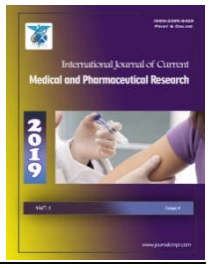
INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH
ISSN: 2395-6429, Impact Factor: 4.656
Available Online at www.journalcmpr.com
Volume 5; Issue 04(A); April 2019; Page No. 4157-4161
DOI: http://dx.doi.org/10.24327/23956429.ijcmpr201904645



Research Article

EVALUATION OF SERUM CALCIUM AND MAGNESIUM STATUS IN PATIENTS OF SUBCLINICAL AND PRIMARY HYPOTHYROIDISM-A MULTI-CENTRIC CROSS-SECTIONAL STUDY IN TERTIARY CARE GOVERNMENT HOSPITALS OF INDIA

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ARTICLE INFO

Article History:
Received 15th January, 2019
Received in revised form 7th February, 2019
Accepted 13th March, 2019
Published online 28th April, 2019

Key words:
Hypothyroidism, Calcium, Magnesium

ABSTRACT

Background: Hypothyroidism is rampant in the urban and semi-urban areas of India and West Bengal is no exception. Additionally calcium and magnesium deficiency is highly prevalent in this state. The effect of thyroid hormones on these minerals has as yet been studied only in a few regions of the country and so far very little among the population of urban and semi-urban West Bengal.
Objectives: To assess the mineral status [calcium and magnesium] among hypothyroid patients and evaluate any association with thyroid hormone levels.
Materials & methods: A multi-centric hospital-based cross-sectional study was conducted with 100 patients diagnosed with hypothyroidism on the basis of their serum TSH and fT4 levels, designated as cases and 100 apparently healthy euthyroid controls. Cases were further sub-classified as subclinical (n=57) or primary (n=43) hypothyroids. Serum levels of TSH, fT4, calcium and magnesium were measured in all study participants.
Results: A significantly higher proportion of cases showed levels of both minerals to be below the lower limit of the reference interval than for the controls (P<0.0001) and this was true for both subclinical as well as primary hypothyroids with lower levels of both elements being more common in the latter sub-group than the former.
Conclusion: Two very elements namely calcium and magnesium are significantly lowered in hypothyroid individuals more so in primary than in subclinical hypothyroidism. Hence, supplementation with these elements along with rectification of their thyroid function may be of immense benefit to such individuals.

INTRODUCTION

Abnormalities pertaining to the thyroid gland are by far one of the most common endocrine disorders prevalent worldwide. Of this, hypothyroidism due to either deficiency of thyroid hormones or from their impaired activity, constitutes the most common type of disorder afflicting the thyroid.[1] Biochemically decrease in triiodothyronine (T3) and thyroxine (T4) concentration leads to hyper secretion of pituitary thyroid stimulating hormone (TSH) and an amplified increase in serum TSH level, constituting the chief laboratory hallmark of the condition.[2]

The condition shows a higher predilection in females with mean annual incidence of 4 out of 1000 in females as opposed to one out of 1000 in males.[3] As of 2013, the estimated worldwide prevalence of hypothyroidism as per the NHANES III study, stood at 4.6% , and a good deal higher in India; at 10%. Furthermore, higher rates were observed in inland than in coastal regions and among all urban centers studied, the highest prevalence was noted in Kolkata at 21.67%.[4]

Calcium and magnesium constitute two very important elements in the body. The levels of both these elements are frequently disturbed in thyroid dysfunctions. Thyroid hormones play an important role in channelizing calcium and phosphorous to serum via osteoclastic bone resorption. This effect is hampered in hypothyroid states accounting for the concomittant mineral deficiency states observed in hypothyroidism.[1] Furthermore, hypothyroidism is often accompanied by hypomagnesaemia, owning to increased fractional excretion of magnesium through urine.[3] Low levels of magnesium can alter the function of Complex V of oxidative phosphorylation, the element being the key player in ATP synthesis through the F_1F_0-ATPase.[5]

In the urban and semi-urban areas of India particularly in the inland areas hypothyroidism is rampant. Added to this is the prevalence of calcium and magnesium deficiency observed

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commonly among the population of these regions. The effect of thyroid hormones on these two elements has as yet been studied only in a few regions of the country and so far very little among the population of urban and semi-urban West Bengal. The aim of the present study was to assess the mineral status [calcium and magnesium] among hypothyroid patients in this geographical region and look for any association with thyroid hormone levels.

MATERIALS AND METHODS

Study design and Subjects

The study was conducted as a multi-centric cross-sectional observational hospital based study in the Department of Biochemistry, Institute of Postgraduate Medical Education and Research, Kolkata and Department of Biochemistry, RG Kar Medical College & Hospital, Kolkata, from January 2018 to December 2018. The study subjects constituted of individuals coming for evaluation of thyroid function to the departmental clinical laboratory. Briefly, 250 patients from clinically suspected hypothyroidism were tested for serum TSH and free thyroxine (fT4) levels, and those found to have subclinical or primary hypothyroidism [based on their serum levels of TSH and fT4] were finally selected as cases. This amounted to 100 cases of whom 57 were found to have subclinical hypothyroidism and the remaining 43, primary hypothyroidism. Simultaneously, 100 age and sex matched apparently healthy euthyroid volunteers were selected as controls.

Selection criteria

***Inclusion criteria*:** Those individuals were chosen as cases who met the following inclusion criteria: a. Age between 18 to 75 years b. Both sexes and c.Clinically suspected and biochemically confirmed either subclinical or primary hypothyroidism. Controls were age and sex matched apparently healthy volunteers who were biochemically proved to be euthyroid.

***Exclusion criteria*:** a. Individuals with history of diabetes mellitus, hepatic disease, bone disease, renal disease, smoking , alcoholism, any other major medical or surgical condition b. Individuals less than 18 and greater than 75 years of age c. Individuals undergoing supplementation with any formulation that could influence the serum levels of calcium and magnesium, were also excluded from the study.

Ethical Considerations: All eligible subjects were provided complete information regarding study and recruited only after obtaining their informed consent for participation.

Collection & Storage of Blood Sample: After an overnight fasting of 12 hours, 3 ml of venous blood, was drawn using full aseptic measures and after obtaining consent for the same. Serum was separated within half an hour by centrifugation at 2000 g for 10 minutes and stored as 1.5 ml aliquots at -20°C until further use.

Analysis of Serum Parameters: Serum TSH was measured by sandwich ELISA [enzyme linked immunosorbent assay] method using commercially available kit. Serum fT4 levels were assayed by competitive ELISA using commercially available kit. All values were obtained using a microplate reader from Biotek. Serum calcium was measured using an ion-selective electrolyte analyzer from Roche. Serum magnesium was assayed by direct colorimetric assay using Xylidyl blue-I (as chelator) which at alkaline pH, yielded a purple colored complex with magnesium. The intensity of the colour formed was proportional to the magnesium concentration in the sample and was estimated spectrophotometrically at 660 nm.

In all serum assays established reference intervals in our laboratory were used viz. serum TSH: 0.5-4.2 µIU/ml, fT4 : 0.8-2.7 ng/dl, serum calcium: 8.0-10.5 mg/dl and serum magnesium: 1.7-2.4 mg/dl.

Statistical Analysis

All statistical test were performed using Graphpad Prism v.5.0. The data were expressed as Mean±S.D. For comparison between serum TSH and calcium levels in cases and controls, serum fT4 and calcium levels in cases and controls, serum TSH and magnesium levels in cases and controls and serum fT4 and magnesium levels in cases and controls, one-way ANOVA was performed with Bonferroni correction. One-way ANOVA with Bonferroni correction was also performed separately for analyzing association between sub-clinical and primary hypothyroidism with mineral status.. In all statistical tests a P value of <0.05 was considered statistically significant.

RESULTS

Patient profile

A total of 250 patients were initially recruited into the study, according to the set inclusion and exclusion criteria, after obtaining informed consent. These patients were all assessed for thyroid function and finally 100 of these study subjects who were diagnosed with hypothyroidism either primary [serum TSH > 10 µIU/ml and serum fT4 ≤0.8 ng/dl] or subclinical [serum TSH 4-10 µIU/ml and serum fT4 within reference interval], were selected as cases. Simultaneously 100 apparently healthy age & sex-matched individuals with normal thyroid function were designated as controls. **Table 1** summarizes categorization of cases and controls along with their serum TSH and fT4 levels [Mean±S.D.]

Table 1 General characteristics of cases and controls along with their serum TSH and fT4 levels

Parameters	Controls	Cases [Subclinical hypothyroidism]	Cases [Primary hypothyroidism]
Number (n)	100	57	43
Gender (m/f)	33/67	17/40	12/31
Age in years (Mean±S.D.)	38.1 ± 12.7	38.8 ± 13.5	39.9 ± 14.5
Serum TSH (Mean±S.D.)	2.4 ± 0.9 µIU/ml	6.4 ± 1.3 µIU/ml	15.9 ± 4.0 µIU/ml
Serum fT4 (Mean±S.D.)	2.0 ± 0.5 ng/dl	1.4 ± 0.36 ng/dl	0.53 ± 0.27 ng/dl

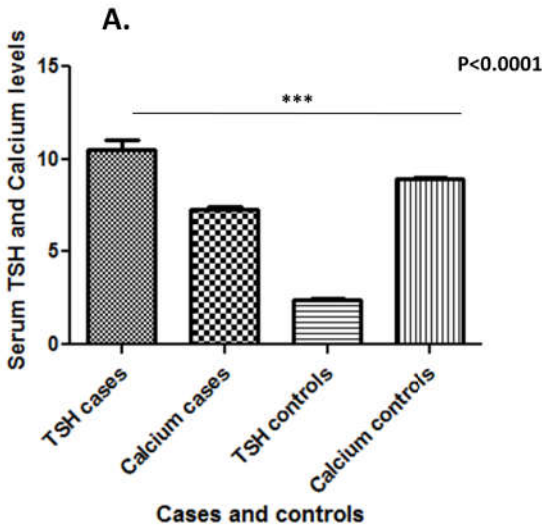
Association between thyroid function and serum levels of minerals [calcium and magnesium] in cases and controls

Serum calcium and magnesium levels were assayed in both groups of cases and controls and analyzed for association with thyroid function ie with serum TSH and fT4 levels. **Table 2** summarizes comparison of serum calcium and magnesium levels between cases and controls along with their serum TSH & fT4 levels. A significantly higher proportion of cases showed serum calcium levels below 8.0 mg/dl as compared to controls [Fig 2.1A & B] and serum magnesium levels below 1.7 mg/dl as compared to controls [Fig 2.2 A & B] and this was true for both the cases of subclinical hypothyroidism [Fig

2.3 A & B] as well as was for primary hypothyroidism [Fig 2.4 A & B].

Table 2 Comparison between serum calcium levels and serum levels of TSH & fT4 among cases and controls .

Parameters	Controls	Cases [Subclinical hypothyroidism]	Cases [Primary hypothyroidism]
Serum calcium levels (Mean±S.D.)	8.9 ± 0.72 mg/dl	7.8 ± 0.72 mg/dl	6.6 ± 0.9 mg/dl
Serum magnesium levels (Mean±S.D.)	2.6 ± 0.4 mg/dl	1.3 ± 0.5 mg/dl	1.0 ± 0.4 mg/dl
Serum TSH (Mean±S.D.)	2.4 ± 0.9 µIU/ml	6.4 ± 1.3 µIU/ml	15.9 ± 4.0 µIU/ml
Serum fT4 (Mean±S.D.)	2.0 ± 0.5 ng/dl	1.4 ± 0.36 ng/dl	0.53 ± 0.27 ng/dl



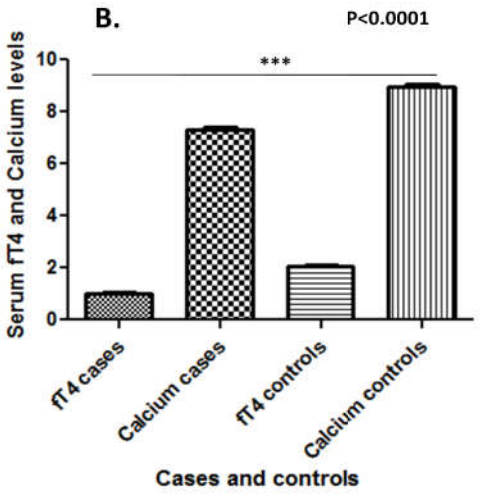
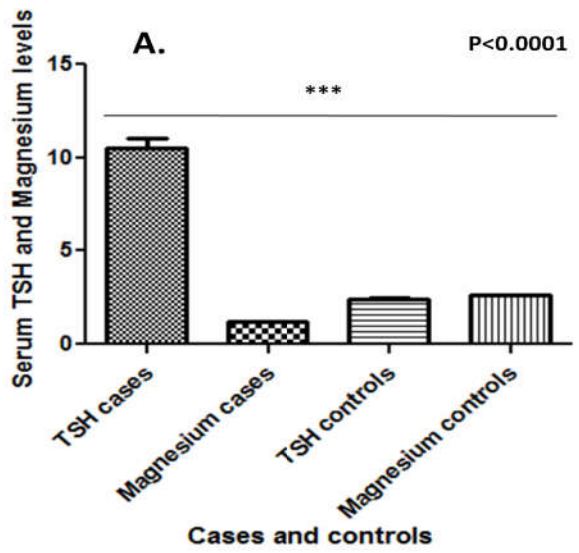


Fig 2.1 (A) Comparison of calcium levels between cases and controls with highly significant inverse association between serum TSH and calcium levels observed; One way ANOVA with Bonferroni correction applied across four groups [P<0.0001] **(B)** Comparison of calcium levels between cases and controls with highly significant direct association between serum fT4 and calcium levels observed; One way ANOVA with Bonferroni correction applied across four groups [P<0.0001].



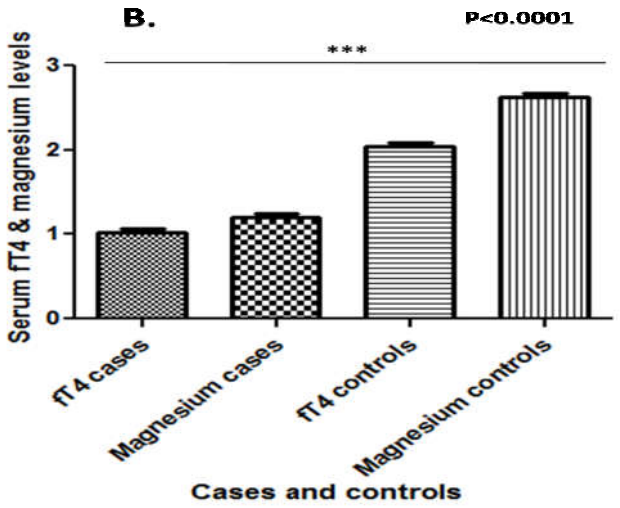
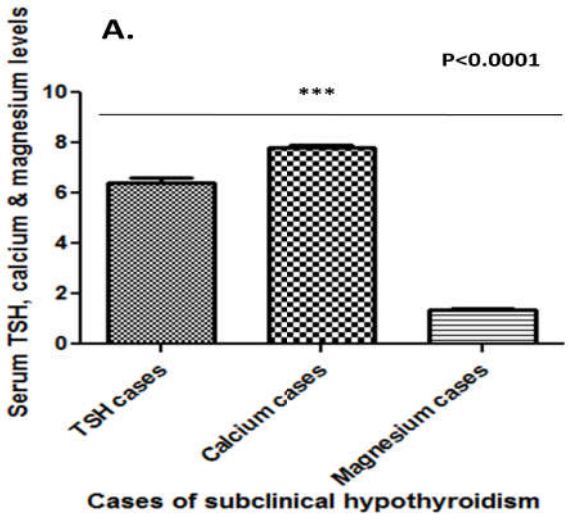


Fig 2.2 (A) Comparison of magnesium levels between cases and controls with highly significant inverse association between serum TSH and magnesium levels observed; One way ANOVA with Bonferroni correction applied across four groups [P<0.0001] **(B)** Comparison of magnesium levels between cases and controls with highly significant direct association between serum fT4 and magnesium levels observed; One way ANOVA with Bonferroni correction applied across four groups [P<0.0001].



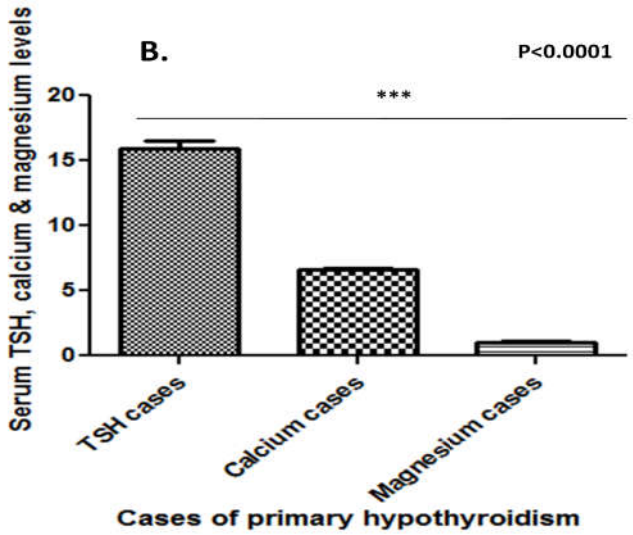


Fig 2.3 Association of calcium and magnesium levels with serum TSH levels in **(A)** and subclinical hypothyroidism and **(B)** primary hypothyroidism. One way ANOVA with Bonferroni correction applied across three groups and an inverse and highly significant association found between serum TSH and serum calcium and magnesium levels in both **(A)** & **(B)** [P<0.0001].

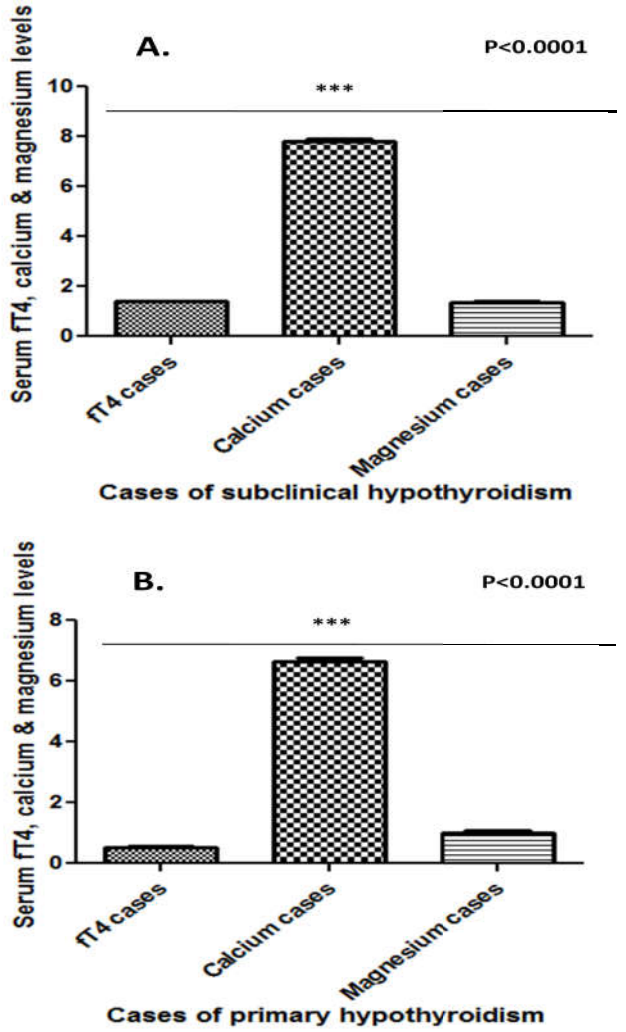


Fig 2.4: Association of calcium and magnesium levels with serum fT4 levels in **(A)** and subclinical hypothyroidism and **(B)** primary hypothyroidism. One way ANOVA with Bonferroni correction applied across three groups and a direct and highly significant association found between serum fT4 and serum calcium and magnesium levels in both (A) & (B) [P<0.0001].

DISCUSSION

Thyroid hormones play a vital role in maintenance of thermogenic, mineral and metabolic homeostasis in humans.[2] Particularly with regard to bone mineral metabolism, thyroid hormones play a pivotal role, mainly stimulating bone resorption;[6] the effect being decreased in patients of hypothyroidism, accounting for lowered serum levels of minerals namely calcium and magnesium, as was also observed in our studies. Furthermore, thyroid hormones are also known to influence the glomerular filtration rate and renal blood flow and has a direct effect on reabsorption of calcium and magnesium.[1]

There have been several studies where hyperthyroidism has been shown to be accompanied by hypercalcaemia. [7][8][9] While we did not recruit any hyperthyroid patients to our study, the calcium levels were below reference interval for a significantly higher proportion of the hypothyroid individuals, recruited as cases., and the effect was more pronounced in the primary hypothyroid sub-group as opposed to the subclinical sub group [mean serum calcium levels 6.6 mg/dl vs. 7.8 mg/dl respectively (Table 2)].

Although some conflicting reports exist, recent studies have demonstrated that depressed thyroid function is often accompanied by hypomagnesaemia. One study conducted in 2018 has reported severely low serum magnesium levels (<0.55 mg/dl) to be associated hypothyroidism, both primary as well as subclinical.[10] Another study has postulated the contribution of physical and psychological stressors in hypothyroidism to augment the daily need for magnesium supply. Such stressors have been shown to promote increased magnesium losses and thereby inhibiting the activity of several magnesium-dependent enzymes.[11] A later report by the same authors pinpoint to the enzymes of oxidative phosphorylation being worst affected accounting for musculoskeletal weakness; and paradoxically such stressors may even be existent in hyperthyroidism necessitating the need for magnesium supplementation in both ends of the spectrum of thyroid dysfunction.[5]

Our study dealing with hypothyroid individuals also demonstrated magnesium levels below the reference interval for a significantly higher proportion of both subclinical as well as primary hypothyroidism subjects [mean serum magnesium level 1.3 mg/dl & 1.0 mg/dl respectively].

However, one drawback of our study was that due to financial constraints, we were unable to measure the serum level of a few other vital elements namely zinc and copper, whose levels are often perturbed in hypothyroidism. Particularly, there have been reports of zinc levels being significantly reduced in hypothyroid subjects.[2][12] We hope to assess for this element in our subsequent studies, as zinc supplementation may also be required in such patients.

Our study has thus tried to emphasize on the existence of depressed circulating levels of two essential elements viz. calcium and magnesium in hypothyroid individuals both in subclinical as well as primary sub-types. Since a significantly higher proportion of the recruited cases demonstrated deficiency of both minerals, we postulate that supplementation of such individuals with both minerals might be helpful in preventing degenerative bone disorders arising out of faulty mineralization and remodeling. This is also significant in the light that majority of these hypothyroid individuals were

women among whom many were perimenopausal and hence more susceptible to degenerative osteopathies such as osteoporosis.

CONCLUSIONS

So far very few studies have been conducted in our part of the country where the status of both calcium and magnesium has been simultaneously studied in hypothyroidism. We have, in our study, assessed the status of both elements in patients of subclinical and primary hypothyroidism as opposed to euthyroid individuals and established a significant relationship between levels of these minerals and thyroid function.. In the coming future we hope to also study the role of these and other minerals such as zinc, copper and phosphate in not only hypo but also in hyperthyroid individuals, so that a wholesome nutritional supplementation scheme might be formulated for patients of both types of thyroid dysfunction.

Acknowledgements

The authors gratefully acknowledge the contribution by all participants of the study. The entire study was conducted with departmental resources and no financial burden was imposed on any of the participants.

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How to cite this article:

Debasmita Bandyopadhyay *et al* (2019) 'Evaluation of Serum Calcium And Magnesium Status in Patients of Subclinical and Primary Hypothyroidism-A Multi-Centric Cross-Sectional Study in Tertiary Care Government Hospitals of India', *International Journal of Current Medical And Pharmaceutical Research,* 05(04), pp 4157-4161.

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