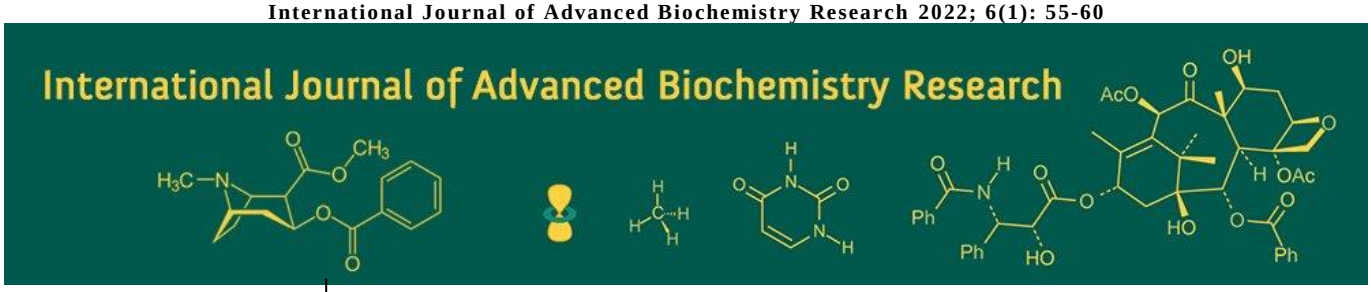

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Study of mineral profile in patients with hypothyroidism

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Abstract
Background: Thyroid hormones play important role in hemodynamic, thermoregulation and metabolism of the body. As a result, renal hemodynamic, glomerular filtration and electrolyte management may be affected in thyroid related problems. Thyroid disease particularly hypothyroidism has recently received more attention because it may be the primary cause of mineral metabolism disturbance due to its direct effect on bone turnover.
Objective: To estimate the serum level of minerals (Calcium, magnesium, phosphorus, iron, zinc and selenium) in hypothyroid patients and compare them with normal healthy controls.
Materials and Methods: The study was carried out from January 2021 to December 2021 in 60 hypothyroid patients and 60 healthy controls (age group 20-60 years), attending the medicine OPD of Teerthanker Mahaveer College & Research Center, Moradabad, Uttar Pradesh. Serum minerals and thyroid profile were measured by using the automated method and their levels were compared with healthy individuals as controls.
Results: The serum magnesium and phosphorus levels were significantly elevated whereas the levels of calcium and zinc were significantly decreased in hypothyroid patients compared to healthy subjects. However, iron (p=0.09) and selenium (p=0.38) levels in hypothyroid patients were found to be less than that of healthy individuals but the difference between them was not statistically significant.
Conclusion: This study concludes that hypothyroid patients should have their serum mineral levels checked regularly, as early diagnosis and intervention can help to avoid further thyroid problems. It will be beneficial in treating hypothyroidism as well.

Keywords: Calcium, iron, hypothyroidism, magnesium, phosphorus, selenium, zinc

Introduction
Thyroid hormones, T_3 (triiodothyronine) and T_4 (tetraiodothyronine) are two main hormones secreted by the thyroid gland. Biologically, T_3 is more active form than T_4 which is synthesized by the process of deiodination of T_4 by 1, 5 deiodinase within the peripheral tissues [1].
Thyroid Stimulating Hormone (TSH) and Thyrotropin Releasing Hormone (TRH) are released by the pituitary gland and hypothalamus and help to regulate T_3 and T_4 [2]. Thyroid hormones have a crucial function in the human body regulating body temperature and regulating the metabolism of carbohydrates, proteins, lipids and minerals [3].
Thyroid hormones play important role in hemodynamic, thermoregulation and metabolism of the body. As a result renal hemodynamic, glomerular filtration and electrolyte management may be affected in thyroid related problems [4].
Thyroid disorders can disrupt bone and mineral homeostasis and they are the main causes of secondary osteoporosis [5]. Among all the endocrine disorders, thyroid disorders are the most common disorders worldwide as well as in India out of which hypothyroidism is the most common [6].
Serum T_3 and T_4 levels are reduced in primary hypothyroidism resulting in pituitary TSH hypersecretion and elevated serum TSH levels. Abnormal activity of thyroid gland slows metabolic processes and causes a variety of clinical psychological and physiological abnormalities [7, 8].
According to the latest researches, India has a high prevalence of hypothyroidism which ranges from 4 to 11% with women suffering from the disorders more than males. Hypothyroidism becomes more common as people get older [1].

Thyroid disease particularly hypothyroidism have recently received more attention because they may be the primary cause of mineral metabolism disturbance due to their direct effect on bone turnover [9].

Thyroid hormones cause bone reabsorption and inhibits parathyroid hormone secretion which leads to raise serum calcium levels but hypothyroidism has the reverse effect. Low calcium level in hypothyroidism is caused by poor calcium mobilization into the bone as evidenced by numerous recent studies. Calcitonin synthesis is increased resulting in the reabsorption of phosphate and excretion of calcium from the renal tubules [10, 11].

Serum calcium level is regulated by thyroid hormones by releasing it from the parafollicular cells of thyroid gland. In primary hypothyroidism thyroxine level is less so calcium outflux from the cells is decreased [12].

Cyclic 3, 5 nucleotide phosphodiesterase enzyme is stimulated by magnesium. In thyroid gland thyrotropin activity is regulated by cyclic AMP and level of magnesium may be influence by thyroid gland in response to pituitary hormone [13]. Thyroid hormones regulate calcium, phosphorus and magnesium which are divalent metal ions required as a cofactor for metalloenzymes. Thyroid hormones also stimulate numerous metabolic pathways [14].

Iron is needed as a cofactor for thyroid peroxidase (TPO) enzymes. This enzyme catalyzes the two reactions in the production of thyroid hormone in thyroid gland. It operates on the apical plasma membrane and is responsible for iodine oxidation and binding to thyroglobulin tyrosine [15, 16, 17].

Zinc too has important roles in thyroid metabolism. It participates in the synthesis and mechanism of action of TRH by assisting T_3 binding to its nuclear receptor. It is also present in thyroid hormone binding transcription factors in which zinc is bound to cysteine residues [18].

Zinc is crucial for the biological activity of thyroid hormones and associated receptors as well as the peripheral conversion of T_3 to T_4 hormones. Enzymes in the synthesis of TRH need zinc as a cofactor [19].

Another micromineral selenium acts as a cofactor of iodothyronine deiodinase enzyme which helps to convert T_4 to T_3 and also important in the regulation of thyroid hormone production. It also serves as a cofactor for glutathione reductase and glutathione peroxidase enzymes which are known to protect the thyroid gland and various other tissues from oxidative stress [20].

Previous research works on the changes in serum mineral levels in hypothyroid patients have yielded mixed outcomes. Some scientists have noticed that there is no change in the circulating levels of these minerals in hypothyroid patients while others have found that the levels of these minerals increase in hypothyroid patients. As a result, this study was done to investigate variations in mineral profile in hypothyroid patients by estimating serum calcium, phosphorus, magnesium, iron, zinc, selenium and analyzing their association with thyroid profile (T_3, T_4 and TSH) levels.

Materials and Methods

The present study has been done on patients of age group 20-60 years including both males and females [8], attending the Medicine OPD of Teerthanker Mahaveer College & Research Center, Moradabad, Uttar Pradesh. The participants having age more than 20 years and less than 60 years diagnosed cases of hypothyroidism (TSH more than 4.5 µIU/ml) [10] were selected from the Medicine OPD of Teerthanker Mahaveer Hospital, Moradabad and who have given written consent form will be included in the study group.

Exclusion criteria

Patients on mineral supplementation, patients with Hepatic disease, Renal disease, Diabetic mellitus, Cardiac disease, pregnant women [21] 8 ml of venous blood sample after overnight fasting for 8-12 hours was collected under all aseptic precautions from all the subjects and dispensed into plain vials. After centrifugation at 3000 RPM for 5 minutes, serum sample was used for analysis of serum minerals and serum thyroid profile by different methods.

- Thyroid hormones (serum T_3, T_4 and TSH) were determined by Enzyme Linked Fluorescent Assay (ELFA) technique [22].
- Serum Calcium was determined by Arsenazo III method [36].
- Serum Magnesium was determined by Calmagite method [24].
- Serum Phosphorus was determined by Ammonium molybdate method [25].
- Serum Iron was determined by Ferrozine method [26].
- Serum Zinc was determined by Colorimetric method [27].
- Serum Selenium was estimated by Spectrophotometric method [28].

Statistical analysis

SPSS version 28.0 for data analysis and Microsoft Word and Excel for graph generation has used. Mean and standard deviation was assessed & comparison was done by using student's t-test & Pearson's coefficient correlation (r-value).

- *p*-value of less than 0.05 was considered as significant [S].
- *p*-value of less than 0.001 was considered as highly significant [HS].

Results

30% of the patients were between the age group of 20-30 years. This indicates a higher prevalence of hypothyroidism in patients between the age group of 20-30 years. Patient's age of 30 to 40 years was seen 18% of cases, 25% of the cases in the age group ranges from 40-50 years and 27% of cases in the age group ranges from 50 to 60 years. In present study, we observed that 78.3% hypothyroid patients were female and 21.6% hypothyroid patients were male. This indicates females have a higher frequency of hypothyroidism than males.

Table 1: Age wise distribution of cases

Age (years)	Number of hypothyroid patients (n=60)	Percentage (%)
20-30	18	30
30-40	11	18
40-50	15	25
50-60	16	27

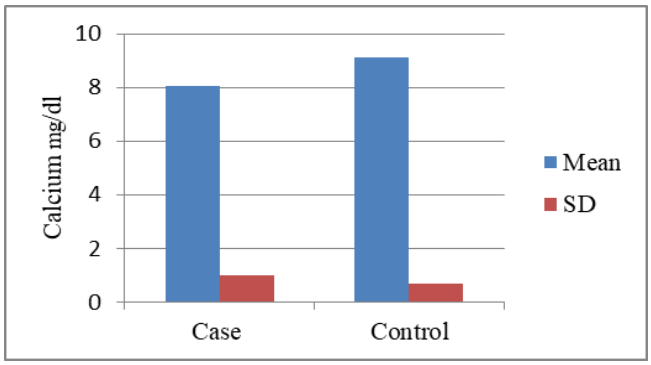
Table 2: Gender wise distribution of cases

Gender	Number of hypothyroid patients (n=60)	Percentage (%)
Female	47	78.3
Male	13	21.6

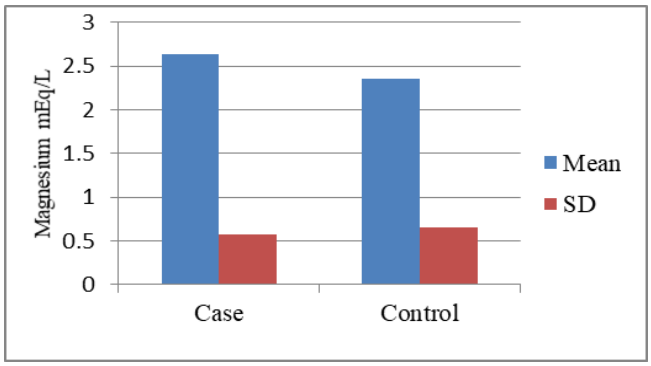
Table 3: Comparison of different parameters in cases and controls

Parameters	Control	Cases	p-value	Significance level
T_3 (0.60-1.85 ng/dl)	1.13±0.46	0.50±0.36	<0.001	HS
T_4 (4.80-11.80 µg/dl)	7.36±1.32	3.91±2.04	<0.001	HS
TSH (0.39-6.16 µIU/ml)	3.03±1.70	14.95±6.83	<0.001	HS
CALCIUM (8.2-10.5 mg/dl)	9.1±0.67	8.06±0.99	<0.001	HS
MAGNESIUM (1.3-2.5 mEq/L	2.36±0.65	2.63±0.57	0.02	HS
PHOSPHORUS (2.5-6.2 mg/dl)	4.22±1.23	6.79±1.04	<0.001	HS
IRON (35-145 µg/dl)	66.61±20.53	59.50±25.29	0.09	NS
ZINC (60-120 µg/dl)	95.79±25.34	52.52±16.55	<0.001	HS
SELENIUM (70-150 ng/ml)	84.85±12.54	82.11±21.04	0.38	NS

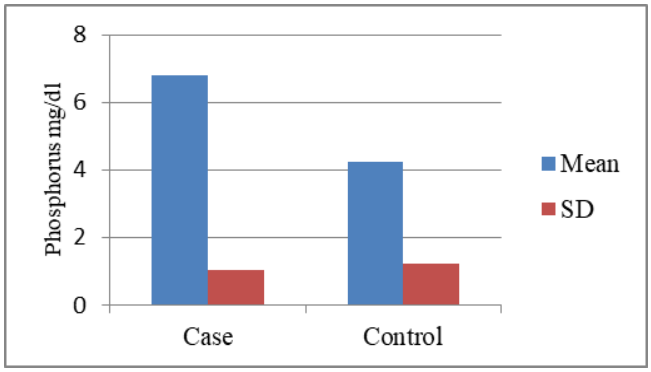
Serum minerals magnesium (p=0.01) and serum phosphorus (p<0.001) levels in the serum were significantly elevated whereas the levels of calcium and zinc were significantly (p<0.001) decreased in hypothyroid patients compared to healthy subjects. However, iron (p=0.09) and selenium (p=0.38) levels in hypothyroid patients were found to be less than that of healthy individuals but the difference between them was not statistically significant.



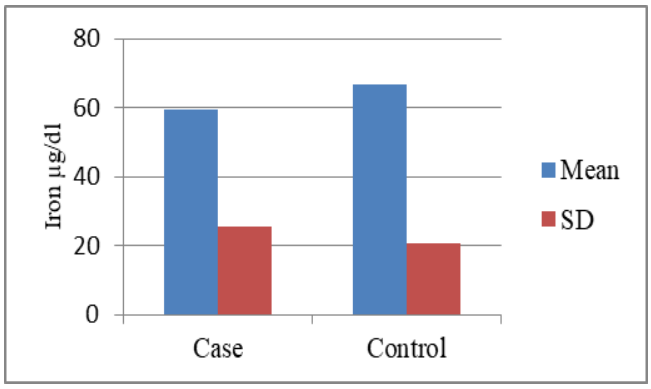
Graph 1: Mean and SD value of calcium among cases and controls



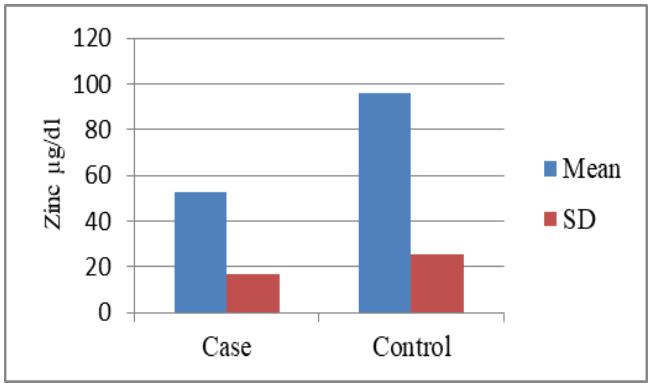
Graph 2: Mean and SD value of magnesium among cases and controls



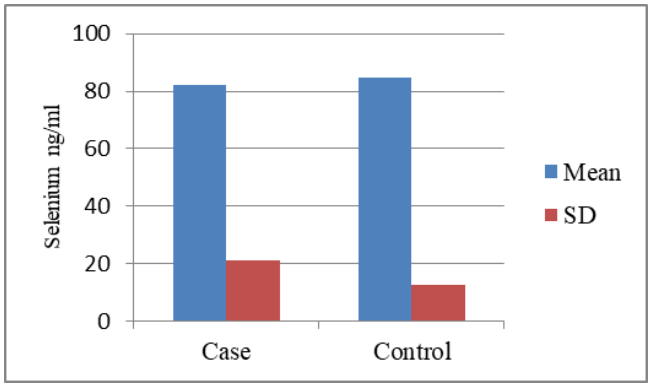
Graph 3: Mean and SD value of phosphorus among cases and controls



Graph 4: Mean and SD value of serum iron among cases and controls



Graph 5: Mean and SD value of zinc among cases and controls



Graph 6: Mean and SD value of selenium among cases and controls

Discussion

Thyroid hormones have vital function in human body which includes regulating body hemodynamic, thermodynamics and metabolism. They also have a significant impact on renal hemodynamic and glomerular filtration rate. Hypothyroidism is the most commonly occurring endocrine

disorder which can lead to different clinical manifestation including minerals disturbance, osteoporosis, congestive heart failure and coma [4].

As a result, this research was carried out to determine the impact of minerals (calcium, magnesium, phosphorus, iron, zinc and selenium) on hypothyroid patients and also found a possible correlation of these minerals with thyroid profile.

In this study maximum number of patients was women and higher prevalence of hypothyroidism was notice in the age group of 20-30 years. Female hypothyroid patients made up almost 78 percent of the total. In table 1 we found that 30% of hypothyroid patients were of the age ranges from 20 to 30 years, 18% in the age group of 30 to 40 years, 25% in the age group of 40 to 50 years and 27% of patients in age group of 50 to 60 years. In table 2 we have seen that 78.3% of hypothyroid patients were female and 21.6% were male. Hence, this shows female patients have a higher prevalence of hypothyroidism than male ones.

In table 3 we compared thyroid profile (T_3, T_4 and TSH) and minerals in hypothyroid cases and controls. We found that the mean value of serum T_3 (0.50±0.36 ng/dl) in hypothyroid patients were significantly (p<0.001) reduced when compared with healthy individuals (1.13±0.46 ng/dl) likewise, serum T_4 level was also significantly (p<0.001) reduced (3.91±2.04 µg/dl) when compared with control subjects (7.36±1.32 µg/dl). While, serum TSH (14.95±6.83 µIU/ml) was found to be significantly (p<0.001) raised when compared with controls (3.03±1.70 µIU/ml).

In the present study we found significant low level of serum calcium with mean concentration of 8.06±0.99 mg/dl in cases when compared with healthy controls 9.1±0.67 mg/dl. Decrease in serum calcium level in hypothyroidism could be related to a low level of thyroxine as thyroid hormones play an essential role in calcium homeostasis through their direct influence on bone turnover [29].

This is similar with a study done by Saxena S, *et al*. 2020 [8] they assessed that in hypothyroid patients, serum calcium level was lowered with a mean value of 8.0±8.4 mg/dl compared with 8.6±9.6 mg/dl healthy individuals. They came to the conclusion that in hypothyroid patients there is a substantial impact of minerals levels.

Our findings are also in similar with Sridevi D, *et al*. 2016 [30] as they found that serum level of calcium (8.58±0.46 mg/dl) was reduced in cases of hypothyroid patients when compared with healthy subjects (10.04±0.56 mg/dl). Their findings showed that thyroid hormones have a substantial effect on minerals and that regular follow up in thyroid hormone deficit might aid in its management.

While the study done by Susanna TY, *et al*. 2016 [14] observed that there is no change in the levels of serum calcium in hypothyroidism (8.98±0.61 mg/dl) compared with controls (8.98±0.49 mg/dl).

Present study shows that significantly (p=0.02) elevated levels of serum magnesium (2.63±0.57 mEq/L) in hypothyroid patients than in controls (2.36±0.65 mEq/L). Because thyroid hormones have great influence on renal tubules, which causes increased reabsorption of magnesium it could be attributable to increased renal retention of magnesium of up to 15 to 30% from the kidneys.

Our findings are consistent with those of Kavita MM, *et al*. 2014 [10] who reported raised level of serum magnesium (2.37±0.46 mEq/L) in hypothyroid patients compared with healthy subjects (2.1±0.28 mEq/L). Their findings showed that serum magnesium levels were elevated in both subclinical and overt hypothyroidism. Early detection and treatment can help to prevent additional bone complications. Kaur J, *et al*. 2014 [6] also found similar result in which level of serum magnesium was significantly (p<0.001) raised in hypothyroid cases 2.23±0.31 mEq/L when compared with healthy subjects 1.27±0.28 mEq/L. Their research found that monitoring serum magnesium levels at routine hypothyroid patient checkups can help individuals with hypothyroidism without any clinical manifestation.

While Nisa F, *et al*. 2013 [18] found that the level of serum magnesium was significantly reduced among hypothyroid patients (0.68±0.37 mEq/L) than in controls (1.01±0.26 mEq/L) which are contradictory to our findings. Their research revealed that serum magnesium levels in hypothyroid patients should be monitored reducing the adverse consequences of magnesium insufficiency in hypothyroid individuals.

In this study, we found a statistically significant increase in serum phosphorus (p<0.001) levels in hypothyroid patients (6.79±1.04 mg/dl) when compared with controls (4.22±1.23 mg/dl) which is similar to the findings done by Kavitha MM. 2014 [10]. They found that serum phosphorus levels were significantly elevated in subclinical and overt hypothyroidism than in controls with mean values of 4.1±0.46 mg/dl, 4.42±0.81 mg/dl and for controls 3.6±0.19 mg/dl, respectively.

Susanna TY, *et al*. 2016 [14] found similar findings showing that serum phosphorus levels in hypothyroid patients (5.56±2.03 mg/dl) were significantly higher (p<0.001) than in healthy people (3.10±0.50 mg/dl).

Increased serum phosphorus levels could be attributed to increased calcitonin synthesis which increases tubular phosphate reabsorption and tubular calcium excretion resulting in hypocalcemia and Hyperphosphatemia as seen in hypothyroidism [10].

In this present study, we found statistically no significant (p=0.09) difference in iron concentration among hypothyroid patients when compared with controls with the mean values of 59.54±25.29 µg/dl and 66.61±20.54 µg/dl, respectively. These findings could be related to hypothyroid patients with impaired iron metabolism and interrupted gastrointestinal absorption of iron resulting in a lower level of serum iron in hypothyroid patients.

These findings in our study of hypothyroidism cases are in similar with the findings of Talebi S, *et al*. [31] they reported that there were no significant changes in the levels of serum iron among hypothyroid patients when compared with healthy individuals.

Our findings are also comparable to those of Banday TH, *et al*. 2017 [32] who found that 34.2% of individuals with primary hypothyroidism had iron insufficiency.

Likewise, we also observed significantly decreased levels of zinc in hypothyroid patients (52.67±16.55 µg/dl) as compared to control subjects (95.79±25.34 µg/dl), which is accordance to the study of Arora M, *et al*. 2018 [34] They observed that the level of serum zinc was decreased in hypothyroid cases than in healthy subjects and their study concluded that patients with hypothyroidism should be given a zinc rich diet to help regulate their thyroid hormone levels and functioning.

However, Nisha F, *et al*. 2013 [18] discovered no statistically significant variation in serum zinc levels between hypothyroid patients and healthy controls in a separate

investigation. Zinc deficiency plays a little role in hypothyroid patients, according to their findings.

The reason behind the low level of serum zinc is due to reduced gastrointestinal absorption and tubular excretion of zinc in hypothyroid subjects.

In the present study, we observed a statistically insignificant (p=0.38) difference between serum levels of selenium among hypothyroid patients (82.11±21.04 ng/ml) and in healthy subjects (84.85±12.54 ng/ml).

The reduction in serum selenium levels in hypothyroidism patients was consistent with previous research by Jinger SK, *et al*. 2012 [35]. They reported that levels of serum selenium were decreased in cases (53.66±10.31 ng/ml) than in controls (114.35±22.5 ng/ml). They also concluded that correlation of serum selenium level would have a helpful effect on treatment and could prevent complications in hypothyroid patients.

This study investigates that serum calcium, iron, zinc and selenium levels were decreased whereas serum phosphorus and magnesium level were increased in cases than in controls. These findings show that altered metabolism of mineral (calcium, magnesium, phosphorus, iron, zinc and selenium) has intimate association with thyroid hormones.

Conclusion

This study concludes that levels of serum magnesium and phosphorus were slightly elevated whereas levels serum, calcium, iron, zinc and selenium were reduced among hypothyroid patients as compared to healthy individuals. In hypothyroidism, it is clear that the lower level of serum T_3, T_4 and elevation in the TSH level is a consistent finding but their effect on the serum levels of calcium, magnesium, phosphorus, iron, zinc and selenium is inconsistent which is due to the variations in the dietary intake of minerals, renal and intestinal absorption some subclinical pathologies and various other complex hormones which may affect minerals metabolism and cellular uptake.

Therefore, hypothyroid patients should have their serum mineral levels checked on a regular basis as early diagnosis and intervention can help to avoid further thyroid problems. It will be beneficial in treating hypothyroidism as well.

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