

Serum ionized calcium and magnesium in normal and hypothyroid females: A comparative study

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Abstract

Introduction: Thyroid disorders exhibit widespread effects on mineral and lipid metabolism. Free or ionized calcium is biologically active but not routinely measured. Previous studies carried out on serum calcium in thyroid disorders reveal conflicting results.

Materials and Methods: The present study conducted in the department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar, consisted of two groups – group 1 (healthy females) and group 2 (newly diagnosed hypothyroid females). Estimation of serum Thyroid stimulating Hormone (TSH) levels were done by 3rd generation assay, FT4 and FT3 were estimated by Enhanced Chemiluminescence using vitros ECI by Orthoclinical Diagnostics. Serum Magnesium was estimated by calmagite method, Serum calcium by Cresolphthalein complexone method and serum total protein was estimated by biuret method, end point. Serum ionized calcium and magnesium was estimated using formulas.

Results: In our study the decrease in serum total calcium was insignificant whereas serum ionized calcium depicted a significant difference in two groups. Serum total and ionized magnesium both showed a significant difference in two groups.

Conclusion: Despite the measurement of free calcium being a better indicator of calcium homeostasis as compared to total calcium, it has not replaced the latter and should be routinely estimated.

Keywords: Total calcium, Ionized calcium, Ionized magnesium, Minerals, Hypothyroidism.

Introduction

Thyroid disease, being one of the most common endocrine disorders, have widespread effect on lipid, carbohydrate, protein, electrolyte and mineral metabolism-most pronounced being mineral metabolism including calcium, magnesium and phosphorus.¹ A decreased turnover of calcium occurs in hypothyroidism, leading to decreased levels in serum due to impaired immobilization. Conversely, impaired mobilization of calcium in hyperthyroid states leads to increase in serum calcium levels. Serum total calcium is maintained within a tight range of 8.5 – 10.5 mg/dl under physiologic conditions.² Stressing on the fact that most of the body calcium (about 99%) rests in the bones and physiologically active form of calcium is the ionized fraction (40%) of the total fraction, rest being unavailable as it is bound to albumin. Thus instead of estimating Serum Total Calcium, Serum ionized calcium estimation would be more useful.^{3,4}

Magnesium apart from being an important mineral also activates many enzymes and regulates the levels of calcium and vitamin D. Magnesium on the other hand is also an important cation that ameliorates cardiac disorders.⁵ It is the second most abundant cation with only 2 % in the extracellular fluid (measurable) and thus these levels do not accurately reflect body stores. Hypomagnesaemia is associated with arrhythmias, coronary vasospasm and high blood pressure. Various studies have reported conflicting results of serum magnesium in hypothyroid patients.^{6,7} Unfortunately there is no ionized magnesium estimation available and has to be calculated.⁸

Though the changes in the calcium and magnesium may be slight in thyroid disorders but disturbances in minerals are important for patients in long run. As metabolic disorders, hypertension and CVD are related to defect in metabolism of cations such as calcium and magnesium.^{8,9} It therefore becomes assessing these minerals of prime importance in hypertension and cardiovascular patients.

Although many studies have estimated the levels of minerals in hypothyroid patients, conflicting results have been reported by all. Our study focussed on the need for estimating ionized calcium and magnesium in newly diagnosed hypothyroid patients in Amritsar.

Materials and Methods

The present cross-sectional comparative study was conducted in the Department of Biochemistry, Sri Guru Ram Das Charitable Hospital, Vallah, Sri Amritsar over a period of one year from June 2017 to May 2018. The study protocol was approved by the institutional ethical committee and informed consent was obtained from study subjects. This study was conducted in the Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar.

The study included female subjects in the age group of 30-60 years. The study consisted of two groups: Group 1:45 healthy females and Group 2:45 newly diagnosed hypothyroid females. Sample size was calculated using G Power 3.1 software and values of the previous study by Mendez D et al¹ were considered. The cases and controls were randomly selected as per the randomization technique on SPSS v.17.0.

Exclusion Criteria

Patients with the history of hepatic diseases, renal diseases, alcohol or critically ill or on calcium or magnesium supplementation, antithyroid drugs or any medication which are known to effect calcium and magnesium were excluded from the present study.

Sample Collection

5 mL of Blood Sample was taken after an overnight fast i.e. approximately on 12 hrs of fasting, in a plain tube (red top vacutainer) under sterile conditions from ante-cubital vein and kept for 30 min then it is centrifuged in the laboratory immediately for serum separation.

The serum thyroid stimulating Hormone (TSH) levels were estimated by 3rd generation assay, FT4 and FT3 were estimated in these subjects by Enhanced Chemiluminescence using vitros Eci by Orthoclinical Diagnostics. It is non competitive immunoassay-sandwich immunoassay.¹⁰⁻¹² Serum sample was used for the estimation of magnesium (Calmagite method),¹³ calcium (Cresolphthalein complexone method),¹⁴ total proteins (Biuret method),¹⁵ total cholesterol (CHOD PAP method)¹⁶, triglycerides (GPO Trinder),¹⁷ high density cholesterol (HDL-C),¹⁸ Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) by Friedwald equation.^{19,20} Serum ionized calcium Level was Calculated by using the following equation:

$$\text{Ionized calcium} = [6.25 \times \text{TC} - (\text{Total protein}) \times 3/8] / (\text{Total protein} + 6.5).^{21}$$

While serum magnesium levels were calculated according to the following formula:

$$\text{Ionized magnesium in mmol/L} = [(0.66 \times \text{Total Mg in mmol/L}) + 0.039].^{22}$$

Statistical Analysis

The data was analyzed using SPSS 17 version software by applying students' t test for unpaired data. Randomization was determined using SPSS 17 version software.

Results

In our study there was a highly significant difference in the mean free T4 and serum TSH levels ($p < 0.05$) between normal and newly diagnosed hypothyroid females whereas no such difference was observed in the levels of Free T3 ($p=0.287$). Mean total serum calcium between the two groups showed an insignificant difference ($p=0.052$) while a highly significant decrease was observed in the levels of mean ionized calcium in hypothyroid patients as compared to controls. Both total and ionized magnesium showed a significant decrease in the two groups ($p < 0.05$). No significant difference was observed in the levels of total proteins and lipid profile among the two groups as illustrated in Table 1.

Table 1: Comparison of biochemical parameters in normal and hypothyroid females

S.No	Parameter	Group 1 Mean \pm SD (n=45)	Group 2 Mean \pm SD (n=45)	P value (2 tailed)
1.	Free T3 (pg/mL)	2.91 + 0.16	3.21 + 0.17	0.272
2.	Free T4 (ng/mL)	1.74 + 0.16	0.98 + 0.07	0.000*
3.	TSH (mIU/L)	2.06 + 0.13	27.83 + 5.53	0.000*
4.	Total Calcium (mg/dL)	9.83 + 0.20	9.49 + 0.49	0.052
5.	Ionized Calcium (mg/dL)	1.32 + 0.04	1.08 + 0.03	0.006*
6.	Total Magnesium (mg/dL)	2.52 + 0.10	1.92 + 0.06	0.003*
7.	Ionized Magnesium (mg/dL)	1.67 + 0.07	1.26 + 0.04	0.000*
8.	Total Proteins (g/dL)	8.73 + 0.17	8.19 + 0.27	0.085
9.	T.Cholesterol (mg/dL)	164.96 \pm 8.16	151.68 \pm 7.69	0.239
10.	Triglycerides (mg/dL)	162.33 \pm 14.46	149.04 \pm 12.77	0.493
11.	HDL Cholesterol (mg/dL)	36.89 \pm 1.93	38.96 \pm 1.70	0.337
12.	LDL Cholesterol (mg/dL)	93.73 \pm 6.99	81.52 \pm 5.87	0.193
13.	VLDL Cholesterol (mg/dL)	32.11 \pm 2.89	29.28 \pm 2.55	0.466

* Significant

Ionized Mg ²⁺		Free T4	Free T3	TSH
	r	-0.326**	0.182	0.201*
	p	0.001	0.078	0.044
Ionized Ca ²⁺				
	r	-0.366**	0.333**	-0.614
	p	0.000	0.001	0.032

** . Correlation is significant at the 0.01 level (2-tailed).
 * . Correlation is significant at the 0.05 level (2-tailed).

There was significant correlation of ionized calcium ($r = -0.614$) and ionized magnesium ($r = 0.201$) with TSH. A negative non-significant correlation of TSH with cholesterol, triglycerides, LDL and VLDL was observed, whereas HDL showed non-significant positive correlation with TSH as illustrated in Table 2.

Discussion

Calcium being the body's major divalent cation has an important role to play in the transmission of nerve impulses, heartbeat, cardiac action potential, clotting mechanisms and many other intracellular functions including action as second messenger etc. Calcium occurs in the body in three important physiochemical states. Ionized calcium, also called free calcium constitutes about 50% of the total serum calcium levels. Rest prevails bound to proteins and other small ions thus not available for action. Ionized calcium homeostasis is tightly regulated by harmony between GI absorption, renal excretion bone resorption and thyroid regulation. The reliability of total serum calcium is usually assumed to reflect ionized calcium levels which may be the case in healthy individuals but not in diseased subjects. Thyroid gland plays a pivotal role in maintaining calcium and magnesium ion balance.²³

Abnormalities in calcium and magnesium homeostasis come under disorders of mineral metabolism. Disorders of thyroid gland have widespread systemic manifestations most pronounced being mineral and lipid metabolism. The calcium sensing receptors (CaSR) represents the molecular mechanisms by which parathyroid cell detects changes in blood ionized calcium concentration and modulate parathyroid hormone secretion to maintain ionized calcium concentrations within a narrow physiological range.²³ Our study showed a more significant decrease in ionized calcium levels in newly diagnosed hypothyroid females as compared to normal females whereas serum total calcium levels were insignificantly decreased. Our study was in accordance with the findings of Hassen et al,²⁴ where a significant difference was found in mean serum ionized calcium levels between test and control groups. Similar findings were also observed by Al Hakeim.²⁵ There was a contrast in the findings of the work done by Mendez et al¹ where no significant difference was observed in both serum total and ionized calcium levels in normal and hypothyroid subjects. The estimation of serum ionized calcium is done by ion selective electrode by direct

potentiometry. A significant difference in total and ionized magnesium was observed within the two groups in our study. No significant difference was observed in the mean serum levels of total proteins and lipid profile in newly diagnosed hypothyroid females as compared to normal females.

Despite the fact that measurement of ionized calcium levels is faster and simpler method as compared to serum total calcium, its measurement is confined only to point of care testing areas and emergency rooms because of difficulty in sample collection and preservation techniques for ionized calcium which requires tight regulation of pH, anaerobic collection and processing of the sample within 30 minutes. Moreover the whole blood for ionized calcium must be collected in heparinised vials with heparin concentration not more than 10 IU/mL to minimize artifactually reduced ionized calcium concentrations.

Conclusion

The alteration in metabolism of cations in hypothyroid patients needs to be regularly evaluated for serum ionized calcium and magnesium as early detection and prevents further complications. Because the free calcium is biologically active and tightly regulated, it is the best indicator for calcium status. Despite the measurement of free calcium being a better clinical indicator, it has not replaced the estimation of total serum calcium, routinely being done in the laboratories. No method for serum magnesium estimation is available and thus has to be estimated by calculation methods.

Conflict of Interest: None.

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