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Original Article

Evaluation of Adjusted Calcium Levels in Patients of Chronic Renal Failure with Hypo-Albuminemia

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ABSTRACT

Objective: To compare adjusted calcium levels with ionised Calcium in chronic renal failure patients with hypoalbuminemia for the correct assessment of their Calcium status.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Mar 2020 to Jan 2021.

Methodology: A total of 304 individuals aged 18 to 90 years, of either gender were included in the study. Participants were differentiated into stages of Chronic renal failure based on the estimated glomerular filtration rate calculated through the Chronic Kidney Disease Epidemiology Collaboration equation. Serum total Calcium, urea and creatinine were analysed on ADVIA 1800R Clinical Chemistry Auto analyser. In addition, ionised Calcium was analysed on Cobas b221 blood gas and electrolyte analyser.

Results: Mean age of the study participants was 56.3±16.03 years. 130(42.5%) individuals had stage-5 while 102(33.3%) had stage 4 chronic kidney disease. Based on ionised Calcium concentration, 37.3% of participants had hypocalcaemia, 57.2% had normocalcaemia, and 4.9% had hypercalcaemia. 57.5% had albumin concentration between 20-29g/L. Cohen's Kappa statistical analysis showed adjusted Calcium to be a poor predictor of the correct Calcium status of the patients with hypoalbuminemia.

Conclusion: Adjusted Calcium, using the Modified Payne formula, overestimates Calcium status in patients of chronic renal failure with hypoalbuminemia when compared with total unadjusted Calcium levels hence misclassifying true hypocalcaemic patients as normocalcaemic.

Keywords: Adjusted calcium, Chronic renal failure, Estimated glomerular filtration rate, Hypoalbuminemia, Ionised calcium.

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INTRODUCTION

Calcium measurement is a frequently requested test in a laboratory setting under different clinical conditions. Calcium levels are tightly regulated in a not-so-wide range (2.1-2.65mmol/L).[1] Disturbed serum Calcium level is not so uncommon laboratory finding in hospitalised patients.[2,3] However, the exact prevalence of altered Calcium homeostasis is unknown, and abnormalities of Calcium concentration can have widespread effects on neurological, renal, gastrointestinal, and bone function.[4] Calcium is among the six most common elements found in the body.[5,6]

Despite the proven fact that it is the ionised or free fraction of Calcium that is physiologically active and should be employed in the interpretation of Calcium homeostasis abnormalities under pathological conditions with low albumin levels such as CKD or in hospitalised patients, especially in those on palliative care, most of the laboratories measure total Calcium levels instead of free Calcium.[7] Most laboratories use dye-binding methods such as *o-cresol phthalein* or arsenazo (III) method for the estimation of total Calcium. The alternative of direct measurement of Calcium is complicated by problems due to pre-analytical considerations including sampling pre-requisites and manual handling precluding full automation, with pH changes due to delayed separation and exposure to ambient air been the most important pre-analytical concerns.[8] The pre-analytical requirements can be accomplished by promptly separating serum from red cells and using properly sealed containers until analysis.[9] There are wide-scale problems associated with total Calcium measurement in the clinical setting, especially in those individuals with hypoalbuminemia states such as in CKD, Liver failure, where there is a decrease in total Calcium level secondary to decreased binding with albumin. This has led to the formulation of different formulas for adjustment of Calcium levels to cater for these clinical states.[10] Adjusted Calcium (albumin dependent), being a calculated parameter, needs evaluation by comparing it with the directly measured parameter of ionised Calcium (albumin

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independent) in order to assess the correct Calcium status in patients of Chronic Kidney Disease with hypoalbuminemia.

METHODOLOGY

The cross-sectional study was carried out at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from March 2020 to January 2021 after ethical approval from the Institutional Review Board (IRB) of AFIP (FC-CHP18-5/READ-IRB/20/465). The sample size was calculated by the WHO sample size calculator taking 5.09% as disease prevalence of Chronic Kidney Disease(CKD).[11]

Inclusion Criteria: Patients having chronic kidney disease (CKD) aged 18 to 90 years, of either gender were included in the study.

Exclusion Criteria: Patients with a pH outside the reference interval of 7.35–7.45 were excluded to reduce any bias in ionised Calcium measurements.

All participants had Chronic Kidney Disease based on their creatinine levels and glomerular filtration rates and were differentiated into different stages of Chronic renal failure based on estimated glomerular filtration rate (eGFR) calculated through Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) using their age, gender, ethnicity and serum creatinine concentration.[12,13]

Internal quality control was run before the analysis of samples. All parameters were analysed according to standard operating procedures. Informed consent was taken from study participants. After collecting serum samples, total serum Calcium was measured by the Arsenazo III method. Serum albumin and creatinine levels were analysed using the bromocresol purple and alkaline picrate methods on the ADVIA 1800R Clinical Chemistry Auto analyser. Venous sample for ionised Calcium (iCa) was collected in a lithium heparin tube and kept at 2-4°C before analysing it on Cobas b221 ABGs and electrolyte analyser using direct ISE technique. Study participants were divided into three categories based on their Calcium status using ionised Calcium and total Calcium, and adjusted Calcium levels. Individuals with total Calcium below 2.1mmol/L and ionised Calcium below 1.13mmol/L were considered hypocalcaemic, with total Calcium between 2.1-2.65mmol/L and ionised Calcium between 1.13-1.32mmol/L taken as normocalcaemic and individuals with total Calcium >2.65 mmol/L or ionised Calcium >1.13mmol/L were classified as hypercalcaemic. Modified Payne formula (Adjusted Calcium (mmol/L)=Total Calcium (mmol/L)+0.018x[40-albumin(g/L)] was used to determine the adjusted Calcium status of individuals. Hypoalbuminemia of study participants was stratified into three groups; group one had albumin concentration <20g/L, Group-2 had albumin concentration between 21-29g/L and Group-3 had albumin concentration between 30-39g/L.

Statistical Package for Social Sciences (SPSS) version 21.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Cohen's kappa was applied to determine the agreement between ionised Calcium and adjusted Calcium levels of study participants.

RESULTS

A total of 304 individuals between having chronic kidney disease (CKD) were included in the study. 130(42.5%) patients had stage-5 CKD, 102(33.3%) had stage-4, 54(17.6%) and 8(2.6%) were suffering from stage 3b and 3a CKD, respectively, and 8(2.6%) had Stage-2, whereas only two individuals had Stage-1 CKD as shown in Figure-1. Distribution for study participants based on ionised, total unadjusted and adjusted Calcium concentration is shown in Figure-2.

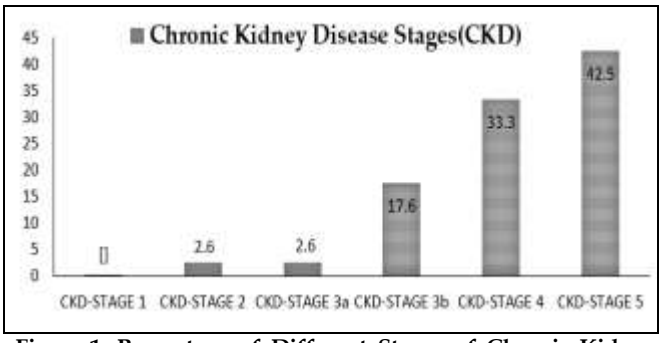

Figure-1: Percentage of Different Stages of Chronic Kidney Disease (CKD) in Sample Population (n=304)

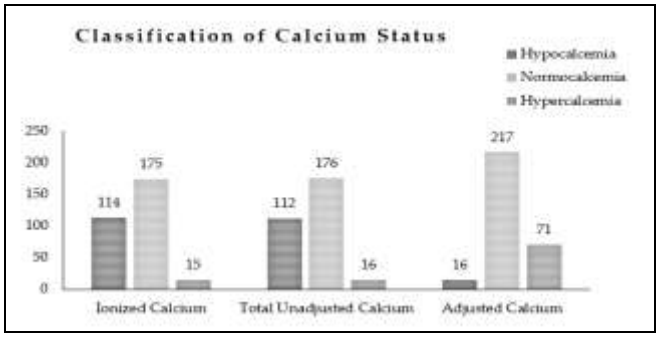

Figure-2: Classification of Calcium Status of Sample Population (n=304)

Cohen's kappa statistic was utilised to show agreement between the study participants' ionised Calcium and adjusted Calcium levels. (Table-I) Out of the 114 individuals with hypocalcemia based on ionised Calcium, only 16(14%) were correctly classified as hypocalcemic by adjusted Calcium levels where, and 91(85.1%) were misclassified as having normocalcemia. 120(68.6%) of the total 175 individuals having normocalcemia according to ionised Calcium were correctly classified as normocalcemic by adjusted Calcium levels. However, 100% of individuals having hypercalcemia were correctly classified as hypercalcemic by the adjusted Calcium levels.

Table-I: Cohen's Kappa Analysis Between Adjusted Calcium and Ionized Calcium (n=304)

Ionized Calcium Status	Adjusted Calcium Status			*p*-value
	Hypocalcaemic	Normocalcaemic	Hypercalcaemic	
Hypocalcaemic	16(14%)	97(85.1%)	01(0.9%)	<0.001
Normocalcaemic	0	120(68.6%)	55(31.4%)	
Hypercalcaemic	0	0	15(100%)	
Total	16(5.2%)	217(71.4%)	71(23.3%)	

κ=0.098 (95% CI, 0.01–0.20)

The application of Cohen's Kappa to find agreement between total unadjusted Calcium and ionised Calcium levels of patients is shown in Table-II. Out of the 114 individuals having hypocalcemia based on ionised Calcium, 110(96.5%) were correctly classi-fied as hypocalcemic by total Calcium levels where, and only 3(2.6%) were misclassified as having normocalcemia. 173(98.9%) of the total 175 individuals having normocalcemia according to ionised Calcium were correctly classified as normocalcemic by total unadjusted Calcium levels. However, 100% of individuals having hypercalcemia were correctly classified as hypercalcemic by the total unadjusted Calcium levels.

Table-II: Cohen's Kappa Analysis between Total Unadjusted Calcium and Ionized Calcium (n=304)

Ionized Calcium Status	Total Unadjusted Calcium Status			*p*-value
	Hypocalcaemic	Normocalcaemic	Hypercalcaemic	
Hypocalcaemic	110(96.5%)	03(2.6%)	01(0.9%)	0.002
Normocalcaemic	02(1.1%)	173(98.9%)	-	
Hypercalcaemic	0	0	15(100%)	
Total	112(36.8%)	176(57.9%)	16(5.2%)	

κ=0.96 (95% CI, 0.81-1.00)

Comparison of Total unadjusted Calcium levels with the Ionised Calcium using Cohen's kappa statistical analysis showed total Calcium to be an excellent predictor of correct Calcium status of the patients with hypoalbuminemia with a κ-value of 0.96(0.81-1.00), indicating an almost perfect agreement between Ionized Ca and total unadjusted Calcium values of individuals.

DISCUSSION

This study has investigated the ability of Adjusted-Calcium to correctly assign Calcium status as compared with ionised Calcium as the gold standard and assessed whether, in CKD patients with hypoalbuminemia, Calcium status estimation by adjusted Calcium may be misrepresented.

As indicated by κ value of 0.098, this study showed poor agreement between the Calcium status of individuals with CKD having hypoalbuminemia based on ionised Calcium and adjusted Calcium levels. Whereas κ-value of 0.96 showed near-perfect agreement between the Calcium status of the same individuals between ionised and total unadjusted Calcium levels. Based on these results, we can safely say that the Calcium status of patients based on adjusted Calcium levels of these patients with CKD having albumin below 39g/L establishes falsely high Calcium concentration of such patients. Our study results indicated that total unadjusted Calcium is a better indicator of the correct Calcium status of CKD patients with hypoalbuminemia compared to total adjusted Calcium using the modified Payne formula, taking ionised Calcium as the gold standard. This was in accordance with another study conducted by Smith *et al*.[14] which showed that adjusted Calcium tends to overestimate the actual Calcium status of individuals with albumin levels below 3g/dl(30g/L) having CKD. Grzych *et al*.[15] concluded that total Calcium with albumin correction wrongly reflects the true Calcium status (as per ionised Calcium) in hospitalised patients with CKD. In contrast, un-adjustment Calcium seems more reliable to assess the Calcium status of such individuals. A study by Lian *et al*.[16] showed that the diagnostic accuracy of total unadjusted Calcium was greater than several formulas used for adjusted Calcium calculation, which coincided with our study results. Similarly, a study conducted by Mateu-de Antonio *et al*.[17] showed that the adjusted serum Calcium values did not precisely classify Calcium status in 41% of the study cases that included hypoalbuminemia patients undergoing hemodialysis due to CKD. The sensitivity and specificity of the formula for corrected Calcium to assess hypocalcaemia were 53% and 85%, respectively, leading to the conclusion that Calcium homeostasis should be assessed by ionised Calcium concentrations instead of total serum Calcium.

Based on these results, modification of total Calcium concentration based on albumin levels has no substantial advantage in assessing the correct Calcium status of individuals with CKD with hypoalbuminemia.[18] Although the Kidney Disease advises: Improving Global Outcomes (KDIGO) that ionised Calcium measurement (iCa) is ideal and that if total Calcium concentration is used as an alternative, then it should be corrected in the case of hypoalbuminemia, however, our study results in conjunction to many others show otherwise. Therefore, instead of using the modified Payne formula for albumin-adjusted Calcium, it is better to derive a community-based correction formula for Calcium adjustment. In our study, we have focused only on CKD patients with albumin levels below 39g/L. Further research needs to be carried out to check the validity of the Modified Payne formula in patients with other pathological conditions and normal albumin levels.

CONCLUSION

Using the Modified Payne formula, adjusted Calcium overestimates Calcium status in patients of chronic renal failure with hypoalbuminemia when compared with total unadjusted Calcium levels hence misclassifying true hypocalcaemic patients as normocalcaemic.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

HH & ZHH: Study design, drafting the manuscript, data interpretation, approval of the final version to be published.

MA & MUM: Conception, drafting the manuscript, approval of the final version to be published.

SI & HJ: Data acquisition, data analysis, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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