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Correlation study between total calcium, ionized calcium, serum albumin and their significance with Vitamin D.

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Abstract

Calcium is an essential but controversial nutrient: there is no consensus on the level of human calcium requirement or the significance of calcium deficiency. Apart from providing rigidity to the skeleton, an alternate metabolic form of calcium viz., ionized calcium plays a key role in the biochemical and metabolic functions. The protection of this critical concentration by parathyroid hormone and vitamin D reflects the vital role that calcium plays in the neuromuscular system in regulation of the heart, in enzyme-mediated reactions and in many other metabolic processes. The objective of this study was to examine and establish the relationship between Total Calcium, Ionized Calcium and Serum Albumin and the significance of these factors with Vitamin D. For the samples, biochemical assay using electro-chemiluminescence technique (for Vitamin D) was used; Total Calcium and Serum Albumin were analyzed using manual dye-binding methods and the readings were acquired using a semi automatic analyzer. Appropriate statistical methods were used to conclude that, a very good correlation exists between Total calcium to VitaminD3 to that of Ionized Calcium to Vitamin D3 ($P<0.0001$) and similar correlation was also observed when the ratio of ionized calcium to albumin was compared. On the whole our study strongly recommends the measurement of all the four parameters for effective diagnosis of Calcium and Vitamin D related disorders.

Keywords: Total Calcium, Ionized Calcium, Serum Albumin and Vitamin D

INTRODUCTION

Calcium is the king of all alkalizing nutrients. The appropriate oxygen type of Calcium effectively douses acid the same way that water douses fire. It destroys oxygen robbing acid in the body fluid there by keeping the body alkaline so that it can prevent disease effortlessly. The Journals of the American Medical Association (JAMA) states that calcium prevents and reverses cancer. The American journal of nutrition states that virtually no major organ system escapes calcium influence. Dietary supplements of calcium along with vitamins prevent further spread of cancer. Chronic calcium deficiency is associated with some forms of hypertension, prostate and colorectal cancer, some types of kidney stones, miscarriage, premenstrual problems, joint and periodontal disease, sleep disturbances, mental depression and cardiovascular diseases. Calcium levels are also associated with arthritic or joint and vascular degeneration calcification of soft tissues, hypertension, and stroke, mood and depressive disorders inhibits the cancer protective effects of vitamins.

A weak heart rate means that calcium is deficient and the contraction phase is weak and short. This results in an increase in heart rate and also irregular heart rate because some contractions are missed entirely. Calcium deficiency can also lead to a fast and irregular heart rate.

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Vitamin D is a steroid vitamin, a group of fat-soluble pro-hormones, which encourages the absorption and metabolism of calcium and phosphorous. Vitamin D is the building block of the hormone calcitriol which works synergistically with parathyroid hormone (PTH). Vitamin D is modified by the liver to become 25-hydroxyvitamin D (also known as 25(OH)D). 25(0H)D is then modified in the kidneys to become calcitriol. This conversion is somewhat regulated by PTH levels. When calcium levels in the blood drop, PTH is released. PTH causes calcium to be released from the bones, thus raising the low calcium levels in the blood. Osteoporosis may result from chronically high levels of PTH. Calcitriol increases absorption of calcium and phosphorus (another major component of bones) from the intestines and decreases their excretion in the urine. In so doing, calcium levels in the blood rise and PTH levels drop.

People are more likely to be deficient in Vitamin D than calcium, moreover Vitamin D also appears to be more important for bones in conjunction with lower calcium intakes (typical in most vegan diets) than in diets that have large amounts of calcium.

Moreover recent studies show that Vitamin D deficiency is associated with allergy and many other diseases [11]. During childhood, vitamin D deficiency can cause growth retardation, skeletal deformities (rickets) and may increase the risk of hip fracture in adulthood [12]. Vitamin D deficiency has been linked to many disorders such as: increased risk for preeclampsia, multiple sclerosis, rheumatoid arthritis, types 1 and 2 diabetes, cardiovascular disease, dementia, some cancers and infectious diseases [13, 14]. Moreover, recent studies suggested that children and adolescents who are vitamin D deficient are at higher risk for certain food and environmental allergies and low vitamin D level is associated with severity of asthma in children [15].

As to be seen there is clearly a distinct relationship between calcium and Vitamin D; two key nutrients which play an important role in the metabolic and biochemical pathways. Thus to better

understand and establish a relationship we propose to study and estimate all the four parameters (total calcium, ionized calcium, serum albumin and Vitamin D) for the purpose of finding association among them, mostly to see correlation between them and to decide which of the three viz., total calcium, ionized calcium and serum albumin gives a better correlation in order to decide which one is the most important analyte to be estimated along with Vitamin D for the evaluation and diagnosis of Vitamin associated disorders.

MATERIALS AND METHODS

After thoroughly going through the review of literature in which total calcium, ionized calcium and serum albumin has a direct relationship to Vitamin D3, we have selected a reasonable number of patients (both male and female , numbering 30). We made use of these patients for our study to establish and understand the relationship among the four analytes studied in this project.

SUBJECTS

30 patients comprising of male and female in the age group of 3 to 83 years, who reported to the outpatient clinic for Master health check up (routine MHC) were enrolled for the study. The laboratory has already established normal values for all the analytes. We have directly estimated all the four parameters (total calcium, ionized calcium, serum albumin and Vitamin D3) for the purpose of finding association among them, mostly to see correlation between them and to decide which of the three viz., total calcium, ionized calcium and serum albumin gives a better correlation in order to decide which one is most important analyte to be estimated along with Vitamin D3 for the evaluation and diagnosis of Vitamin associated disorders.

Sample collection

As the analytes mentioned (Total calcium, ionized calcium, serum albumin and Vitamin D3) will not be affected due to non fasting status, blood sample collection was done between 8– 10 am when the patients report to collection center, which is the standard time for the blood collection for all biochemical investigations.

All possible standard precautions were taken while collecting blood samples. The use of sterile disposable needle and vaccutainer were employed for sample collection. All blood collections were done by qualified phlebotomists. Correct procedure was followed at every step such as correct site for vein puncture and pressure used to transfer into vaccutainer. On the whole occurrence of hemolysis was prevented.

Sample processing

All the blood samples were allowed to clot at room temperature for 30 minutes. The tubes were gently tapped to displace clot adhering to the tube and then centrifuged with the cap on in each tube for 10 minutes at 2500 rpm. After centrifugation, serum was transferred to clean glass tube using disposable pipettes. The samples were either analyzed immediately using Olympus AU640 or preserved at a temperature less than -15°C until analysis.

Biochemical assays

Olympus AU640 Discrete selective analyzer was used to measure Total calcium and albumin. While Calcium measurement was carried out using Arsenazo 111 Dye Binding method, the Albumin measurement was done using bromocresaol green (BCG). Established Standard Operating Procedures were used to carry out the above two measurements. Bio-Rad, USA accuracy controls were used to validate the accuracy of all analytes.

Using the latest electro-chemiluminescent analyzer, (Updated version of Enzyme Immuno Assay), used for the assay of Vitamin D3. Extensive quality control measures were done so as to get accurate values. Roche 9180 Ion Selective Calcium Analyzer was used for the estimation of ionized calcium.

Statistical analysis

A software downloaded from the website www.easycalculation.com was used to calculate correlation coefficient(r), students distribution (t) values and the probability (p), all of which are presented in Tables 1, 2 and 3, for all patients, males and females respectively. Each Table contains the pair of analytes compared, the correlation coefficient, students distribution values and probability.

RESULTS

The primary data was obtained for all the 30 patients studied for the measured analytes viz., Calcium, Ionized calcium, Albumin and VitaminD3 along with 6 calculated parameters (ratios) by finding the ratios among any two analytes so as to calculate correlation coefficient(r), student's distribution (t) and probability (p) by comparing two parameters at a time.

The mean values obtained for all the 4 analytes lies well within the normal values used in the laboratory, suggesting that the population selected did not represent either calcium or Vitamin related disorders, but represent patients attending the Master Health Check up. All the calculated ratios between any two analytes were also within the normal range.

Similar results were obtained for the 15 Females patients studied. The mean values obtained for the measured as well as for the calculated ratios are well within the normal range, suggesting that segregating the patients, sex wise did not show any difference since all these 15 Females also attended the Master Health Check up.

Data obtained from the 15 males included in this study, with the entire pattern of analyte results as well as the calculated ratios are similar to the one observed for all the patients as well as Female patients.

As the sole aim of this study was to find out the association between any two analytes out of 4 measured as well as 6 calculated, we did not select patients diagnosed of having either calcium or Vitamin related disorders.

Table 1 shows the statistical parameters viz,; correlation coefficient(r), student's distribution(t) and Probability(p) for the 30 patients studied. Good correlation is observed between calcium to ionized calcium as well between calcium to albumin and also ionized calcium to albumin there by linking all the three as being associated. A very high level of significance is observed when ratios between two analytes are compared with a set of ratios obtained with other two analytes.

Table 1.Correlation and Probability coefficients for "All Patients"

S.No	Pairs Compared	R	t	P
1.)	Ca vs Ca++	0.694	5.1	<.0001
2.)	Ca vs Alb	0.808	7.25	<.0001
3.)	Ca++ vs Alb	0.368	2.1	0.0225
4.)	Ca++/Vit D vs Ca	0.332	1.86	0.0365
5.)	Alb/Vit D vs Ca	0.338	1.9	0.0338
6.)	Ca/Ca++ vs Age	-0.368	-2.1	0.0226
7.)	Ca/Alb vs Age	0.324	1.81	0.0403
8.)	Ca++/Alb vs Age	0.374	2.13	0.0208
9.)	Ca/Ca++ vs Ca++/Alb	-0.805	-7.1	<.0001
10.)	Ca/Vit D vs Ca++/Vit D	0.999	138.62	<.0001
11.)	Ca/Vit D vs Alb/Vit D	0.999	134.41	<.0001
12.)	Ca/Alb vs Ca++/Alb	0.832	7.93	<.0001
13.)	Ca++/Vit D vs Alb/Vit D	0.998	93.56	<.0001

Calcium, ionized calcium and albumin all shows a highly significant correlation to Vitamin D3 suggesting that alteration in any one of the 4 analytes will affect the metabolic function of the other three. An excellent correlation(r=0.999, t=138.62 and p= <0.0001) was obtained between calcium/vitamin D3 to ionized calcium/VitaminD3 suggesting that all three analytes should be measured to diagnose all calcium and Vitamin related disorders. The next best correlation is between calcium/vitaminD3 and albumin/VitaminD3 followed by ionized calcium/Vitamind3 and albumin/VitaminD3.

Table 2. Correlation and Probability coefficients for "Female Patients"

S.No	Pairs compared	R	t	P
1.)	Ca vs Ca++	0.646	3.16	0.0035
2.)	Ca vs Alb	0.785	4.75	0.0002
3.)	Ca/Ca++ vs Age	-0.394	-1.6	0.0654
4.)	Ca/Alb vs Age	0.365	1.47	0.0824
5.)	Ca/Vit D vs Age	0.429	1.78	0.0484
6.)	Ca++/Al vs Age	0.424	1.75	0.0509
7.)	Ca++/Vit D vs Age	0.497	2.14	0.025
8.)	Alb/Vit D vs Age	0.353	1.41	0.0898
9.)	Ca/Ca++ vs Ca++/Alb	-0.845	-5.92	<.0001
10.)	Ca/Vit D vs Ca++/Vit D	0.98	18.63	<.0001
11.)	Ca/Vit D vs Alb/Vit D	0.984	20.63	<.0001
12.)	Ca/Alb vs Ca++/Alb	0.839	5.78	<.0001
13.)	Ca++/Vit D vs Alb/Vit D	0.95	11.35	<.0001

Table 2 presents similar statistical parameters for the 15 Females included in this study. In this group too significant correlations are obtained which are comparable to the one observed for all patients, but the t values are less although the p values are similar.

Table 3.Correlation and Probability coefficients for "Male Patients"

S.No	Pairs compared	R	t	P
1.)	Ca vs Ca++	0.766	4.12	0.0007
2.)	Ca vs Alb	0.827	5.1	0.0001
3.)	Ca++ vs Alb	0.533	2.18	0.0248
4.)	Ca++/Vit D vs Ca	0.392	1.48	0.0828
5.)	Alb/Vit D vs Ca	0.398	1.5	0.0792
6.)	Ca/Ca++ vs Age	-0.4	-1.51	0.0783
7.)	Ca++/Alb vs Age	0.374	1.4	0.0937
8.)	Ca/Ca++ vs Ca++/Alb	-0.746	-3.88	0.0011
9.)	Ca/Vit D vs Ca++/Vit D	0.999	156.23	<.0001

10.)	Ca/Vit D vs Alb/Vit D	0.999	133.2	<.0001
11.)	Ca/Alb vs Ca++/Alb	0.833	5.22	0.0001
12.)	Ca++/Vit D vs Alb/Vit D	0.999	112.6	<.0001

Table 3 shows similar statistical data for the 15 Males studied. It is interesting to observe that both the r and t values are very high indicating that men have greater muscle mass and bone density than females, but the overall conclusion is similar to the one observed for all patients.

DISCUSSION

A recent study showed that an elevated total to ionized calcium ratio was detected in 12% of all patients. 33% percent of liver failure patients demonstrated an elevated total to ionized calcium ratio. The study group demonstrated significantly high mean total calcium levels, significantly lower mean ionized calcium levels, and significantly higher mean total to ionized calcium ratios than controls. As a result, the study group also had significantly increased mean calcium chloride replacement requirements in comparison with controls. The mean calcium to citrate infusion ratio was elevated in the study group in comparison with controls. An elevated total to ionized calcium ratio was associated with increased mortality in comparison with controls. No patients suffered complications from ionized hypocalcemia or elevated serum total calcium.

The ionized calcium concentration is tightly regulated by parathyroid hormone and vitamin D. The wide range in the normal total plasma calcium concentration is probably due to variations in the plasma concentration of albumin among normal healthy individuals and to variations in the state of hydration that can alter the concentration of albumin.

The net effect is that measurement of the total plasma calcium concentration alone can be misleading, since this parameter can change without affecting the ionized fraction. This problem can occur in hypoalbuminemia and multiple myeloma

In general, the plasma calcium concentration falls by 0.8 mg/dL (0.2 mmol/L) for every 1.0 g/dL (10 g/L) fall in the plasma albumin concentration. The measured plasma calcium concentration can be corrected for the presence of hypoalbuminemia from the following equation:

Corrected [Ca] = Measured total [Ca] + 0.8 x (4.5 - [alb])

where plasma calcium and albumin concentrations are measured in units of mg/dL and g/dL, respectively. Thus, if the measured values are 7.6 mg/dL and 2.5 g/dL: The corrected calcium will be 9.2 mg/dL

In the settings noted above, measurement of the total plasma calcium concentration may not be sufficient to determine the presence or absence of a disturbance in calcium homeostasis. Thus, the plasma calcium concentration should be correlated with simultaneously measured concentrations of albumin and phosphate. Direct measurement of the ionized calcium concentration should be obtained in patients with borderline hypercalcemia and in those with symptoms of hypocalcemia but a normal total calcium concentration.

Calcium-binding protein is an intracellular protein, and that its synthesis may be a consequence of the raised intracellular calcium content of the intestinal epithelial cells resulting from 1,25-dihydroxycholecalciferol-stimulated Ca2+ transport. We propose that calcium-binding-protein synthesis is necessary for maintaining the stimulated rate of Ca2+ transport, which is initiated by other factors.

The adverse effects of excessive calcium intake may include high blood calcium levels, kidney stone formation and kidney complications. Elevated calcium levels are also associated with arthritic/joint and vascular degeneration, calcification of soft tissue, hypertension and stroke, and increase in VLDL triglycerides, gastrointestinal disturbances, mood and depressive disorders, chronic fatigue, and general mineral imbalances including magnesium, zinc, iron and phosphorus[16]. Thus calcium has a very vital role in the biochemical and metabolic pathways.

In this study we have established very good correlation between Total calcium to VitaminD3 to that of Ionized Calcium to Vitamin D3 ($P<0.0001$) and similar correlation was also observed when the ratio of ionized calcium to albumin was compared. On the whole our study strongly recommends the measurement of all the four parameters for effective diagnosis of calcium and Vitamin related disorders.

CONCLUSION

Our study has demonstrated that a strong relationship exists between calcium and Vitamin D3 metabolism since very good correlations were found between any two analytes compared. Some previous studies have also suggested such relationship but none have presented data similar to the one that we presented in this project work.

To have a better understanding of calcium homeostasis, one need to measure not just the total calcium but ionized calcium as well as serum albumin levels too. Similarly to get a better understanding of Vitamin related disorders, we need to measure the above three analytes mentioned along with VitaminD3 levels since both calcium as well as VitaminD3 metabolism are interlinked.

As the sole aim of this study was to establish the relationship between VitaminD3 and the calcium homeostasis related analytes, we did not select established diseased patients.

Further studies are required to see if calcium or Vitamin supplements are required in case one of the analytes measured levels really low. Further studies on experimental animals by inducing Avitminosis as well as hypoalbuminemia , hypocalcemia and hypercalcemia and finding its effect on bone metabolism will give a much better understanding about calcium, albumin as well as Vitamin D3 metabolism and their disorders.

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