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Full Length Research Paper

Derivation of a formula for adjusting the total serum calcium in Nigeria environment

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The total calcium concentration, total protein, albumin and globulin were estimated for 302 patients that reported for serum calcium estimation at the clinical biochemistry laboratory of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria. Based on regression analysis, three formulae were derived for adjusting the total serum calcium concentration. It was observed that the total serum concentration correlated closely with albumin (α = 0.919 70) but poorly correlated with total protein (α = -0.25 960); where α = correlation coefficient. Adjusted calcium = total calcium – (0.91972 x albumin) + 3.70429 was obtained.

Key words: Total serum calcium, adjusting formula, serum albumin.

INTRODUCTION

Calcium is necessary for several physiological processes. These include neuromuscular transmission, smooth and skeletal muscle contraction, cardiac automaticity, nerve function, cell division and movement. It is also a cofactor for many steps during blood coagulation. Intracellular calcium is involved as a second messenger in many intracellular responses to chemical and electrical stimuli and required by many enzymes for full activity. Many different calcium binding proteins have been described, but the two with well established functions are troponin and calmodulin. Troponin is involved in muscle contraction, whereas calmodulin causes configurational changes to proteins and enzyme activation (Ganong, 2003).

The maintenance of a constant free ionised calcium concentration is very important for physiological functions. Normal calcium concentrations are maintained as a result of tightly regulated ion transport by the kidneys, intestinal tract and bone. Parathyroid hormone, and active form of vitamin D, are involved in calcium homeostasis. Calcium sensing receptors have also been identified. The total serum calcium is accounted for as calcium bound to protein, ionized calcium and calcium complexed to citrate, lactate, sulphate, carbonate and phosphate. The calcium bound to protein and ionized calcium is roughly in equal proportion (Payne et al., 1973).

It is the ionized calcium that is clinically important (Mahendra, 2001); therefore ionized calcium should be measured directly if a disturbance of a calcium homeostasis is suspected. However, this is not possible in many hospitals in Nigeria because of the cost of the instruments involved; hence some other screening procedures are necessary for estimating ionized serum calcium.

Many proposed formulae are available in literature for estimating ionized calcium. All of them are derived outside Nigeria. Such formulae have been based on total serum protein (Payne et al., 1973), on plasma specific

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Table 1a. Shows concentrations of Total Calcium, Total Protein, Albumin and Globulin in the 302 subjects for the study, as well as the reference range.

S/No	Parameter	Concentration Obtained	Reference Range
1	Total Calcium	6.40-12.0mg/100ml	9.0-11.mg/100ml 2.25mmol/L – 2.75mmol/L
2	Total Protein	3.0-9.8gm/100ml	4.5-9.0gm/100ml
3	Albumin	1.0-4.9gm/100ml	2.4-4.5gm/100ml
4	Globulin	2.0-5.0gm/100ml	2.1-4.5gm/100ml

Table 1b. Distribution of serum calcium status as observed in the 302 subjects, in relation to the reference range. *The entire study population and the observed result.

Number Among The Study Population	Parameter	Concentration Obtained	Reference Range
302*	100%*	6.40-12.0mg/100ml *	9.0-11.0mg/100ml
47	15.56%	Low Calcium level	
234	77.48%	Normal Calcium level	
21	6.96%	Elevated Calcium level	

gravity (Berry et al., 1973), or on plasma albumin (Khan and Desborough, 2001). Various adjusting factors have been derived from various adjusting formulae and they are expressed as mmol/L change in calcium for each 1 g/dL change in albumin. The following factors have been obtained by various workers; 0.018 (Orrwel, 1971), 0.21 (Wills and Lewin, 1971), 0.25 (Payne et al., 1973), 0.22 (Pain et al., 1975) and 0.20 (Khan and Desborough, 2001)

Adjusting total serum calcium relieves anxiety about the patients whose abnormal total calcium value results from abnormal protein values and have not reflected a disorder of calcium metabolism. The aims of this study are to derive an adjusting formula for total serum calcium estimation in Nigeria environment and test the suitability of the derived formula in the environment.

MATERIALS AND METHODS

A total of three hundred and three (302) patients were used for deriving the adjusting formula. These patients were chosen out of the three hundred and thirty-seven (337) patients who were randomly selected from the patients that reported at the clinical biochemistry laboratory of Olabisi Onabanjo University Teaching Hospital Nigeria. The remaining thirty-five (35) patients were excluded from the study, as they were considered unsuitable, because a few of them had renal problems (as indicated by renal function tests), and others with history suggestive of a bone disease, endocrine disorder, unpredictable acid-base status and poor nutrition. The 302 patients were within age range 23 – 52.

The 302 selected patients were bled, taking 10.0 ml of whole blood in each case and the serum specimens obtained. The serum specimens were then analysed for total calcium, total protein, albumin, globulins and phosphate. Cresolphthalein complexone was used to determine total calcium (Gitelman, 1967), biuret method for total protein (Henry et al., 1957), bromocresol green for albumin (Webster and Bignell, 1974) and globulins values was obtained by subtracting the value of albumin from the value of the total protein. Stannous chloride hydrazine reagent was used for phosphate (Berenblum and Chain, 1938).

The correlation coefficients, standard errors and regression coefficients of total calcium on total protein, total calcium on albumin and total calcium on globulins were calculated with the aid of computer. Thereafter, based on the computed parameters, three adjusting formulae for serum total calcium were derived (Little et al., 1974). The derived adjusting formulae were then applied on the estimated total calcium of the 302 patients used for this study. The formulae for adjusting total calcium was further applied, for their suitability in the study environment, on 902 serum specimens which were randomly selected from the serum specimens of the routine laboratory work, for serum calcium estimations, of the Teaching Hospital Laboratory over a period of two years.

RESULTS

The various observations are as presented below. Table 1a shows the range obtained for total calcium concentration, total protein concentration, albumin concentration and globulin concentration in the 302 subjects used for this study. When the range of total calcium concentrations obtained in the 302 subjects were compared with the reference range for total calcium concentrations; it was found that 47 (15.56%) of the subjects had low calcium concentration, 21 (6.96%) had elevated calcium concentration and 234 (77.48%) had normal calcium concentration (see Table 1b).

Relation between total calcium and total protein

There was a significant ($P<0.001$) but poor positive correlation between total calcium and total protein concentrations ($\alpha = 0.44250$). The standard error in the correlation coefficient was 0.002 and the regression coefficient was 0.44245. The regression equation of total calcium on total protein was obtained as total calcium =

Table 2. Shows total calcium concentration in the 302 subjects before and after adjusting on total protein, albumin, and globulins concentrations.

Measured total calcium for 302 subjects	Mean mg/100ml	SD mg/100ml	95%Limits mg/100ml
(i) Unadjusted total calcium values	9.59	0.77	8.05 -11.13
(ii) Values of total calcium adjusted on total protein(formula A)	9.99	0.70	8.89-11.39
(iii) Values of total calcium adjusted on albumin (formula B)	9.99	0.45	9.09 -10.89
(iv) Values of total calcium adjusted on globulin (formula C)	9.99	0.75	8.49 -11.49

Table 3. Total serum calcium in 902 patients before and after adjusting on albumin concentration.

Measured total calcium for 902 patients	Mean Conc. mg/100ml	S.D. mg/100ml	95%Limits mg/100ml
(i) Unadjusted values	9.41	0.80	7.81 – 11.01
(ii) Values after adjusted on albumin concentration	9.87	0.43	9.01 – 10.73

Table4. Shows the percentages of the 902 patients (on which the albumin conc. Adjusting formula for total calcium conc. was tested) having low values, raised values, and normal values for total calcium conc. before and after adjusting the value.

Measured total calcium value	No. of patients with low values (<9.0mg/100ml)	No. of patients with raised values > 11.0mg/100ml	No. of Patients with normal values (9-11mg/100ml)
(i) Unadjusted	229 (25.39%)	51 (5.65%)	622 (68.96%)
(ii) Values adjusted on albumin concentration	104 (11.53%)	34 (3.79%)	764 (84.70%)

(0.44245 x total protein) + 6.25809. Hence:

Adjusted calcium = total calcium – (0.44245 x total protein) + 3.74191

(Formula A)

Relation between total calcium and albumin

There was a significant (P<0.001) good positive correlation between total calcium and albumin concentrations (α =0.91970). The standard error in the correlation coefficient was 0.001. The correlation coefficient was significantly greater than that of total calcium on total protein. The regression coefficient of total calcium on albumin was 0.91972 and the regression equation was obtained as total calcium = (0.91972 x albumin) + 6.29571. Hence:

Adjusted calcium = total calcium – (0.91972 x albumin) + 3.70429

(Formula B)

Relation between total calcium and globulins

There was a significant (P<0.001) but very poor negative correlation between total calcium and globulin concentration (α = –0.25960). The standard error in the correlation coefficient was 0.001 and the regression equation was obtained as: total calcium = (–0.25962 x globulins) + 10.61167. Hence:

Adjusted calcium = total calcium + (0.25962 x globulin) – 0.61167.

(Formula C)

Application of the derived formulae on the total calcium of the 302 subjects in this study gave the results presented in Table 2. From Tables 2 and 1a, formula B (total calcium adjusted on albumin as shown in table 2) is the most appropriate. Here, the 95% limits (Table 2) gave a value of 9.09-10.89 mg/100 ml, compared with the normal reference range (Table 1a) of 9.0 -11.0 mg/100 ml (2.25 -2.75 mmol/L). Finally, the suitability of formula B (total calcium adjusted on albumin) in our environment was then tested on 902 sera randomly selected and the result is presented in Table 3. Further analysis revealing the statistical distribution of the study population (the 902 sera) before and after adjusting on albumin is shown in Table 4.

DISCUSSION

The various results shown in this study revealed that many a time serum total calcium is estimated roughly.

More accurate results would be obtained if the adjusting formula could be adopted. For instance, Table 1b shows that while 77.48% of the 302 patients had total calcium that fell within the reference range, there are 15.56% that had low levels and 6.96% with elevated levels. The patients indicated to be hypocalcaemia or hypercalcaemia here are not all correctly diagnosed. This is evident from the results in Table 2, which revealed different mean values for total serum calcium for unadjusted and the adjusted values. Also different are the values of the 95% limits for the various adjusting formulae and the unadjusted. A clearer picture is seen with the results of tables 3 and 4. It is revealed here that formula B is the most suitable one (formula based on albumin concentrations). Out of the 902 sera on which the suitability of the adjusting formula was tested, 229 patients had low total serum calcium values, 51 had raised values and 622 had normal values prior to adjustment. After adjustment, using albumin concentration adjusting formula, 104 had low total serum calcium, 34 had raised values and 764 had normal values. This shows that 125 were regarded as hypocalcaemia in error and 17 as hypercalcaemia in error.

Among the various adjusting formulae, the one based on albumin concentration is the most suitable, and it gives a satisfactory adjustment (Payne et al., 1979). In calculating serum total calcium, the use of an average regression coefficient as applied in this work had been challenged because it is correctly argued that patients with high or low ionized calcium concentration would have higher or lowers regression coefficients (Marshall and Nordin, 1974). However, it has been revealed that in adjustment based on albumin, values that are higher than should be when ionized calcium was high and lower when ionize calcium was low, fulfilled the intended function as a screening procedure (Payne et al., 1974). Better still, flame photometric determination could be carried out in lieu of our method. However, most hospitals in Nigeria especially those in semi-urban and rural areas do not have access to flame photometric determinations. It has also been argued that the use of an average regression coefficient is inappropriate because tourniquet and posture experiments show that there are significant differences even between normal individuals in regression of calcium on albumin (Pain et al., 1975).

However Payne et al. (1979) proved that there are good grounds for using a common regression coefficient that has been derived from normal and abnormal albumin concentrations. They, however, emphasized that a single co-efficient will not make appropriate adjustments for the variable changes that may follow venous stasis and it is therefore still important to take blood samples with a free flow technique; this was the situation in this study.

Our adjusting formula is necessary to avoid wrong labeling of patients as being hypocalcaemia or hypercalcaemia. In conclusion, the use of our formula: adjusted calcium = total calcium – (0.91972 x albumin) + 3.70429 is recommended for use in routine laboratory estimation of total serum calcium in Nigeria and in those countries having similar health setting with Nigeria.

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