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CORRELATION OF SERUM VITAMIN B12,IRON AND ZINC LEVELS IN PATIENTS WITH HYPOTHYROIDISM AND HYPERTHYROIDISM.

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ABSTRACT

Background Hypothyroidism is a clinical entity resulting from deficiency of Thyroid Hormones. Several Minerals & trace elements are needed for normal Thyroid Hormone metabolism .Theirdeficiencies impair the Thyroid Hormone Metabolism. Present study was conducted to find out the possible correlation between Vitamin B12,Iron and Zinc in Patients with Hypothyroidism & Hyperthyroidism and compared with the normal controls.

Materials & Methods-Our study included 100 patients with Thyroid disorders and 50 normal control subjects. In all subjects T3,T4,TSH were measured by CLIA and iron by Ferrene-s method, zinc by Flame Emission Spectrophotometry.

INTRODUCTION

The deficiency of micronutrients such as vitamins and minerals is of great concern in public health. The World Health Organization (WHO) reported more than

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twobillion people are affected by micronutrient deficiency and its related health consequences [1].

Elimination of micronutrient deficiencies through nutrition supplementation programs is widely seen as the most promising and cost-effective way to eradicate nutrition deficiency. The optimal metabolic functioning of an individual requires a proper supply of micronutrients such as vitamins, coenzymes, and intracellular elements. Micronutrients play a crucial role in catalyzing various enzymatic reactions, regulating the permeability of cell membranes, and various other physiological activities [3]. Optimal metabolic functioning of an individual requires a proper supply of micronutrients such as Vitamins, coenzymes& intracellular elements.

Thyroid hormone (TH) regulates metabolic processes essential for normal growth and development as well as regulating metabolism in the adult. Patients with deficiency of vitamin B12 and hypothyroidism usually have symptoms of fatigue, weakness, poor memory retention, itching and loss of sensation [4,5].

Ferritin is the storage form of iron present in the body. Iron deficiency impairs thyroid hormone synthesis. Low serum ferritin is one of the most overlooked causes of low thyroid function. Micronutrients play a crucial role in catalyzing various enzymatic reactions, regulating the permeability of cell membranes, and various other physiological activities

The pathogenesis of thyroid disorders has also been shown to be highly influenced by dietary factors, i.e., the availability of micronutrients such as iodine, vitamin D, iron, selenium, copper, zinc, vitamin B12, etc. Micronutrients are involved in physiological functioning like hormone synthesis, hormone transportation, and its binding to a target receptor. Micronutrients. Previous studies have shown the importance of nutrients in regulating the metabolism of thyroid hormones. Zinc in particular, is essential for the synthesis of thyroid hormones as well as its actions on target tissues. Zinc is required for synthesis of the thyrotropin releasing hormone (TRH), plays an important role in the binding of T3 to its nuclear receptor, participates in the synthesis of the thyroid stimulating hormone (TSH) in

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the anterior pituitary, and acts as an inhibitor or cofactor of type 1 and type 2 deiodinases [5,6,7,8,9].

Zinc is also important for proper operation of the immune system, antioxidant activity, sensorineural function and structural stability of membranes, transcription/translation of polynucleotides, and endocrine function, especially thyroid hormone metabolism [8.9]. A healthy adult has 2-3 g of zinc distributed in all tissues, fluids and secretions, with approximately 90% in skeletal muscles and bones, 11% in liver and skin, and the remaining in other tissues. Only a small portion (about 0.5%) of total zinc content in the body is found in the blood. From this total, 80% are present in erythrocytes and approximately 16% in plasma [10.11]. Inside the cells, about 50% of the zinc in the cytoplasm is 30 to 40% in nucleus and 10% in the plasma membrane

Materials and Methods

The present study was carried out in 100 patients suffering from Thyroid disorders who were attending the OPD of our Hospital. Informed consent was obtained from all the individuals. Persons between age of 20-60 yrs. were included. Persons with Diabetes mellitus, Hypertension and Secondary Hypothyroidism were excluded from the study.

The Sample was collected by overnight fasting ,5ml venous blood was collected from individuals and was allowed to clot completely at Room temperature and then centrifuged for ten min at approx. 3500rpm.

Measurement of serum Vitamin B12 was done Chemiluminescence Immunosorbent Assay (CLIA).Serum Zinc was estimated by Flame Emission Spectrophotometry and Serum Iron by Ferrene-S method.

The Statistical analysis was done using MS Excel(Microsoft 365),python 3.10.12 and IBMSPSS Statistics 27.The association between the two variables was analyzed using Pearson’s correlation coefficient .For all Tests ,a p value of <0.05 (two tailed)was considered statistically significant. Normality was checked using the Shapiro-Wilk normality test. Levene's Test was employed to check the Equality

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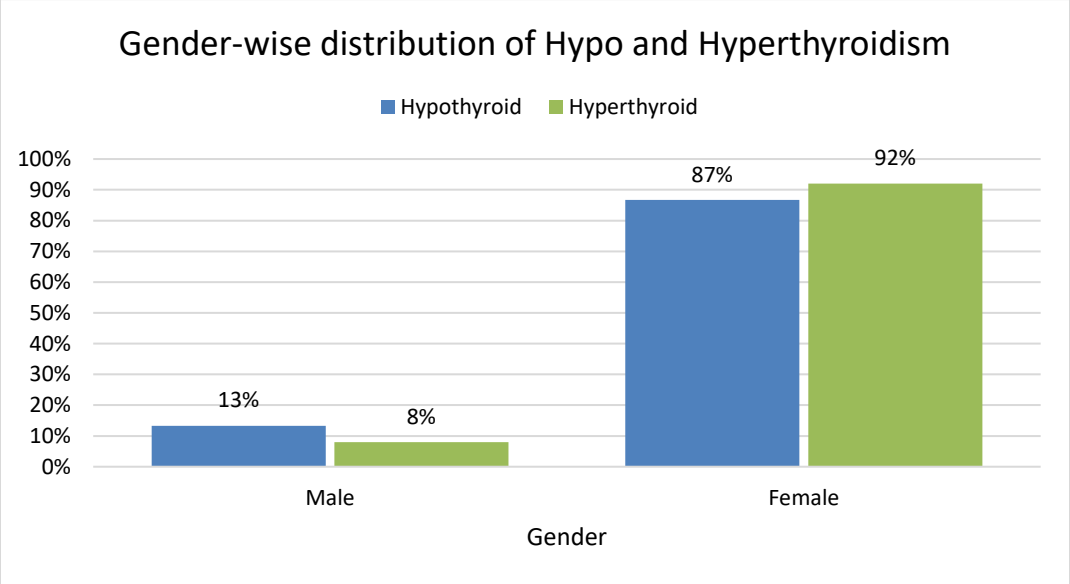
of Variances. Differences between the hypothyroid, hyperthyroid and normal groups were analysed using One-Way ANOVA for normally distributed variables or Independent Samples KruskalWalli's test for the assumptions violating the assumptions. The association between the two variables was analysed using Pearson's correlation coefficient. For all the tests, a p-value of<0.05 (two-tailed) was considered statistically significant.

Results:

Total number of subjects included in the present study were 150, out of which 75 were Hypothyroid, 25 were Hyperthyroid and 50 were the normal controls.

Demographic Parameters:

Graph 1: Gender-wise distribution of Hypothyroid and Hyperthyroid patients



Graph one shows that 87% of the female population had Hypothyroidism & 92% had Hyperthyroidism. as compared to the male patients

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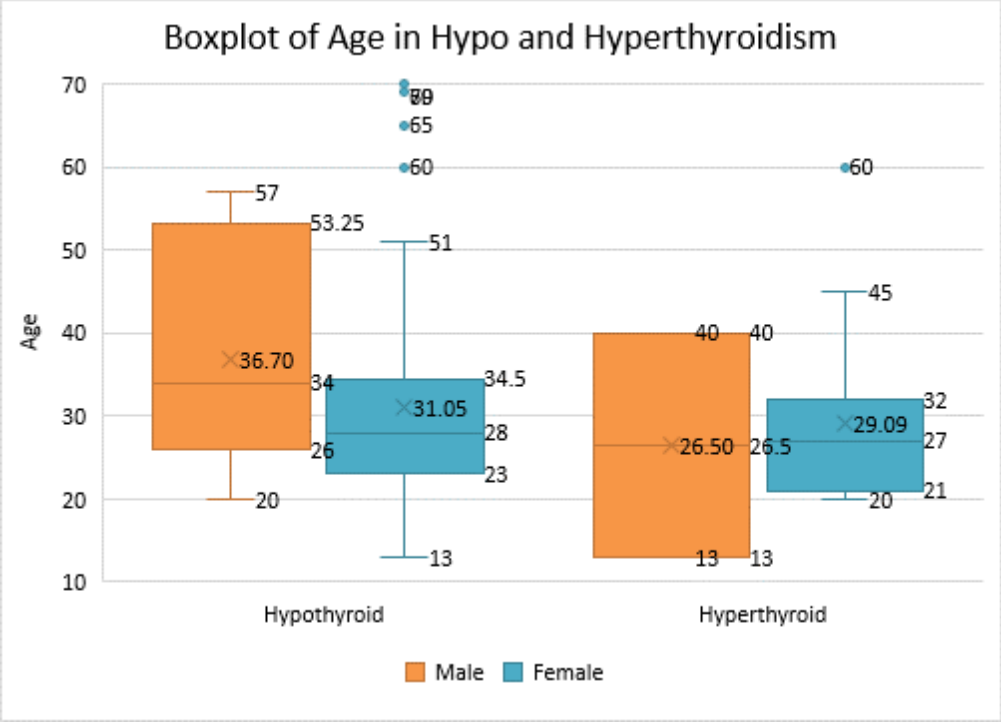
Graph 1 - shows genderwise distribution of thyroid patients.By statistical analysis we found a significant difference in the two groups indicating that 87% of Females were Hypothyroid in Graph 1 as compared to 13% Males.

Graph 2: Boxplot of Age in Hypothyroid and Hyperthyroid patients



Graph 2- shows age wise distribution of thyroid patients.In Hypothyroid patients the age grp (31.80 ± 13.50) & in Hyperthyroid patients (28.88 ± 9.70)

Results:

Table 1: Laboratory findings of Hypothyroid, Hyperthyroid and Normal group

	Hypothyroid Mean ± SD (n = 75)	Hyperthyroid Mean ± SD (n = 25)	Normal Mean ± SD (n = 50)	P-value
Age	31.80 ± 13.50	28.88 ± 9.70	30.40 ± 12.89	0.761
TSH	56.56 ± 38.48	0.04 ± 0.04	1.07 ± 0.79	*<0.001**

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Total T3	0.93 ± 0.61	1.66 ± 0.74	5.46 ± 4.28	*<0.001**
Total T4	6.26 ± 4.67	13.69 ± 5.17	6.21 ± 1.75	*<0.001**
Vitamin B12	226.63 ± 275.29	794.72 ± 474.64	569.96 ± 179.68	*<0.001**
Sr Zinc#	59.36 ± 22.44	88.60 ± 15.80	77.84 ± 12.33	*<0.001**
Sr Iron#	60.59 ± 33.43	122.32 ± 30.60	107.38 ± 19.35	*<0.001**

Values represented are mean ± SD for quantitative variables; test used: One-Way ANOVA#or Independent Samples KruskalWalli's test. P-value<0.05; statistically significant.*

Table 1- In normal individuals group,all the serum values were in normal range.Levels of TSH were higher(56.56 ± 38.48) in Hypothyroid patients as compared to Hyperthyroid (0.04 ± 0.04).Serum Zinc (59.36 ± 22.44), Serum Iron(60.59 ± 33.43) & Vitamin B12(226.63 ± 275.29) were reduced in Hypothyroid patients with significant p value < 0.001.

Table 2: Pearson Correlation Coefficient among the Vitamin B12, Sr. Zinc, Sr. Iron with TSH in hypothyroid

		TSH	Vitamin B12	Sr Zinc	Sr Iron
TSH	Pearson Correlation	1	-0.514**	-0.587**	-0.570**
	Sig. (2-tailed)		<0.001	<0.001	<0.001
	N	75	75	75	75
Vitamin B12	Pearson Correlation	-0.514**	1	0.423**	0.498**
	Sig. (2-tailed)	<0.001		<0.001	<0.001
	N	75	75	75	75
Sr Zinc	Pearson Correlation	-0.587**	0.423**	1	0.539**
	Sig. (2-tailed)	<0.001	<0.001		<0.001
	N	75	75	75	75
Sr Iron	Pearson Correlation	-0.570**	0.498**	0.539**	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	
	N	75	75	75	75

Test used: Correlation test. P-value<0.05; statistically significant.*

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Table 3: Pearson Correlation Coefficient among the Vitamin B12, Sr. Zinc, Sr. Iron with Total T3 in hypothyroid

		T3	Vitamin B12	Sr Zinc	Sr Iron
T3	Pearson Correlation	1	0.255*	0.16	0.386**
	Sig. (2-tailed)		0.027	0.171	0.001
	N	75	75	75	75
Vitamin B12	Pearson Correlation	0.255*	1	0.423**	0.498**
	Sig. (2-tailed)	0.027		0	0
	N	75	75	75	75
Sr Zinc	Pearson Correlation	0.16	0.423**	1	0.539**
	Sig. (2-tailed)	0.171	0		0
	N	75	75	75	75
Sr Iron	Pearson Correlation	0.386**	0.498**	0.539**	1
	Sig. (2-tailed)	0.001	0	0	
	N	75	75	75	75

Test used: Correlation test. P-value<0.05; statistically significant.*

Table 4: Pearson Correlation Coefficient among the Vitamin B12, Sr. Zinc, Sr. Iron with Total T4 in hypothyroid

		T4	Vitamin B12	Sr Zinc	Sr Iron
T4	Pearson Correlation	1	0.293*	0.359**	0.328**
	Sig. (2-tailed)		0.011	0.002	0.004
	N	75	75	75	75
Vitamin B12	Pearson Correlation	0.293*	1	0.423**	0.498**
	Sig. (2-tailed)	0.011		<0.001	<0.001
	N	75	75	75	75

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Sr Zinc	Pearson Correlation	0.359**	0.423**	1	0.539**
	Sig. (2-tailed)	0.002	<0.001		<0.001
	N	75	75	75	75
Sr Iron	Pearson Correlation	0.328**	0.498**	0.539**	1
	Sig. (2-tailed)	0.004	<0.001	<0.001	
	N	75	75	75	75

Test used: Correlation test. P-value<0.05; statistically significant.*

Table2, 3 &4-Pearsons correlation coefficient between variables in Hypothyroid & Hyperthyroid patients.

Table 5: **Association between T3, T4 and TSH with all the parameters in Hypothyroid cases.**

Pearson Correlation	**T3**	**T4**	**TSH**
Age	-0.03	0.041	-0.072
T3	1	0.635	-0.319**
T4	0.635**	1	-0.624**
TSH	-0.319**	-0.624**	1
Vitamin B12	0.255*	0.293*	-0.514**
Sr Zinc	0.16	0.359**	-0.587**
Sr Iron	0.386**	0.328**	-0.570**

*Values represented are Pearson's correlation coefficient. Correlation is significant at the 0.01 level** (2-tailed) and at 0.05 level* (2-tailed)*

Table 6: **Association between T3, T4 and TSH with all the parameters in Hyperthyroid cases.**

Pearson Correlation	**T3**	**T4**	**TSH**
Age	0.307	0.273	-0.079
T3	1	0.629**	-0.401*
T4	0.629	1	-0.217
TSH	-0.401*	-0.217	1
Vitamin B12	0.267	0.07	0.161
Sr Zinc	0.116	0.28	-0.077
Sr Iron	-0.132	0.109	0.26

*Values represented are Pearson's correlation coefficient. Correlation is significant at the 0.01 level** (2-tailed) and at 0.05 level* (2-tailed).*

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Table 5 &6 -Showed association between all the parameters in Hypothyroid & Hyperthyroid cases.

Table 7: Correlation of thyroid profile with Vitamin D, Vitamin B12, serum zinc and iron in Hypo and Hyperthyroidism

Correlation between	Hypothyroid	Hyperthyroid
Serum T3 and Vitamin B12	r value = 0.255 p-value = 0.027*	r value = 0.267 p-value = 0.197
Serum T4 and Vitamin B12	r value = 0.293 p-value = 0.011*	r value = 0.070 p-value = 0.739
Serum TSH and Vitamin B12	r value = -0.514 p-value < 0.001**	r value = 0.161 p-value = 0.442
Serum T3 and Serum Zinc	r value = 0.160 p-value = 0.171	r value = 0.116 p-value = 0.582
Serum T4 and Serum Zinc	r value = 0.359 p-value = 0.002**	r value = 0.280 p-value = 0.175
Serum TSH and Serum Zinc	r value = -0.587 p-value < 0.001**	r value = -0.077 p-value = 0.714
Serum T3 and Serum Iron	r value = 0.386 p-value = 0.001**	r value = -0.132 p-value = 0.530
Serum T4 and Serum Iron	r value = 0.328 p-value = 0.004**	r value = 0.109 p-value = 0.605
Serum TSH and Serum Iron	r value = -0.570** p-value < 0.001**	r value = 0.260 p-value = 0.209

*Values represented are Pearson's correlation coefficient (indicated by r-value). Correlation is significant at the 0.01 level** (2-tailed) and at 0.05 level* (2-tailed).*

Table 7- Vitamin B12 shows significant and positive correlation with T3(**r value = 0.255p-value = 0.027*),**
T4 (r value = 0.293 p-value = 0.011*) &TSH(r value = -0.514.p-value < 0.001)**

Serum Zinc Showed positive & significant correlation with T4(**r value = 0.359 p-value = 0.002**) &TSH(r value = -0.587.p-value < 0.001*}**

Serum Iron showed significant & positive correlation with T3(**r value = 0.386 p-value = 0.001**},T4.(r value = 0.328 p-value = 0.004**) & TSH(r value = -0.570****

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p-value < 0.001}**

DISCUSSION

Thyroid Hormones are critical for the regulation of Basal metabolic rate of organs like liver, kidney, heart,brain.Abnormality in Thyroid function may also cause due to Iron deficiencyAnemia, decreasing the plasma T4 levels reducing the peripheral conversion of T4to T3 & increase in TSH.

In the present study ,serum TSH levels were significantly higher & total T3 & T4 levels were at lower levels in Hypothyroid In hyperthyroid patients T3 & T4 levels were higher & TSH levels were lower as compared to normal individuals.

In the present study,B12 was observed significantly low(226.63 ± 275.29) in Hypothyroid patients with p value < 0.001 . as compared to Normal individuals(569.96 ± 179.68)

Another study, conducted in Turkey, reported Vitamin B12 subclinical Hypothyroidism as 25.6 % among 100 patients with Subclinical Hypothyroidism & 18.6% among 100 patients with overt Hypothyroidism.

In our study, we found significantly decreased level of zinc in patients of hypothyroidism as compared to control subjects, which is in line with the findings of Mohammed et al. [10], Baloch et al. [11], Al-Juboori et al. [12], Ali et al. [13], Jinger et al. [14], Rashid et al. [15]. The possible mechanism for the decreased level of zinc in hypothyroidism patients is that gastrointestinal absorption of zinc is severely impaired in these patients[16]. Another explanation, according to the study done by Bellisola, is due to the significant influence of TSH in the variations of concentration of iodine, selenium, and zinc in normal and altered human thyroid tissues [17]. In addition to this, the tubular excretion of Zn also leads to low levels of plasma zinc [18].

Another study also reported, Iron deficiency in significant portions of Patients with Hypothyroidism.

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Pearson's coefficient analysis between the Vitamins, Micronutrients& Thyroid parameters of the candidates elucidated the possible correlation between micronutrients such as zinc ,Iron with thyroid parameters.

To our knowledge ,this is the first retrospective study of the association of wide range of micronutrients & the incidence of total thyroid parameters.The study provides baseline data on the potential micronutrients in thyroid health.

Conclusion

In our study Females have higher percentage of Hypothyroidism as compared to males. Patients with Hypothyroidism have lower levels of serum Iron, Zinc& Vitamin B12 as compared to Hyperthyroid patients. Thyroid hormone plays a crucial role in Hemopoieticsystem,and hence may lead to Anemic condition. It is recommended that Hypothyroid patients should be tested for serum Iron, Zinc and Vitamin B12 levels.

Conflict of Interest

The Authors declare no conflicts of interest

Limitations- The Sample size was relatively smaller, further studies on larger population are required to determine the diagnostic utility of these trace elements in screening& planning treatment of thyroid disorders

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