SERUM CALCIUM MEASUREMENT : TOTAL VERSUS FREE (IONIZED) CALCIUM

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ABSTRACT

Measurement of serum free (ionized) calcium (Ca^{++}) reflects true calcium status of the body in health and disease. Present study evaluates efficacy of Ca^{++} over total calcium (CaT) in serum for calcium status. 52 subjects were enrolled for study. Anaerobic fasting blood sample for Ca^{++} measurement and autoclaved plain bulb for estimation of CaT, Total protein (TP) and Albumin was used. CaT, Corrected CaT, Ca^{++} , Calculated Ca^{++} were measured and correlated. Corrected CaT and calculated Ca^{++} were derived from the measured parameters. Study group showed significant difference between CaT and corrected CaT ($p < 0.006$), Ca^{++} and calculated Ca^{++} ($p < 0.001$). Negligible correlation was observed between Ca^{++} and serum protein. Positive correlation was observed between CaT and calculated Ca^{++} , TP and albumin. Findings indicate that Ca^{++} levels are independent of serum protein status. With scrupulous sampling, Ca^{++} may be a better parameter than presently used CaT for assessing calcium status in serum.

KEY WORDS

Albumin, Anaerobic Sampling, Calculated Total Calcium, Corrected Free Calcium, Free Calcium, Total calcium.

INTRODUCTION

Calcium, the king of minerals is the fifth most common element and the most prevalent cation found in the body which has a very important role to play in skeletal mineralization, blood coagulation, neuromuscular conduction, maintenance of normal tone and excitability of skeletal and cardiac muscle, stimulus secretion of exocrine glands and preservation of cell membrane integrity and permeability, particularly in terms of sodium and potassium exchange (1).

Hence calcium estimation has great clinical importance in many diseases. The oldest procedure for determination of total serum calcium concentration is that of Clark and Collip. The Clark-Collip procedure has in recent years been replaced by a number of more convenient and accurate methods involving flame atomic emission spectrometry, photometry and flurometry methods (2).

Along with this came the methods of measurement of "the physiologically important" fraction of calcium i.e. free calcium. Its significance was well established way

back in 1935 when McClean and Hastings observed that the amplitude of contraction of isolated frog heart is proportional to free calcium concentration (3, 4, 5, 6).

Free calcium is useful index than total calcium and provides the better indication of calcium status because, Ca^{++} , and not total calcium, is biologically active and tightly regulated by calcium binding hormones. Because calcium is predominantly transported bound to serum proteins, total calcium levels are greatly influenced by protein concentration especially albumin. Albumin is the principle transport and depot protein for calcium in blood plasma and the albumin bound fraction constitutes about half of the total concentration of calcium in plasma (7, 8).

Though the measurement of free calcium is clinically more useful, it could not substitute total calcium for want of quicker and accurate technology. This problem is now solved due to availability of ion-selective electrodes, for free calcium measurements (6). It is high time that clinicians realize the importance of free rather than total calcium determination for actual body calcium status. The rapidity and accuracy of Ion Selective Electrode (ISE) technique currently used compensates for the cost of the investigation.

In our country, malnutrition is quite a common problem, thereby influencing total protein concentration including albumin. The measured total calcium value is affected by total protein concentration particularly albumin. In this scenario false low calcium levels may be reported. This instigated the present study to be

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taken up, to determine whether free (ionized) calcium levels was a better index of calcium status of the body, as compared to measured total calcium levels.

MATERIAL AND METHODS

A prospective study was undertaken from December 2002 to September 2003 on 52 subjects which included 30 normal healthy individual and 22 osteoporotic subjects at Padmashree Dr. D.Y. Patil Medical College, Hospital and Research Centre. Fasting blood sample was collected from antecubital vein without tourniquet into two separate containers. (i) A 3 ml plain conical centrifuge capped tube filled till the brim. (ii) A plain bulb. The centrifuge tube was used for the estimation of free calcium for the purpose of anaerobic sampling which is a pre-requisite or crux of estimation of free calcium and the plain bulb sample was used for the estimation of Total Calcium, Total Protein and Albumin.

The measured biochemical parameters included -

- 1) Free (ionized) calcium - AVL 9180 Electrolyte analyzer (ISE) (8, 9).
- 2) Total Serum Calcium - Arsenazo III Method (10).
- 3) Total Serum Protein - Biuret Method (11).
- 4) Serum Albumin - Bromocresol Green (BCG) Method (12).

Values for Total Protein, Albumin and Globulin was estimated for derivation of **calculated parameters** which include -

- 5) Corrected Total Calcium (13).
- 6) Calculated Free Calcium (14).

Formulae for calculated parameters -

- 1) Corrected Total Calcium (mg/dl) = Total Calcium (mg/dl) + 0.8 (4 - Albumin(g/dl))
- 2) Corrected Total Calcium in mmol/Litre = Corrected Total Calcium (mg/dl) x 0.25
- 3) Calculated Free Calcium in mmol/Litre
 - i) % Protein bound = 0.8 x Albumin (g/L) + 0.2 x Globulin (g/L) + 3
 - ii) Calculated Free Calcium (mg/dl) = Total calcium - Protein bound Calcium
 - iii) Calculated Free Calcium in mmol/Litre = Calculated Free Calcium (mg/dl) x 0.25

The data so obtained was statistically analyzed using student's paired 't' test.

RESULTS

The values for measured and calculated biochemical parameters are illustrated in Table 1. Corrected total calcium showed a significant increase than measured total calcium ($p < 0.006$). Calculated free calcium also showed a significant increase than measured free calcium ($p < 0.001$).

Positive negligible correlation was observed between measured total calcium and calculated free calcium ($r = 0.27$) whereas considerably positive correlation was observed between measured total calcium and total serum protein ($r = 0.30$) and albumin ($r = 0.34$). Measured free calcium showed a negligible negative correlation with total serum protein ($r = -0.16$) while albumin showed a negligible positive correlation ($r = 0.02$).

DISCUSSION

The experimental and clinical findings of McLean and Hastings demonstrated unequivocally in the mid 1930s that Ca^{++} rather CaT is of primary physiological and clinical importance (3, 4, 5). Over the past 50 years, numerous investigators have repeatedly confirmed and extended this central role of Ca^{++} (18, 21, 22, 23). About half the calcium in serum is bound to protein or complexed. It is the other half, ionized and biologically active, that interests the clinician (8,15). Decrease in serum ionized calcium can cause tetany (involuntary muscle contraction) and related neurological symptoms, regardless of the total serum calcium concentration (20).

Clinical studies have also 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, multiple myeloma, renal stones and renal insufficiency (16, 17, 22). James et al in their findings showed that diagnostic accuracy was enhanced when C-terminal immuno-reactive parathyrin (i-PTH) and free calcium data were used because fewer patients were considered hypercalcemic, in keeping with the increased accuracy of Ca^{++} in defining hypercalcemia previously reported (21, 24).

In present study, corrected total calcium showed a significant increase than measured total calcium ($p < 0.006$). Calculated free calcium also showed a significant increase than measured free calcium ($p < 0.001$). This increase is probably due to variation in total protein concentration and variable binding of calcium to protein (albumin) in different individuals. Variations in calculated free calcium and corrected total calcium were observed because of the change in total protein concentration especially albumin so calculated parameters (calculated free calcium, corrected total calcium) may not reflect actual calcium status in hypoproteinemic or hyperproteinemic

Table 1. Measured and calculated biochemical parameters and Pearson's correlation co-efficient of measured and calculated parameters with serum proteins

Biochemical Parameters	Mean±S.D (n = 52)	Measured Total Calcium r-value	Measured Free Calcium r-value
Measured Total Calcium (mmol/L)	2.20 ± 0.20 *	NA	NA
Corrected Total Calcium (mmol/L)	2.32 ± 0.18 *	NA	NA
Measured Free calcium (mmol/L)	1.29 ± 0.12 **	NA	NA
Calculated Free Calcium (mmol/L)	1.41 ± 0.15 **	0.27	NA
Total Serum Protein (gm/dl)	7.5 ± 1.0	0.30	- 0.16
Serum Albumin (gm/dl)	3.6 ± 0.6	0.34	0.02
Results are expressed as Mean±S.D. n = number of subjects studied, NA - Not Applicable, * p<0.006 Statistically highly Significant ** p<0.001 Statistically highly Significant			

conditions. These findings are in accordance with the work of Thode *et al.* (25).

In present scenario the correlation between measured total calcium and total protein and albumin was considerably positive but weakly correlated with calculated free calcium, while measured free calcium showed a negligible correlation with total protein and albumin. Findings of the present study were also corroborated by the work of Sorva *et al.* (26).

Measurement of total calcium for the diagnosis of 'normocalcaemic' Hyperparathyroidism may be replaced by measurement of free calcium (27). The data provided by George *et al.* (6) and the many studies that support them clearly indicate that Ca⁺⁺ is the test of choice in nearly all-medical diagnostic and treatment situations. Thus, the sound analytical performance of today's second generation Ca⁺⁺ analyzers and the inherent superiority of Ca⁺⁺ measurements over CaT measurements permit us to state that Ca⁺⁺ is a test for the clinical service laboratory whose time has come!

SUMMARY

- 1) Measurement of free calcium by Ion Selective Electrode is a simple, rapid and accurate method for assessing calcium status.
- 2) With scrupulous anaerobic sampling, free calcium may be a better indicator than presently used total calcium for assessing calcium status in serum.

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REFERENCES

1. Alan, H. Gowenlock, Janet, R. McMurray and Donald, M McLanchlan (2002) Varley's Practical Clinical Biochemistry, 6th Ed., pg 601.
2. Robertson, W.G. and Marshall, R.W. (1979) Calcium measurement in serum and plasma : Total and Ionized. Crit. Rev. Clin. Lab. Scan. vol. 11, 271-304.
3. McLean, F.C. and Hastings, A.B. (1934) A biological method for the estimation of calcium ion concentration. J. Biol. Chem. vol. 107, 337-350.
4. McLean, F.C. and Hastings, A.B. (1935) The state of calcium in the fluids of the body. The conditions affecting the ionization of calcium. J. Biol. Chem. vol. 108, 285-322.
5. McLean, F.C. and Hastings, A.B. (1935) Clinical estimation and significance of Ca⁺⁺ ion concentration in the blood. Am. J. Med. Sci. vol. 189, 601-613.
6. George, N.B. Jr., Catherine, Brassard and Salvador, F. Sena (1986) Measurement of ionized calcium in serum with ion selective electrodes : A mature technology that can meet the daily service needs. Clin. Chem. vol. 3218, 1437-1447.
7. Kragh, Hansen V. and Vorum, H (1993) Quantitative analysis of the interaction between calcium ions and human serum albumin., Clin. Chem. vol. 39, 202-208.

8. Carl, A. Burtis and Edward, R. Ashwood (1990) Tietz , Clinical guide to laboratory tests, 2nd Ed, (Philadelphia : W.B. Saunders, 60), 98-99, 118-119, 456-459, 510-511, 720-721.
9. Bishop, M.L., Duben-Engelkirk, J.L. and Fody, E.P. (1992) Clinical chemistry principles procedures correlations. 2nd Ed. (Philadelphia : J.B. Lippin Cott Co.), 281.
10. Farrell, C.E. (1984) "Electrolytes" in clinical chemistry theory. Analysis and correlation. The C.V. Mosby Company. Kaplan L.A., Pesce. A.J. (Ed), Chap 55, 1054.
11. Carl, A. Burtis and Edward, R. Ashwood (1986) Tietz Text book of clinical chemistry, W.B. Saunders, 579.
12. Carl, A. Burtis and Edward, R. Ashwood (1986) Tietz Text book of clinical chemistry, W.B. Saunders, 589.
13. Carl A Burtis and Edward, R. Ashwood (1999) Tietz Text book of clinical chemistry, W.B. Saunders (Ed 3rd), 1400.
14. Isselbacher, Braunwad, Wilson, Martin, Fanci and Kasper (1994) Harrison's principles of internal medicine, 13th Ed. vol. 2, 2139.
15. Conceicao, S.C., Weightman, Doris, Smith, P.A. Luno, J. Ward, M.K. and Kerr, D.N.S. (1978) Serum ionized calcium concentration : Measurement versus calculation. B.M.J. vol. 1, 1103-1105.
16. Crottogins, A.J. and Siggaard, Anderson O. (1981) Plasma ionized calcium in the critically ill on total parental nutrition. Scand. J. Clin. Lab. vol. 41, 49.
17. Transhol, I. (1977) On the diagnosis of so called normally calcaemic hyperthyroidism. Acta. Med. Scand. vol. 202, 481.
18. Robertson, W.C. and Marshakk, R.W. (1981) Ionized calcium in body fluids. Crit. Rev. Clin. Lab. Sci. vol. 15, 85-125.
19. Burritt, M.F., Pierides, A.M. and offord, K.P. (1980) Comparative studies of total and ionized serum calcium values in normal subjects and patients with renal disease. Mayo Clin. Proc. vol. 55, 606-613.
20. Bhagavan, N.V. (2002) Medical biochemistry, 4th Ed, pg 874.
21. Ladenson, J.H., Lewis, J.W., McDonald, J.M., Slatopolsky, E. and Boyd, J.C. (1979) Relationship of free and total calcium in hyperglycemic conditions. J. Clin. Endocrinol. Metab. vol. 48, 393-397.
22. Ladenson, J.H. and Bowers, J.R.G.N. (1973) Free calcium in serum II. Rigor homeostatic control, correlations with total serum calcium and review of data on patients with distributed calcium metabolism. Clin. Chem. vol. 19, 575-582.
23. Toftaletti, J. (1986) Ionized calcium through its first decade : A review of the analytical aspects and clinical importance of ionized calcium measurements. NOVA Biomedical (Available from NOVA Biomedical Inc., 200 propects St., Waltham, M.A. 02254-9141).
24. James, C.B., John, W.C., Eduards, S. and Jack, H. Ladenson (1981) Parathyryn measured concurrently with free or total calcium in the differential diagnosis of hypercalcaemia. Clin. Chem. vol. 27/4, 574-579.
25. Thode, J.J., Jorgenson, B., Bhatia, H.M., Kjaerulf-Nielson, M., Bartels, P.D., Fogh-Anderson, N. and Siggaard-Anderson, O. (1989) Comparison of serum total calcium, albumin-corrected total calcium and ionized calcium in 1213 patients with suspected calcium disorders. Scand. J. Clin. Lab Invest. vol. 49 (3), 217-223.
26. Sorva, A., Elfving, Pohja P. and Tibis, R.S. (1988) Assessment of calcaemic status in geriatric hospital patients : Serum ionized calcium versus albumin-adjusted total calcium. Scand. J. Clin. Lab. Invest. vol. 48 (6), 489-494.
27. Editorial (1979) Serum calcium. Lancet I, 858.