ORIGINAL ARTICLE

Comparison of Ionized Calcium and Albumin Corrected Total Calcium Concentration in Renal Failure Patients at Shalamar Hospital Lahore

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ABSTRACT

Background: Calcium plays many crucial functions including skeletal mineralization, blood coagulation, neuromuscular conduction, preservation of cell membrane integrity and permeability. Free calcium is useful index than total calcium and provides better indication of calcium status. Many laboratories still use Total calcium measurements with correcting formulas which lack accuracy, and reports of new equations still occasionally appear in the literature.
Aim: To find the correlation between measured serum calcium(total), calculated serum calcium and ionized serum calcium in renal patients.
Methods: A total of 160 patients of renal failure and 80 healthy subjects were included in the study. The tests performed included the renal function tests (urea & creatinine), total calcium, albumin and ionized calcium. The ionized calcium was performed on electrolyte analyzer Diestro.
Results: Total calcium shows a positive correlation with albumin, corrected calcium and ionized calcium. While albumin is not correlated with corrected calcium and ionized calcium. **Conclusion:** The use of ionized calcium rather than albumin-corrected calcium would influence the calcium classification of the individual patient.
Keywords: Renal failure, corrected calcium, ionized calcium.

INTRODUCTION

The physiological importance of calcium is far-reaching. It has got some essential and distinctive but interdependent intracellular and extracellular activities. Intracellular calcium is a crucial regulator of numerous cellular events, including muscle contraction, hormone secretion, glycogen metabolism, and cell division. Extracellular calcium not only provides a steady supply of calcium for intracellular use but also plays an important role in clotting and membrane integrity[1]

Calcium in plasma or serum exists in three forms: 1) ionized or free calcium, 2) calcium bound to proteins (primarily albumin), and 3) complexed or chelated calcium, bound to a variety of anions (phosphate, bicarbonate, sulfate, citrate, and lactate)[1,2]. Together, the ionized and complexed calcium constitute the diffusible fraction of calcium. This portion may also be called the ultrafilterable calcium, since it passes through biologic membranes. About 90% of the protein-bound calcium is linked to albumin with the remaining 10% bound to a variety of globulins. Because approximately half of the calcium is protein bound, the interpretation of total calcium depends on the values for serum albumin and total protein[3].

Experiments by Moore[4] and McLean and Hastings[5,6] confirmed that ionized calcium accounts for the biologically active form of serum calcium and is involved in calcium homeostasis of healthy individuals and patients with parathyroid abnormalities.

Many equations have been proposed to determine ionized calcium concentrations[7,8]. These equations are essentially empirical equations based on a correlation between ionized calcium concentration measured by the ion selective electrode method and an observed concentration for albumin or another substance [9].

Laboratories routinely determine the total calcium concentrations which measure all three forms of calcium. Corrected calcium concentration estimates the total concentration as if the albumin concentration was normal. Clinical laboratories use these equations to calculate corrected calcium values. Some involve the albumin level, while others use total protein[1].

Precision in ionized calcium measurement was revolutionized after the introduction of ion-selective electrodes[3]. Numerous studies have identified that the direct measurement of ionized calcium in cases of patients receiving transfusions with citrated blood;

in critically ill patients; and in patients with the late stages of chronic kidney disease (CKD), hyperparathyroidism, and hypercalcemia of malignancy is clearly superior to its calculation from total calcium and albumin. Similarly in the later stages of CKD, pH and albumin fluctuations may also alter relative calcium fractions unpredictably[3].

Although the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines recommend the use of albumin-corrected total calcium, published equations do not accurately predict ionized calcium[10,11]. Direct measurements of ionized calcium, which are rarely done in this patient population, are important for optimal clinical decision-making. Hypercalcemia may be falsely reported, when total calcium and albumin measurements are used to estimate ionized calcium[10]. Given the superiority of direct measurements of ionized calcium in identifying patients with primary hyperparathyroidism compared with estimates based on corrected total calcium, it is likely that estimates based on total calcium will be similarly inaccurate in identifying patients with CKD and secondary and tertiary hyperparathyroidism.

Clinical guidelines for the treatment of disturbances in mineral and bone metabolism in patients with chronic kidney disease have recently been published[12]. The main factor for regulation of parathyroid hormone (PTH) secretion is the extra cellular ionized calcium concentration[13]. In clinical guidelines, the target level for serum calcium is given as albumin corrected total calcium concentration and no target level is given for the concentration of ionized calcium[10,12]. It is time that clinicians realize the importance of free rather than total calcium determination for actual body calcium status.

In the present study, concentration of total calcium, ionized calcium and corrected total calcium based on the serum albumin level were evaluated in renal failure patients and the correlation between these biochemical parameters was assessed so as to determine whether free (ionized) calcium levels was a better index of calcium status of the body, as compared to measured total calcium levels.

MATERIALS AND METHODS

A cross sectional study was undertaken from June 2012 to December 2012 on patients who reported to the outpatient dialysis clinic of the Shalamar Hospital. A total of 190 patients of renal failure were included in the study. 90 healthy subjects as controls were also included in this study. Patients of renal failure were included in group A whereas group B comprises of healthy subjects. Standard precautions were taken while collecting blood samples. The use of sterile disposable needle and vaccutainer were used for sample collection. The tourniquet was not applied as it can affect serum calcium levels. The vacutainers for estimation of ionized calcium was transported immediately to the laboratory. All the tests were performed within 2 hours of sample collection.

Methodologies: Serum Total Calcium, albumin, ionized calcium, urea and creatinine were performed. Ionized Calcium was performed on Diestro 103 AP Electrolyte Analyzer by employing Ion Selective Electrode. Total Calcium, Albumin, Urea and Creatinine were performed on Mindray BS-400 which is a fully automated chemistry analyzer. Serum total calcium was performed by Arsenazo III Method and serum albumin by Bromocresaol green (BCG) method. Serum urea was performed by using urease method and serum creatinine by creatinine PAP method. The data was analyzed using SPSS version 19.0. For calculating corrected calcium following formula was used[8].

Corrected Serum Calcium(mg/dl)= Total serum calcium (mg/dl) + 0.8 [4- serum albumin (g/dl)]

RESULTS

One hundred and ninety patients of renal failure and ninety healthy control subjects were included in this study. The patients were recruited from Shalamar Hospital Dialysis unit. The mean age range of patients and controls was 54.65±15.4 years and 40.96±19.9 years respectively. The values for measured and calculated biochemical parameters are illustrated in table 1.

In renal failure patients a positive correlation was found between albumin and total calcium (r=.286, p value=.001). A negative correlation was observed between albumin and corrected calcium (r=-.201, p value=.005). Similarly negative correlation was observed between albumin and ionized calcium(r=-.191, p value=.007).

Total calcium showed a positive correlation with ionized calcium (r=0.489, p value= < 0.001) and corrected calcium(r=.871, p value <0.001). Similarly ionized calcium has a positive correlation with corrected calcium (r=0.6, p value<0.001)

Table 1: Mean levels of albumin, corrected calcium, total calcium and ionized calcium in renal failure patients and controls

Parameter	Patient (Mean±SD)	Control (Mean±SD)	P Value
Albumin	**3.35±0.78**	**3.63±0.80**	0.008
Total calcium	7.71±1.2	7.84±1.3mg/dl	0.440
Corrected calcium	8.24±1.27	8.14±1.3mg/dl.	0.559
Ionized calcium	4.43±0.69mg/dl	4.51±0.64 mg/dl	0.377

Fig. 1: Relationship between Total calcium and ionized calcium

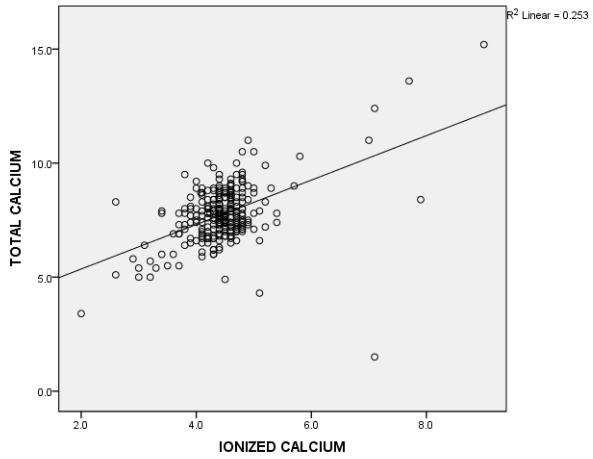
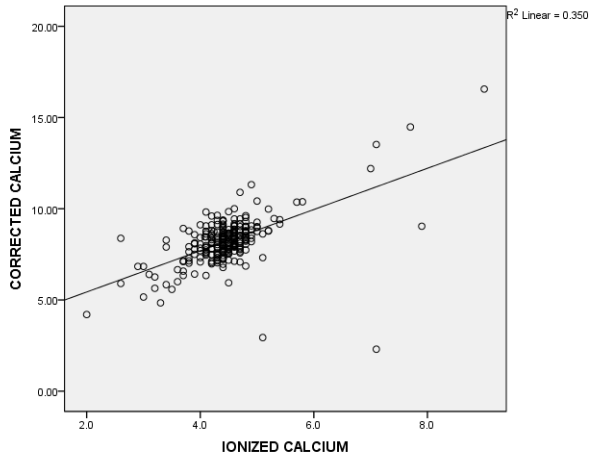


Fig. 2: Relationship between Ionized with corrected calcium



DISCUSSION

Accurate measurement of serum calcium in patients with chronic renal failure is very important as both hypo and hypercalcemia are considered as the predictor of mortality. The vitamin D metabolites which are used for the normalization of serum calcium levels and in the prevention and treatment of hyperparathyroidism in patients with CRF is associated with increase in serum calcium levels[11].

Clinical studies have revealed that the free calcium is a better indicator of the calcium metabolic disturbances than total calcium in diseases such as acute acid-base disturbances, dysproteinemia, hyperparathyroidism, renal stones and renal insufficiency[14]. James et al in their findings showed that diagnostic accuracy of CRF patients was enhanced when i-PTH and free calcium data were used. In keeping with the increased accuracy of ionized Ca in defining hypercalcemia previously reported[14]

In present study, corrected calcium showed a significant increase than measured total calcium in renal failure patients. Corrected total calcium showed a significant increase than ionized calcium. This increase is probably due to variation in total protein concentration and variable binding of calcium to protein (albumin) in different individuals. Variations in corrected total calcium were observed because of the change in total protein concentration especially albumin, so corrected total calcium may not reflect actual calcium status in hypoproteinemic or hyperproteinemic conditions. These findings are in accordance with the work of Thode *et al*[14].

Direct measurements of ionized calcium, which are rarely done in our patient population, are important for optimal clinical decision-making.[10] In particular, hypercalcemia may be over diagnosed when total calcium and albumin measurements are used to estimate ionized calcium, leading to potentially inappropriate clinical choices regarding the use of vitamin D and its analogues[10].

In patients with renal failure, additional studies comparing the direct measurement of ionized calcium with that of estimated ionized calcium using published algorithms showed different results.

Citrate also binds calcium thus lowering the ionized calcium and inhibiting blood coagulation. Direct measurements of ionized calcium are routinely necessary in patients treated with continuous venovenous hemofiltration used for dialysis, especially when citrate is used as the anticoagulant. In this case ionized calcium must be measured not only in the systemic circulation but also in relation to the dialyzer to determine adequacy of anticoagulation and to detect citrate toxicity. Because direct determinations of citrate are rarely performed, it is not possible to correct for the reduction of ionized calcium caused by the binding of calcium to citrate; in this setting, it is imperative that ionized calcium be measured directly[3].

Abundant evidence establishes the importance of ionized calcium in renal failure patients. The algorithms to predict ionized calcium from total calcium have not proved accurate, especially in patients with complex illness. In the critical care setting, ionized calcium should be the routine measurement as well as where procedures such as continuous hemofiltration. In the outpatient setting, estimating ionized calcium from measurements of total calcium and albumin remain more feasible; however, direct measurement of ionized calcium is now suggested in several ambulatory conditions, including patients in the later stages of CKD as well as in patients with suspected hyperparathyroidism.

With time, the number of these conditions will almost certainly expand and measurements of ionized calcium will become the routine, preferred method for determining the level of calcium in all patients. This beneficial evolution in a clinical measurement should lead to demonstrable improvements in patient care[3].

Use of albumin-corrected calcium concentrations may lead to inappropriate clinical decisions with withdrawal of vitamin D, calcium containing phosphate binders and reduction of calcium concentration in the dialysis fluid of a patient classified as hypercalcaemic.

In our study, fewer patients would have been classified as hypercalcaemic using ionized calcium than would have been the case if albumin-corrected calcium was used for calcium classification. In our study albumin-corrected calcium could not substitute for ionized calcium in classifying patients as hypo-, normo- or hypercalcaemic. In present scenario the correlation between total calcium and albumin was considerably positive while negative correlation was observed between albumin and ionized calcium(r=-.191, p value=.007). Findings of the present study were also supported by the work of Sorva *et al*[15]. Measurement of total calcium for the diagnosis of 'normocalcaemic' Hyperparathyroidism may be replaced by measurement of free calcium [16]. The data provided by George *et al*[17] and the many studies that support them clearly indicate that ionized Ca is the test of choice in nearly all-medical diagnostic and treatment situations.

Thus, the sound analytical performance of today's electrolyte analyzers and the inherent superiority of ionized Ca measurements over total Ca measurements permit us to state that ionized Ca is a test for the clinical laboratory.

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