RESEARCH PAPER

Evaluation of Serum Zinc Levels in Newly Diagnosed Autoimmune Hypothyroidism in Avadh Region of UP, India

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ABSTRACT

Introduction: Dysfunction and metabolic abnormalities of thyroid gland are the most common endocrine disorder in India and worldwide. Thyroid hypofunction is associated with low serum zinc level, but limited data available in autoimmune thyroid dysfunction and findings are highly contradictory. The present study aimed to evaluate serum zinc levels in patients with anti TPO positive AITD and to find correlation with thyroid hormones.

Materials and Methods: A comparative cross-sectional study was conducted at tertiary care hospital, Ayodhya, involving 80 newly diagnosed autoimmune hypothyroid patients (aged 25–55 years) and compared with 80 healthy controls. Biochemical parameters including fasting plasma glucose (FPG), thyroid profile, and serum zinc were analysed. Data were statistically evaluated using SPSS version 29.0.

Results: The mean serum ferritin level was significantly lower in the hypothyroid group (65.42±16.56 µg/dl) compared to controls (94.77±16.26 µg/dl), with a p-value <0.0001. A strong negative correlation was found between TSH and serum zinc levels (r = –0.74) and positive correlation observed between serum T4 with serum zinc (0.36).

Conclusion: The present study established the presence of distinct and significant changes in serum zinc levels in overt hypothyroidism. So, the trace element zinc often result in development of thyroid dysfunction and thyroid hypofunction may result disturbances in zinc metabolism.

Keywords: Trace element, zinc hypothyroidism, AITD

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Introduction:

Thyroid dysfunction is among the most common disease of endocrine gland which affects a considerable portion of the population. [1] The burden of thyroid disease in general population of India is enormous and data from various studies suggest that in India approximately 42 million people suffer from thyroid abnormalities.[2] Thyroid diseases may be caused by qualitative or quantitative alterations in hormonal secretion, the increase in size of the gland or both mechanisms. Hypothyroidism is a disorder in which the thyroid gland is unable to synthesize and secrete sufficient amount of thyroid hormones to meet the requirement of the peripheral tissue. [3] Approximately 95 % of hypothyroidism is caused by thyroid malformation which is regarded as primary hypothyroidism. Environmental iodine deficiency is the most common cause of primary hypothyroidism on a worldwide basis.[4] In area of iodine sufficiency the most common case of hypothyroidism is chronic autoimmune thyroiditis (Hashimoto's thyroiditis Autoimmune thyroid disease have been estimated to be 5-10 times more common in women than in men. Various studies suggested that the prevalence of hypothyroidism in developed countries is 4-5%. [5] Study conducted on Indian populations in eight different cities by Unnikrishnan er al. revealed high prevalence of hypothyroidism (10.95%), affecting approximately 1 in 10 adults in the population studied.[6] Thyroid hormone deficiency in hypothyroid patients causes slowing of a wide variety of metabolic processes, which results in decrease resting energy

expenditure, oxygen consumption, and use of substrates, reduced thermogenesis, increase body weight.[7]

Zinc is a necessary trace element for human body which is required in limited amount. It is crucial for immune system, various enzyme activity, cell stimulation, neural transmission and also for normal functioning of metabolic homeostasis and also essential for sensitizing tissues to thyroid hormones.[8,9] Kotecha PV in 2008 study revealed that nearly two third of the 2 billion Indian population have zinc deficiency which result in development of associated metabolic disorders.[10] Thyroid hormone directly affect zinc metabolism and study conducted on nephrectomised rats suggested that diminished thyroid function was strongly associated to low serum zinc level.[11] In another epidemiologic study conducted on subjects older than 40 years stated that there was significant negative relationship exist between thyroid volume and serum zinc levels in hypothyroid patients.[12] Study revealed that zinc deficiency resulted in enhanced expression of hepatic thyroxine 5' monodeiodinase enzyme activity, which inactivates thyroid hormone.[13] Zinc is very essential in synthesis of type I and type II deiodinase enzyme, thyrotropin releasing hormone and also important for T4 to T3 conversion. [13] Zinc acts as linker for T3 nuclear receptor in hypothalamus, which stimulates the synthesis of TRH. Zinc transporters are available in hypothalamus, pituitary and thyroid glands for the release of respective hormones.[14] In addition T3 receptors are believed to belong to the group of nuclear receptors that contain zinc binding protein motif.[15] Study of Ihnatowicz P et al. suggested that deficiency of this trace element has been associated to change in tissue structure of thyroid gland, apoptosis and higher increase of TPOAb and Tg Ab.[16] There are studies which suggest that zinc transport in intestine and renal tubules are dependent on thyroid hormones which was observed in rat model.[17] Furthermore Zn deficiency may cause hypothyroidism and hypothyroidism may alter serum zinc levels. Some study revealed that overt hypothyroidism is associated with low serum zinc level. [18,19,20] But study conducted by Joanna Szczepanik et al. in 2021, revealed that there was no change in serum zinc levels in Hashimoto's thyroiditis which contradict other findings.[21] So studies regarding serum zinc level in autoimmune thyroid dysfunction are very limited and contradictory and no study conducted in Avadh region of eastern Uttar Pradesh, which drag our interest to evaluate serum zinc in autoimmune thyroid dysfunction.

Material and Method:

A comparative cross-sectional study was carried out in Biochemistry department in collaboration with General Medicine department for approximately 16 months duration from January 2024 to April 2025 at Rajshree Dashrat Autonomous State Medical College, Ayodhya. The study was performed on the patients attending the Medicine OPD. Ethical clearance was obtained from Institutional Ethical Committee. After calculating sample size, 80 cases of newly diagnosed hypothyroid patients age group between 25 to 55 years were selected. Samples were taken in the basis of inclusion criteria. Inclusion criteria were newly diagnosed overt hypothyroid patients age group between 25 to 55 years and both sexes were included. Hypothyroid patients selection criteria of the cases were based on biochemical laboratory investigation and clinical sign and symptoms. Hypothyroid subjects were primarily diagnosed on the basis of total T3, T4 levels below the normal reference range and TSH above normal reference range. 80 apparently healthy subjects were selected as control whose thyroid profiles were within normal reference range. All the biochemical parameters (FPG, T3, T4, TSH, Anti TPO, serum zinc) were analysed at central Biochemistry laboratory. The following patients were excluded from our study setting. Subjects taking any kind of medication affecting thyroid hormone levels. Sub clinical hypothyroidism and secondary hypothyroidism. Diabetic, Tuberculosis, hypertension, cancer and known cases of HIV. Pregnant women and women with contraceptive pills. Obese subjects BMI > 35.

All the subjects were explained about aim and objective of the study. Written consents were obtained from all along with detail history. Height and weight of all the subjects were measured.

After overnight fast approximately 4 ml of blood was collected from an antecubital vein with full aseptic precaution without anticoagulant and allowed it to clot. Clotted blood was centrifuged and clear serum was collected. The serum was preserved at -20^{0}C until analysed. Thyroid profile (T3, T4, TSH,) test was done using Architech Chemiluminescence analysers I1SR1000. Anti TPO test was performed by ELISA method using Erba Lisa Scan EM instrument with Calbiotech ELISA kit. Serum zinc was analysed by colorimetric assay by Erba Chem 7 semi analyser using coral clinical system. Fasting plasma glucose was estimated by GOD-POD method by Selectra Pro M autoanalyzer to rule out diabetic patients as exclusion criteria. All the test protocols were standardized and calibrated before quantization of all biochemical parameters as per NCCLS (National Committee for Clinical Laboratory

Standards)[22] guidelines. Modified OSHA (Occupational safety and health act, 1970) guidelines were implemented in all steps of sample collection, processing & handling of bio-medical waste product.[23]

Statistical analysis:

Statistical analysis was performed using SPSS version 29.0 All data was expressed as mean ± standard deviation. Comparison of serum levels of biochemical parameters between two groups was performed by unpaired Student 't' test. P value < 0.05 considered as statistically significant. Receiver Operative Characteristic curve (ROC) analysis was performed for serum zinc to obtain cut off value with maximum sensitivity and specificity to find out diagnostic efficacy of serum zinc.

Results:

group is shown in Table -1. The mean age of hypothyroid patients was 40.86±7.58 years and control group was 40.15±7.42 years. The mean BMI of hypothyroid group was significantly elevated than the control group (BMI for hypothyroid 25.92±2.07; control 23.37± 1.54) with p value ,0.0001 which is statistically highly significant. Table 2 shows the age and sex distribution of hypothyroid case and control group. Thae maximum number of subjects in our study was between 41 to 45 years (n=23). Number of female patients (42/80) were more than that of males (38/80) in my study population.

Table -3 refer to normal values of T3, T4, TSH and anti TPO, and also observed values of hypothyroid group and control group. It was observed that TSH value was significantly raised in hypothyroid group (42.87±23.32 µIU/ml) with p value highly significant (< 0.0001). Mean anti TPO value of hypothyroid group was 125.35± 63.41 IU/ml as compared with control group it was found 11.41±6.66 IU/ml.

Plasma glucose level in both hypothyroid and control group was within normal reference range as all the non-diabetic subjects were selected. Though in hypothyroid group the FPG was slightly raised (87.24±7.18) in comparison with control group (83.38±10.98). The mean serum zinc level in hypothyroid group was 65.42±16.56 µg/dl and control group was 94.77±16.26 µg/dl as referred to table 4. On comparing with student 't' test, p value was < 0.0001 which is highly statistically significant. Figure-2 shows significant negative correlation of serum zinc with TSH in hypothyroid patients with r value =-o.74. There was no significant correlation was observed between serum zinc and total T3 (r=0.14). Significant positive correlation was observed between serum zinc and total T4 (r=0.36).

Table 1: Demographic characteristics of study population & BMI

Parameters	Controls (mean±SD)	Hypothyroid cases (mean±SD)
Age Yrs)	40.15±7.42	40.86±7.58
Mean age of Males	40.32±7.14	40.28±7.19
Mean age of Females	41.07±7.31	39.78±7.92
BMI	23.37± 1.54	25.92±2.07

Table 2: Age and Sex distribution of Hypothyroid cases and controls

Age (Years)	Controls (Total=80)		Hypothyroid cases (Total =80)	
	Males	Females	Males	Females
25-30	4	5	4	4
31-35	8	4	6	10
36-40	13	10	10	9
41-45	9	11	11	12
46-50	1	3	2	3
51-55	3	9	5	4
Total	38	42	38	42

Table 3: Thyroid profile in hypothyroid cases and control

Parameter	Normal range	Controls	Hypothyroid cases	p-value
T3 (ng/ml)	0.58-1.59	1.24±0.32	0.50±0.19	<0.0001
T4 (µg/dl)	4.8-11.7	7.12±2.01	2.87±1.26	<0.0001
TSH (µIU/ml)	0.35-5.9	2.66±1.29	42.87±23.32	<0.0001

Anti-TPO (IU/ml)	< 45 IU/ml	11.41±6.66	125.35±63.41	<0.0001

Table 4: Comparison of biochemical parameters between two groups

Parameters	Controls (mean ±SD)	Range (Control)	Hypothyroid cases (mean ±SD)	Range (Hypothyroid cases)	p-value
Fasting Plasma glucose (mg/dl)	83.38 ±10.98	69-106	87.24± 7.18	73-105	0.009
Serum Zinc (µg/dl)	94.77 ±16.26	67.8-132.5	65.42± 16.56	31.3-102.4	<0.0001

Figure -1 shows Box Plot demonstrating distribution of upper quartile, median and lower quartile of serum zinc concentration (ng/ml) of hypothyroid and control group. Mean ± SD of serum zinc in hypothyroid group was 65.42±16.56 µg/dl. Median for hypothyroid group was 67.3. Mean ± SD of serum zinc in control group was 94.77±16.26 µg/dl and median for control group was 92.15

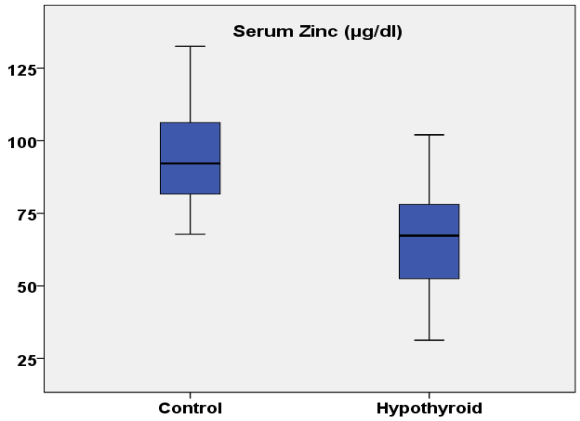

Fig-1: Box plot demonstrating upper quartile, median and lower quartile with
max and min concentration values obtained for serum zinc concentration (µg/dl)

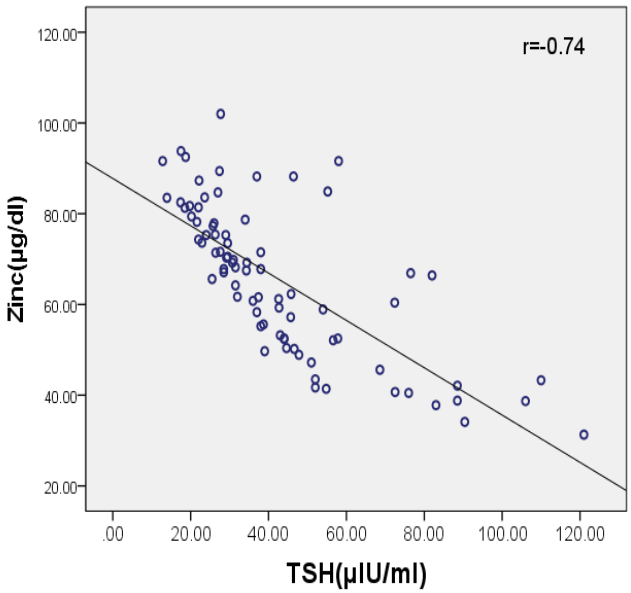

Fig-2: Correlation serum Zinc with TSH in hypothyroid cases.
Fig-3 shows ROC curve analysis of serum zinc concentration (ng/ml) in hypothyroid and control group. Area under curve was 0.897; standard error as per Hanley & McNeil was 0.019; 'p' value,0.00; sensitivity was 82% and specificity was 78% with best cut off value 81.0 µg/dl.

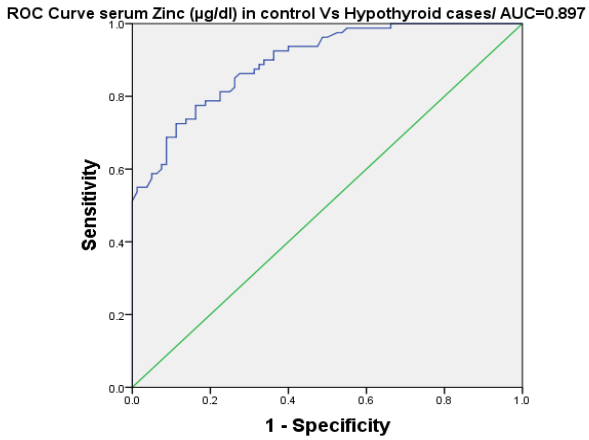

Fig-3: ROC curve analysis of serum Zinc concentration (µg/dl) in hypothyroid and control group.

Discussion:

Zinc is a vital trace element that plays an important role in body's mineral coenzyme cycle. The present study aims to evaluate the relationship between serum zinc and thyroid profile in autoimmune overt hypothyroid patients. According to our study the mean ± SD levels of serum zinc level in hypothyroid group was 65.42±16.56 µg/dl and in control group was 94.77±16.26 µg/dl which clearly suggest that in our study the hypothyroid group had significantly lower level of serum zinc in comparison to control group

which was statistically highly significant with p value <0.0001. Similar finding was reported by Shahnaz Khatun et al.(2019), where they evaluated serum zinc in 80 hypothyroid patients and compared with 80 healthy subjects and found mean±SD of serum zinc in hypothyroid group was 85.20±36.11 µg/dl and in control group was 100.85±23.86 µg/dl, which clearly suggest lower serum zinc levels(p=0.002) in hypothyroid patients.[24] Our present study was in accordance to animal model study conducted by Simsek G et.al.(1997) where methimazole induced hypothyroid animal showed significant decrease plasma zinc concentration.[25] Another study conducted by Bassam A et al. (2022) who evaluated serum zinc levels in 38 hypothyroid patients and compared with 12 healthy controls and confirmed significant changes in serum zinc levels in hypothyroid patients.[26]. The significance reduction of serum zinc in hypothyroid patients in comparison to normal subjects were observed by other different researchers (Saima Khan et.al 2022, Amrendra Mani Patel et.al. 2023, Hernando Vargas Uricoechea et. al. 2024)[19,18,20] which was in agreement with our present study. Survey conducted by Napolitano G et. al. suggested that zinc deficiency is common in children with Down's syndrome, who also have higher incidence of hypothyroidism.27

Zinc affects thyroid hormone secretion at several levels. Zinc deficiency inhibits TRH synthesis and decreases extrathyroidal conversion of T4 to T3 and zinc is an important cofactor for deiodinases I and II.[13] It has been observer that zinc deficiency results in structural tissue changes of thyroid gland and increased apoptosis.[16] In our current study the significantly decreased zinc levels in hypothyroidism can be explained by few possibilities. The most possible mechanism is that decrease gastrointestinal zinc absorption is highly impaired in severe hypothyroidism.[28] The link between zinc deficiency and hypothyroidism can be established by another fact that body zinc distribution is altered in those patients and zinc absorption is increased by other tissues like liver, which attributed to low serum zinc.[29] Another reason, according to the study conducted by Bellisola et. al (1998) is due to the significant influence of TSH in the various concentration of iodine, selenium and zinc in normal and altered thyroid tissue.30

In our present study highly, significant negative correlation was observed between TSH and serum zinc levels (r=-0.74). Significant positive correlation was observed between serum T4 and serum zinc levels (r=0.36) but no significant correlation was observed between serum zinc and total T3. Above findings are in accordance with similar study conducted by Bassam A et al. (2022) and Saima Khan et. al. (2022).[26,19] Our correlation study findings contradict the another research carried out by Manisha A et. al. who observed significant positive correlation of zinc with T3 level, but there was no significant correlation with other hormones like T4 and TSH.[31]

Few studies reported contradiction where no alteration was observed in serum zinc levels in overt hypothyroidism. Study conducted by Fakhar Un Nisa et. al.(2013) revealed that there was no change of serum zinc in hypothyroidism. [32] Another recent study conducted by Joanna Szczepanik el.al. (2021) where they evaluated serum zinc in 42 Hashimoto's thyroiditis patients and compared with 30 healthy controls.They stated that that there was no differences in the concentration of zinc in patients with Hashimoto's disease which contradict our findings. 21 Study conducted by Hernando Vargas et. al. (2024) revealed that a serum zinc level < 70 µg/dl was associated with higher frequency of AITD. [20] They suggested that the high prevalence of zinc deficiency could be one of the underlying causes of the increased susceptibility to AITD in general population. By ROC curve analysis we also found similar cut off value of serum zinc level (81 µg/dl). To our knowledge this is the first study evaluating a cut off point for zinc levels and its association with auto immune thyroid disease (AITD) in Indian population. Our study finding suggest that the zinc and thyroid metabolism have reciprocal relationship because insufficient zinc supplementation causes hypothyroidism and hypothyroidism in turn induces zinc deficiency.

Conclusion:

The present study revealed that serum zinc levels are significantly reduced in overt hypothyroidism. Significant negative correlation was observed between TSH and serum zinc level and positive correlation observed between T4 and serum zinc level. So, we can conclude that trace element zinc deficiency often lead to development of hypofunction of thyroid gland and thyroid dysfunction in turn affect the zinc metabolism homeostasis. Due to various contradictory findings the exact role of low levels of serum zinc in hypothyroidism is not fully understood, so further studies are needed with larger sample size to understand the clinical significance of this abnormality and the role of zinc supplementation in thyroid disease.

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Authors contribution: Mohua Roy Mukherjee conceived the study and performed the experiments and analyzed the data and prepared the manuscript. Dr Anupama Sharma guided in writing the manuscript and corrected and edited the manuscript. All other author reviewed and approved the final manuscript.

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Availability of data: The data will be available on request by the corresponding author.

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