifuging at 4,000 x g for 5 minutes. Pretreatment serum calcium, FPG, TG, HDL, LDL, TC, and albumin were detected by Beckman AU5832 automatic biochemical analyzer. All tests were performed with original reagents produced by the manufacturers according to standard operation procedures, and calibration and quality control were performed sequentially before testing to ensure the accuracy of the test.

Table 1. Clinical features and metabolic parameters of included patients.

Variables (n = 180)	Values
Age (years)	58.1 ± 11.5
≤ 60	99 (55.0)
> 60	81 (45.0)
Gender	
Male	144 (80.0)
Female	36 (20.0)
BMI (kg/m^2)	
Normal (≤ 25)	123 (68.3)
Overweight (> 25)	69 (31.7)
History of diabetes	
Yes	30 (16.7)
No	150 (83.3)
History of hypertension	
Yes	49 (27.2)
No	131 (72.8)
HBsAg	
Negative	37 (20.6)
Positive	143 (79.4)
Cirrhosis	
Yes	137 (76.1)
No	43 (23.9)
NAFLD	
Yes	34 (18.9)
No	146 (81.1)
Alcohol intake (> 5 g/day)	
Yes	24 (13.3)
No	156 (86.7)
Tumor size (cm^3)	
≤ 5	115 (63.9)
> 5	65 (36.1)
Differentiation	
Low/Moderate	118 (65.6)
High	62 (34.4)
Vessel invasion	
Yes	71 (39.4)
No	109 (60.6)
Distant metastasis	
Yes	28 (15.6)
No	152 (84.4)
Metabolic parameters	
FPG (mmol/L)	6.04 ± 2.40
TG (mmol/L)	1.14 ± 0.64
HDL (mmol/L)	1.05 ± 0.30
LDL (mmol/L)	2.58 ± 0.76
TC (mmol/L)	4.11 ± 0.92
Serum calcium	
Albumin (g/L)	39.24 ± 5.09
Total serum calcium (mmol/L)	2.26 ± 0.12
Corrected serum calcium (mmol/L)	2.28 ± 0.09

Results are presented as number (%) or mean ± SD. BMI - body mass index, HBsAg - hepatitis B surface antigen, NAFLD - nonalcoholic fatty liver disease, FPG - fasting plasma glucose, TG - triglycerides, HDL - high-density lipoprotein, LDL - low-density lipoprotein, TC - total cholesterol, SD - standard deviation.

Statistical analysis

All analyses were conducted with GraphPad Prime 5.01 (GraphPad Software Inc, CA, USA) or SPSS 20.0 software (IBM Corporation, Armonk, NY, USA). All data are displayed as mean ± standard deviation (SD). Unpaired *t*-tests were used to compare the clinical indicators between different age groups, gender groups, BMI groups, and some other clinical features at the time of diagnosis. Multivariable covariance analysis adjusted for confounders in cirrhosis or distant metastasis were carried out. Simple correlation analyses were conducted to evaluate the correlation between total calcium or corrected calcium and metabolic profiles. Partial correlation analyses adjusted for cirrhosis and distant metastasis were carried out as well. All statistics were two-tailed, and a p-value < 0.05 was considered statistically significant.

RESULTS

Comparison of serum calcium in HCC patients with different clinical features

Serum calcium in patients with different clinical features were compared by unpaired *t*-test. Patients with cirrhosis had lower total serum calcium than those without cirrhosis (2.25 ± 0.12 vs. 2.29 ± 0.10 mmol/L; p = 0.037). For other clinical features, there were no significant differences between paired groups. In addition, we also performed comparisons on corrected serum calcium. It was found that patients with distant metastasis had higher corrected serum calcium than those without distant metastasis (2.32 ± 0.06 vs. 2.27 ± 0.09 mmol/L; p < 0.0001). Other paired groups showed no significant differences.

To determine the influence of cirrhosis and distant metastasis, covariance analyses were performed. After adjusting for confounders, total serum calcium was still lower in patients with cirrhosis than those without cirrhosis (p = 0.035), and corrected calcium was still higher in patients with distant metastasis compared to those without distant metastasis (p = 0.003) (Table 2).

Table 2. Comparison of serum calcium in HCC patients with different clinical features.

Characteristics	Total calcium (mmol/L)	p	Corrected calcium (mmol/L)	p
Age (years)				
≤ 60	2.27 ± 0.11	0.258	2.28 ± 0.09	0.793
> 60	2.25 ± 0.12		2.28 ± 0.08	
Gender				
Male	2.25 ± 0.12	0.051	2.28 ± 0.09	0.961
Female	2.30 ± 0.75		2.28 ± 0.07	
BMI (kg/m^2)				
Normal (≤ 25)	2.25 ± 0.12	0.075	2.27 ± 0.09	0.231
Overweight (> 25)	2.28 ± 0.10		2.29 ± 0.08	
History of diabetes				
Yes	2.26 ± 0.12	0.936	2.30 ± 0.08	0.150
No	2.26 ± 0.12		2.27 ± 0.09	
History of hypertension				
Yes	2.25 ± 0.13	0.403	2.27 ± 0.09	0.278
No	2.27 ± 0.11		2.28 ± 0.08	
HBsAg				
Negative	2.27 ± 0.11	0.594	2.28 ± 0.09	0.933
Positive	2.26 ± 0.12		2.28 ± 0.09	
Cirrhosis				
Yes	2.25 ± 0.12	0.037 * ‡	2.28 ± 0.08	0.267
No	2.29 ± 0.10		2.26 ± 0.10	
NAFLD				
Yes	2.28 ± 0.10	0.325	2.27 ± 0.08	0.751
No	2.26 ± 0.12		2.28 ± 0.09	
Alcohol intake (> 5 g/day)				
Yes	2.23 ± 0.13	0.123	2.28 ± 0.07	0.984
No	2.27 ± 0.11		2.28 ± 0.09	
Tumor size (cm^3)				
≤ 5	2.26 ± 0.12	0.983	2.27 ± 0.09	0.116
> 5	2.26 ± 0.11		2.29 ± 0.08	
Differentiation				
Low/ Moderate	2.26 ± 0.12	0.777	2.28 ± 0.09	0.275
High	2.27 ± 0.11		2.27 ± 0.09	
Vessel invasion				
Yes	2.27 ± 0.12	0.677	2.29 ± 0.09	0.113
No	2.26 ± 0.11		2.27 ± 0.08	
Distant metastasis				
Yes	2.27 ± 0.12	0.659	2.32 ± 0.06	< 0.0001 * &
No	2.26 ± 0.12		2.27 ± 0.09	

Results are presented as mean ± SD. BMI - body mass index, SD - standard deviation. * p < 0.05, ‡ Multivariable analysis adjusted for distant metastasis (p = 0.035 *), & Multivariable analysis adjusted for cirrhosis (p = 0.003 *).

Table 3. Correlation analyses between serum calcium levels and metabolic parameters.

Parameters	Total calcium				Corrected calcium			
	r *	p *	r †	p †	r *	p *	r †	p †
FPG	-0.224	0.003 ‡	-0.221	0.003 ‡	-0.045	0.544	-0.045	0.547
TG	0.227	0.002 ‡	0.210	0.005 ‡	0.065	0.386	0.040	0.595
HDL	0.154	0.040 ‡	0.158	0.036 ‡	-0.128	0.087	-0.110	0.144
LDL	0.309	< 0.0001 ‡	0.298	< 0.0001 ‡	0.159	0.033 ‡	0.126	0.095
TC	0.347	< 0.0001 ‡	0.332	< 0.0001 ‡	0.102	0.172	0.083	0.273

FPG - fasting plasma glucose, TG - triglycerides, HDL - high-density lipoprotein, LDL - low-density lipoprotein, TC - total cholesterol. * Pearson's correlation coefficients between serum calcium and metabolic parameters, † Partial correlation coefficients between serum calcium and metabolic parameters, adjusted for cirrhosis and distant metastasis, ‡ $p < 0.05$.

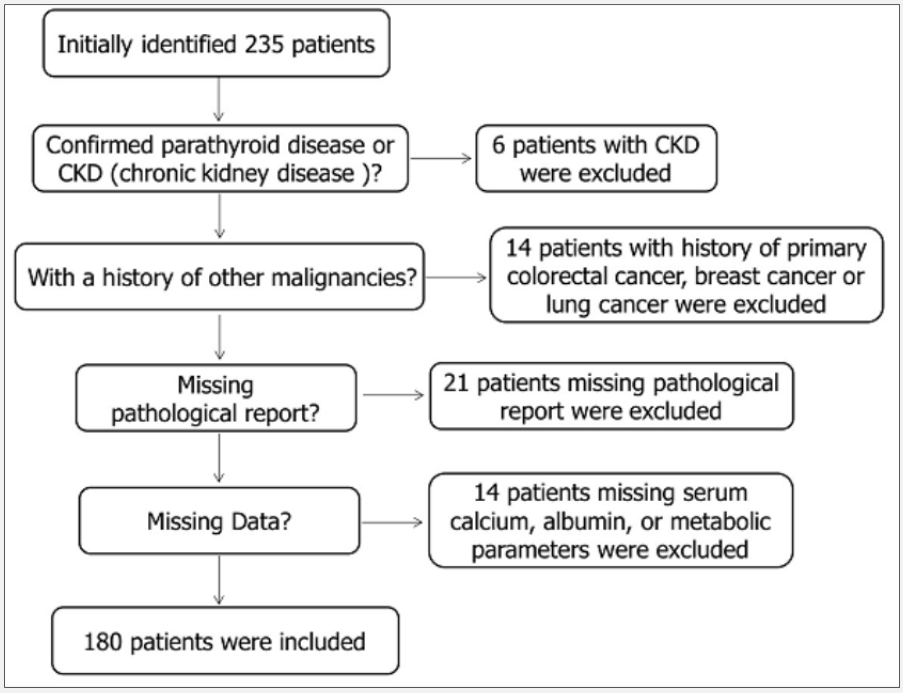

Figure 1. The diagram of patient selection process.

Correlation analyses between serum calcium and metabolic parameters

Total serum calcium showed significant and negative correlation with FPG ($p = 0.003$), and significant positive correlation with TG ($p = 0.002$), HDL ($p = 0.040$), LDL ($p < 0.0001$), and TC ($p < 0.0001$). However, corrected serum calcium was only correlated with LDL ($p = 0.033$), but not other metabolic parameters (FPG, $p = 0.544$; TG, $p = 0.386$; HDL, $p = 0.087$; TC, $p = 0.172$) (Table 3).

Next, partial correlation analyses were performed between serum calcium and metabolic parameters, adjusted for cirrhosis and distant metastasis. The significant correlations of total serum calcium with FPG ($p = 0.003$), TG ($p = 0.005$), HDL ($p = 0.036$), LDL ($p < 0.0001$), and TC ($p < 0.0001$) were still observed after adjustment for these variables. However, there was no correlation between corrected serum calcium and FPG

and serum lipids in the partial correlation analysis (FPG, p = 0.547; TG, p = 0.595; HDL, p = 0.144; LDL, p = 0.095; TC, p = 0.273) (Table 3).

DISCUSSION

In this study, we found that total serum calcium was negatively correlated with FPG and positively correlated with serum lipids in HCC patients, and these correlations were still significant after adjusting for multiple variables. However, the correlation was not obvious regarding corrected serum calcium. HCC patients with cirrhosis had significantly lower total serum calcium than those without cirrhosis, and corrected serum calcium was significantly higher in patients with distant metastasis compared to their counterparts.

As Ca^{2+} is a critical modulator of the cell cycle and indispensable for cell proliferation, glucose and lipids might be critical for tumorigenesis by regulating Ca^{2+} and Ca^{2+}-mediated signaling pathways. It has been demonstrated that a high glucose concentration causes an acute rise in cytosolic Ca^{2+} due to an increased calcium influx into cardiac myocytes [17]. In addition, cholesterol has been reported to activate the Ca^{2+} channel and thereby increases Ca^{2+} entry, which results in upregulated cell proliferation and migration of prostate cells [18]. The effect of glucose control and lipid-lowering drugs on HCC has also been studied. It has been reported that metformin, a classical glucose control drug, inhibits hepatocellular carcinogenesis and decreases HCC risk [19, 20], and statins used to reduce the level of blood cholesterol are associated with a reduced risk of HCC as well [21]. Basic research suggests that these drugs may exert anticancer activity via Ca^{2+} [22,23]. Therefore, there is a tight relationship between Ca^{2+} and metabolic profiles. We assumed that serum calcium has an influence on metabolism-impaired HCC by regulating cytosolic Ca^{2+} concentration. In the future, more fundamental research is needed to prove this hypothesis.

Interestingly, this study suggested lower total serum calcium in HCC patients with cirrhosis and higher corrected calcium in HCC patients with distant metastasis. The cirrhosis and distant metastasis state of HCC patients has been shown to be associated with metabolic syndrome. HCC in patients with features of MetS is likely to occur in the absence of significant fibrosis and cirrhosis in the liver [24,25] and at early stages without distant metastasis at diagnosis [26,27]. Therefore, serum calcium was likely to be associated with dysregulated FPG and serum lipids in HCC patients with cirrhosis and distant metastasis. Unfortunately, HCC patients with different cirrhosis or distant metastasis states had similar levels of FPG and most lipids, and only LDL was higher in HCC patients with distant metastasis (data not shown). The reason for the inconsistency might be that our patients have used glucose control and lipid-lowering drugs to control abnormal glucose and lipids before being diagnosed with HCC, but we could not trace back the complete medication history of the patients.

This study has some limitations. First, it was a single-center study and only 180 patients were involved, so selection bias is inevitable. In addition, medication history might also be a confounder in this study, but we could not trace back the complete medication history of the patients and were therefore not able to include it into our analyses.

CONCLUSION

Despite these limitations, our study is the first to demonstrate correlations between serum calcium and FPG as well as serum lipids in HCC patients. Total serum calcium or corrected serum calcium, but not FPG or most lipids, was found to be associated with cirrhosis and distant metastasis state of HCC patients, indicating that serum calcium, especially total serum calcium, might be a novel and more sensitive indicator when assessing the effect of MetS on HCC. Multi-centered clinical studies and basic research are needed to further verify our findings.

Acknowledgment/Financial Support:
This work was supported by National Natural Science Foundation of China grant 81702788.

Declaration of Interest:
The authors have declared no conflicts of interest.

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