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ASSESSMENT THE RELATIONSHIP OF S. IRON LEVEL AND T3, T4, TSH IN HYPOTHYROID PATIENTS

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ABSTRACT

The present work attempts to investigate S. Iron level in sample of patients complaining from hypothyroidism. One hundred and five blood samples for hypothyroidism patients including 17 males and 88 females have been collected during the period November 2018 up to May 2019 from the patient visiting the specialized center for Endocrinology and Diabetes. Their age ranged between 10 to 70 years. The results of the investigations have been compared with those for 37 apparently healthy controls who matched patients in the age and gender. Evaluation of serum concentration of T3, TSH was done by ELASA technique application and T4 was estimated by using ELFA methods, while serum Iron was determined by Bathophenanthrolin method. This study showed that there was a highly significant prevalence of hypothyroidism among age 31-50 years in comparison with other age groups $P < 0.01$. Moreover, this study revealed that the majority of patients were females 84% in comparison with males 16% with highly significant differences between their frequencies $P<0.01$. Additionally, highly significant elevation was observed in mean of TSH (17.490µIu\ml) in comparison with control group (2.883µIu\ml) $P < 0.01$. S. Iron was at the visit (60.561±26.80) with no significant differences in comparison with control (67.054±18.22) $P > 0.05$. The current study also revealed to that the mean of UIBC and TIBC (269.793±74.555, 327.084±51.258 respectively, and had highly significant differences in comparison with control group (222.621±39.121, 302.027±47.501, respectively. Finally TSH has a weak positive correlation with S. Iron ($r=0.038$) with anon significant differences $P=0.704$.

KEYWORD: T3, T4, TSH, S. Iron, Relationship.

INTRODUCTION

Iron deficiency anemia is the most common type of anemia and the most common cause of microcytic anemia. Iron deficiency ranges from Iron depletion to Iron deficiency anemia which can affect the function of numerous organ systems.[1] Iron deficiency anemia may be caused by diets low in iron, body changes, gastrointestinal tract abnormalities and blood loss. The most common symptoms of iron deficiency anemia are abnormal paleness of the skin, irritability, lack of energy (fatigue), increase heart rate (tachycardia) and enlarged spleen. It is usually discovered during a medical examination through a blood test that measures the amount of hemoglobin and the amount of iron in the blood.[2] Iron deficiency anemia is more common in people with hypothyroidism.[3]

Iron deficiency anemia impairs thyroid hormone metabolism because the first steps in thyroid hormone synthesis are catalyzed by thyro- Peroxidases, which are iron requiring enzymes, iron deficiency lowers plasma T3 and T4 concentration, reduces the rate of conversion of T4 to T3 and increase the thyrotropin concentration because of these impairments in Iodine metabolism, goiter in anemic individuals may be less responsive to Iodine treatment.[4] Hypothyroidism caused by the inability of thyroid gland to make thyroid hormone thyroxene T4 and triiodothyonine T3 is called primary hypothyroidism.

The most common cause is the deficiency of the element iodine and the destruction of thyroid gland by the immune system a condition called Hashimatos thyroiditis, also called chronic thyroidit.[5] Other causes include surgical removal of part or all of the thyroid gland, radioactive iodine used for treatment of hyperthyroidism, radiation exposure to the neck, special X- ray's dyes and certain drugs such as Lithium. Since the thyroid gland is regulated by the pituitary gland and hypothalamus, disorders of these hypothyroidism affects the whole from early symptoms like weakness, fatigue, cold intolerance, weight gain and late symptoms like slow speech, dry flaky skin thickening of the skin, and decrease sense of taste and smell.[6] The function of

thyroid gland is to make thyroid hormone, which in turn regulates the body's metabolism.[7] Normal thyroid secretion depends on thyroid stimulation hormone TSH.[8]

It is also known as thyrotropin , it's a glycoprotein in the anterior pituitary gland which regulates the endocrine function of thyroid gland.[9] It does this by producing a protein called thyroglobulin and then attaching Iodine to portion of it in order to produce the two forms of thyroid hormone called Triiodothyronine T3 and thyroxin T4.[10] Thyroxin (tetraiodthyronine) is produced as a precursor thyroglobulin (Tg) which is cleaved by thyroid peroxidase (TPO) enzyme to produce active T4. This hormone contains four iodine atoms. Triiodothyronine is identical to T4 but it has one less iodine atom per molecule.[11] Iodine is actively absorbed from the blood stream and concentrated in the thyroid follicles via a reaction with the enzyme thyroperoxidase , iodine is covalently bound to tyrosine residues in the thyroglobulin molecules forming monoiodothyrosin (MIT) and diiodotyrosin (DIT) linking two particles of DIT produces thyroxine. The combination of one particle of MIT and one particle of DIT results in triiodothyrorine. Proteases digest iodinated thyroglobulin releasing the hormones T4 and T3 because of T3 is more active than T4 and it's the final form of hormone , its present in less quantity than T4.[12,13] Deficiencies of iron and iodine are major overlapping public health problems in the developing world.[14,15] Iron status affects thyroid metabolism and iodine deficiency disorders. The initial steps of thyroid hormone synthesis are catalyzed by thyroperoxidase and are dependents on iron. In addition iron deficiency may alter central nervous system control of thyroid metabolism and modify nuclear triiodthyronine binding.[16,17] Iron deficiency anemia is associated with lower plasma thyroid hormone concentrations in rodents and in some studies in human.[16] Sever deficiencies of iron (Fe) and Iodine (I) affects more than one third of the world's population. Table, salt, fortified with I and Fe, would be useful in areas in which anemia and goiter coexist.[18] This study aimed to assess the relationship of S. Iron level and T3, T4, TSH among hypothyroid patients during the period study.

MATERIALS AND METHODS

This study was performed during the period from November/ 2018 to May / 2019. One hundred and five hypothyroid subjects were selected from people attending the specialized Center for Endocrinology and Diabetes. Their ages range from 10-70 years. Careful history was obtained from patients including age, sex. All patients were free from iron medication affecting on iron level. For each patient the following tests were carried out:

1. Thyroid Function tests which include T3, T4 and TSH using special kits of ELISA.
2. Serum Iron Level.
3. Total Iron binding Capacity level (TIBC).

For blood sampling, ten ml of blood sample has been collected from one of the big veins in the ante-cubital fossa of each hypothyroid patient and control group which were undergone, sera is isolated for subsequently T3, T4, TSH, S. Iron, and TIBC.

Kits	Company	Origin
1. Total Thyroxin T_4 kit	Biocheck	France
2. Triiodothyronine T_3 kit	Biocheck	France
3.Thyroid Stimulating Hormone (TSH)	Biocheck	France
4. Iron Kit.	Fluitest Iron-B	Germany
5. TTBC kit	Cinnagen Inc.	Iran

Total thyroxin t_4 estimate

Principle

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and predispensed in the sealed reagent strips. All the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of SPR several times. The sample is taken and transferred into the well containing T4 antigen labeled with alkaline-phosphatase (Conjugate). Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T4 antibodies coated on the interior of the SPR. During final detection step, the substrate (4-methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to calibration curve stored in memory and then printed out.[19]

Content of the kit

T4 Strips (STR) ……...… 60
T4 SPR (SPR) ……...…... 2X30
T4 Control (C1)…… 1X3 ml (Liquid)
1 MLE Card ..…… 1X1
1 Package4 insert. …….. 1X1

Description of t4 strip

Wells	Reagents
1	Sample well
2-5	Empty wells
6	Conjugate: Alkaline Phosphatase labeled T4 derivative + ANS (0.8 m mol / L) + Sodium Salicylate (9.3 m mol / L + 1 g / L Sodium Azide (400 µL)
7	Wash Buffer:Tris-Tween, NaCl (0.05 mol / L) pH 7.4 + 1 g /L Sodium Azide (600 µL)
8	Wash Buffer:Tris-Tween, NaCl (0.05 mol / L) pH 7.4 + 1 g /L Sodium Azide (600 µL)
9	Wash Buffer: Di-ethanolamine (1.1 mol /L or 11.5%) pH 9.8 +1 g / L sodium Azide (600 µL).
10	Cuvatte with Substrate: 4-methyl-umbelliferyl phosphate (0.6 mmol /L) + Di-ethanolamine (0.62 mol / L or 6.6% pH 9.2) + 1 g / L Sodium Azide (300 µL).

Test procedure

1. The required reagents were removed from the refrigerator and allowed to reach the room temperature for at least 30 minutes.
2. Only one T4 strip and one SPR were used for each sample, control or calibrator to be tested.
3. T4 was selected on the instrument to enter the test code. The calibrator must be identified by "S1" and tested in triplicate if the control was tested, it should be identified by "C1".
4. The calibrator, Control and sample were mixed using vortex type mixer.
5. Two hundred µL of calibrator, sample, or control was added into the sample well.
6. SPRs and strips were inserted into the instrument provided the color labels with the assay code on the SPR and the reagent strips were matched.
7. The assay was initiated as directed in the operator manual. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes.
8. After the assay was completed, The SPR and strips were removed from the instrument.
9. The used SPRs and Strips were disposed into an appropriate recipient.

RESULTS

Once the assay was completed, results were analyzed automatically by the computer. Fluorescence was measured twice in the reagent strips reading cuvette for each sample tested. The first reading was a background reading of the substrate cuvette before the SPR was introduced into the substrate. The second reading was taken after incubating the substrate with the enzyme remaining on the enterior of the SPR. The Relative Fluorescence Value (RFV) was calculated by subtracting the background reading from the final result. Results were calculated automatically by the instrument in relation to the calibration curve stored in memory (4-parameter logistic model) and were expressed in n mol / L.

Normal range =60-120 nmol/L

Triiodothyronine (T3) estimation

Principle of the assay: In this test, a second antibody (goat anti-mouse IgG) is coated on the microtiter wells. A measured amount of patient's serum, a certain amount of mouse monoclonal antibody (mAb) anti-T3 antibody, and a constant amount of T3 conjugated with horse radish Peroxidase are added to the microtiter wells. During the incubation period, the mouse anti-T3 Ab is bound to the second Ab on the wells; and T3 & conjugated T3 compete for the limited binding sites on the anti-T3 Ab. After 60 minutes of incubation at room temperature, wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB reagent is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with addition of the stop solution, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color is proportionated with the amount of unlabeled T3 standards assayed in the same manner, and the concentration of T3 in the unknown sample is then calculated.[20]

Reagents

1. Goat anti –mouse IgG coated microtiter wells, 96 wells
2. Enzyme conjugate concentrate 1.3ml
3. Enzyme conjugates diluents, 13ml
4. T3 reference standards, O, 0.75, 1.5, 3.0, 6.0, and 10.0 nglml, 1 set, 1.0ml each ready to use
5. Antibody reagent, 7ml
6. TMB reagent (one –step) 11ml
7. Stop solution (1N HCL) 11ml

Procedure

1. The desired No. of coated wells was secured in the holder. A data sheet was made for sample identification.
2. Fifty µl. of Standard, samples and Controls were pipetted into appropriate wells.
3. Fifty µl. of the Ab reagent was dispensed into each well, mixed thoroughly for 30 sec.
4. One hundred µl. of working conjugate reagent was added into each well and mixed thoroughly for 30 seconds.
5. The plate was incubated at room temperature for 60 minutes and then the incubated mixture was removed by flicking the plate contents into a waste container.
6. The microtiter wells were rinsed and flicked 5 times with DW or deionized water.
7. The wells were stroked sharply onto absorbent paper to eliminate the residual water droplets.

8. One hundred µl. of TMB reagent was added to each well and mixed gently for 10 seconds.
9. The plate was incubated at room temperature in the dark for 20 minutes without shaking.
10. The reaction was stopped by the addition of 100 µl. of Stop solution to each well and mixed gently for 30 seconds.
11. The absorbancy was read at 450 nm using the ELISA reader and within 15 minutes.

Calculation of the Results

1. The average absorbance values (A450) were calculated for each set of reference Standards, Controls and samples
2. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical axis (Y) and concentration on the horizontal axis (X).
3. Using the mean absorbance value for each sample, and determining the corresponding concentration of T3 in ng / ml from the standard curve

Normal range = 0.9-2.33ng/ml

Thyroid stimulating hormone (TSH) estimation

Principle: This test is based on the principle of a solid phase enzyme-linked immunosrobent assay ELISA.[21] The assay system utilized a unique monoclonal antibody directed against a distinct antigenic determinant on the intact molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-TSH antibody is the antibody enzyme (Horse Radish Peroxidase)-conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the RSH molecule being sandwiched between the solid phase and enzyme-linked antibodies, then after two hours of incubation at room temperature, and wells are washed with water to remove unbound labeled antibodies. A Solution of TMB reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, changing the blue color to yellow. The concentration of TSH is directly proportionated to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Reagents

1. Murine monoclonal anti- TSH coated microtiter wells
2. Set of reference standards, O, 0.1, 0.5, 2.5 and 10MIU/ml, lyophilized
3. Enzyme conjugate reagent, 13ml
4. TMB reagent (one-step), 11ml
5. Stop solution (1N HCl), 11ml

Assay procedures

1. The desired number of coated wells is secured in the holder
2. one hundred µL of standards , specimens , and controls were dispensed into appropriate wells
3. One hundred µL of enzyme conjugate reagent were dispensed into each well and mixed thoroughly for 30 seconds.
4. The plate was incubated at room temperature (18-25^{0}C) with shaking at 175 RPM, for 120 minutes.
5. The incubated mixture was removed by flicking plate contents into a waste container.
6. The microtiter wells were rinsed and flicked 5 times with distilled or deionized water
7. The wells were stroke sharply onto absorbent paper to remove residual water droplets
8. one hundred µL of TMB reagent are dispensed in to each well Mix gently for 10seconds
9. The plate was incubated at room temperature for 20 minutes
10. The reaction was stopped by adding 100 µL of stop solution to each well and then mixed gently for 30 seconds
11. The absorbance was read at 450 nm within 15 minutes

Calculations

1. The mean absorbance values (A450) were calculated for each set of reference standards , control , and samples
2. A standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in µ IU /ml on graph paper , with absorbance values on the vertical (Y) axis and concentration on the horizontal (X) axis
3. The mean absorbance values for each specimen were used to determine the corresponding concentration of TSH in µ IU/ ml from the standard curve

Normal range =0.28-6.82 µ IU/ ml

Serum iron level estimation

Principle of the test

Serum iron is liberated from its complex with Transferrin by the action of surfactants at acid pH values, once liberated it is reduced to Fe^{+2} and reacts with bathophenanthroline to produce colored complex which is photometrically determined.[22]

Reagent concentration

R l:

Acetate buffer, pH 4.7	0.2 mol/ L
Hydroxylamine	0.06 mol/ L
Bathophenanthroline	0.2 m mol/ L

R 2:

Acetate buffer pH 4.7	0.2 mol / L
Hydroxylamine	0.06 mol / L

R 4:

Iron	166 µ g / dl

Procedure

1. Six hundred µL of R_1 was dispensed on to Reagent blank, sample of standard tubes

2. Six hundred µL of R_2 was pipetted on to sample blank tube
3. one hundred µL of distilled water was pipetted on to reagent blank tube
4. one hundred µL of sample was dispensed on to sample and sample blank tube
5. one hundred µL of standard was pipetted on to standard tube and then mixed vigorously, until serum proteins have been completely dissolved
6. The mixture was let stood for 30 minutes at room temperature.

Calculation

$$\frac{dA_2 \text{ sample} - dA_1 \text{ Sample blank}}{dA_2 \text{ standard} - dA_1 \text{ reagent blank}} \times 166 = \mu g. \text{ iron/100m1}$$

d A = density absorbance.

Normal range: women: 73-145 µg/dl
Men: 59-158 µg/dl

Unsaturated iron binding capacity (UIBC) determination

Principle of the test

A known amount of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferring at unsaturated iron binding sites. The additional unbound ferrous ions are measured using the ferrozine reaction. The difference between the amount of ferrous ions added and the unbound ions measured is the unsaturated iron-binding capacity (UIBC).[23]

Reagents

R l. Buffer
R2. Saturating Solution
R3.Ferrozine Reagent

Procedure

1. Two m1 of R_1 was dispensed into test, standard and blank tube
2. Fifty µL of distilled water was pipetted on to standard tube and 1m1 was pipetted onto blank tube
3. Fifty µL of R_2 was dispensed on to test , and standard tube
4. Fifty µL of sample was pipetted on to test tube and then mixture was mixed well and was read the initial absorbance A_1 against blank at 560 nm.
5. Fifty µL of R_3 were dispersed on to test, standard and blank tube
6. Mixed well and incubated at 37^0C for 10 minutes, then the absorbance A_2 was read against blank at 560 nm.

Calculation

$$\text{UIBC} = \frac{A_2 \text{ (Test)} - A_1 \text{ (Test)}}{A_2 \text{ (Standard)} - A_1 \text{ (Standard)}} \times 500 \quad \mu g / dl$$

Normal Value= 160-310 µg / dl

Total iron binding capacity (TIBC) determination

The TIBC is equal to the serum iron concentration plus the UIBC:

TIBC = UIBC + Serum Iron.[24,25]

Normal Value= 210-380 µg / dl

Statistical analysis

The suitable statistical methods were used in order to analyze and assess the results, they include the followings:

Descriptive statistics

A. Statistical tables including observed frequencies with their percentages.
B. Summary statistic of the readings distribution (mean, SD& SEM).
C. Graphical presentation by (bar - charts).

Inferential statistics:- These were used to accept or reject the statistical hypotheses; they include the followings:

A) Chi-square ($\chi 2$).
B) Kruskal Wallis test.
C) Matched paired t-test for repeated measurements.
D) Person correlation coefficient (r).

Note: The comparison of significant (P-value) in any test were:

S= Significant difference ($P<0.05$).
HS= Highly Significant difference ($P<0.01$).
NS= Non Significant difference ($P>0.05$).

Computer & programs

All the statistical analyses were done by using Pentium-4 computer through the SPSS program (version-10) and Excel application.[26]

RESULTS AND DISCUSSION

Hypothyroidism results from the failure to maintain adequate tissue levels of thyroid hormones, and if thyroid hormone T3,T4 decreased it would cause an increase in secretion of TSH by pituitary gland, this case is called hypothyroidism.[27] The Iron–deficiency anemia is a condition that has been associated with hypothyroidism often the causal relationship is not clear.[28,29] Iron-deficiency anemia was listed among one of the abnormalities that may occur with hypothyroid state but it is not necessary to be associated with hypothyroidism for many hypothyroid patients had normal Iron level.

Results in table[1] represented the distribution of patients according to age group. The table shows that the majority of patients were between[31-50] years (51.4 %), also the age's frequency of patients at[51-70] years was (26.7%). Moreover, the table showed that the number of hypothyroid patients[10-30] years was (21.9%) only. The data analysis revealed a highly significant difference between the number of patients in the different age groups with ($P<0.01$). The above observation somewhat disagreed with the result of Morganti, *et al*[30] who stated an increased prevalence of hypothyroidism demonstrated in the elder population, while increased incidence of

primary hypothyroidism shown in table [1] in age group[31-50] years has no interpretation in available references, but may be due to older people's come for checking up less frequently than younger people.

All forms of thyroid disease were four to five times more common in females than in males. The reason was not clear.[31] Table [1] revealed that the distribution of patients was according to gender. It was obvious that women were at high risk for developing disease (84%) in comparison with men (16%) with highly significant difference between number of females to males (P<0.01). These results were in agreement with the observation of Bjoro *et al*[32] and the study performed in West Indians, Jamaica in which it was represented that females were more suspected to have hypothyroid disease than males.[33]

In spite of data supporting the use of serum TSH concentration as the best test to detect abnormal thyroid function, measurement of circulating thyroid hormones with or without serum TSH continued to be frequently requested to evaluate thyroid function.[34] Highly significant difference (P<0.01) was observed in mean of T3 and T4 (0.914 ± 0.216, 35.155 ± 26.846) respectively in the case comparison with the control group (2.213 ± 0.249, 80.202 ± 10.431) respectively .There was a highly significant difference in the mean of TSH level in the case comparison with control group (17.490 ± 10.614) (2.883 ± 1.935) respectively (P< 0.01). The current results were in agreement with the study of Hind[35] who found that TSH is a good marker for laboratory diagnosis and patients' follow up with primary hypothyroidism [Table 2]. Iron is a very critical mineral for individuals with thyroid disease, anemia in hypothyroidism is more often due to iron deficiency.[36] Most patients with hypothyroidism had normal iron level. In Table 4.3 showed that S. Iron was (60.561 ± 26.80) in the case with no significant difference in comparison with control (67.054 ± 18.22) (P> 0.05). The same table revealed too that the mean of UIBC and TIBC (269.793 ± 74.555, 327.084 ± 51.258 respectively) had highly significant difference in comparison with control group (222.621 ± 39.121, 302.027 ± 47.501) respectively. Date in table [4] showed the correlation between T3 and T4 in the case which revealed that there was strong positive correlation (r=0.542) with a highly significant difference (P=0.00). Correlation between T3 and TSH in this table showed that there was a weak negative relationship (r =-0.116) with anon significant difference (P=0.236). These results provide that normal thyroid secretion depends on (TSH),[8] and this was the basis of mechanism between these two variables. In correlation between T4 and TSH there was a weak negative correlation (r= -0.033) with a highly significant difference (P= 0.734) This study is in agreement with Watanakunakorn,[37] who found that T4 was low and TSH level high in patients with hypothyroidism and this was the basis of mechanism between these two variables and this was considered as final diagnosis of primary hypothyroidism.

Table [5] showed that there was a weakly Negative correlation (r= - 0.142) between T3 and S. Iron with a non significant difference (P = 0.149) and there were a weakly Negative correlation (r = -0.064) between T4 and S. Iron with a non significant difference (P = 0.520) while TSH with S. Iron there are a weakly Positive correlation (r = 0.038) with a non significant difference (P = 0.704) in the case, the results of this study was agree with Siavash *et al* [38]. Who found in his study there were no significant correlation between serum-iron with T4.

Table 1: Characteristics of studied samples according to age and gender.

Studied groups		No.	%	Comparison of significant	
				P-value	Sig.
Age groups (Year)	10-30	23	21.9	0.00	HS
	31-50	54	51.4		
	51-70	28	26.7		
Gender	Male	17	16	0.00	HS
	Female	88	84		
	25-29.9	31	29.5		
	>30	51	48.6		
	Positive	29	27.4		

Table 2: Mean distribution of serum thyroid hormone levels among studied groups.

Thyroid hormone	Studied groups	N	Mean	Std. Deviation	Std. Error	Comparison of significant	
						P-value	Sig.
T 3 ng/ ml	Control	37	2.213	0.249	0.041	-	-
	case	105	0.914	0.216	4.683	0.00	HS
T 4 n mol/L	Control	37	80.202	10.431	2.35	-	-
	case	105	35.155	26.846	4.550	0.00	HS
TSH µ Iu/ mL	Control	37	2.883	1.935	0.318	-	-
	case	105	17.490	10.614	2.002	0.00	HS

Table 3: Mean distribution of hematological exam among studied groups.

hematological exam	Studied groups	N	Mean	Std. Deviation	Std. Error	Comparison of significant	
						P-value	Sig.
S. Iron	Control	37	67.054	18.22	2.995	-	-
	case	105	60.561	26.80	2.615	0.193	NS
U.IBC	Control	37	222.621	39.121	6.431	-	-
	case	105	269.793	74.555	7.241	0.00	HS
T.IBC	Control	37	302.027	47.501	7.809	-	-
	case	105	327.084	51.258	5.002	0.005	HS

Table 4: Correlation between serum chemicals in visit patients.

Pearson Correlation		T 4 nmol/L	TSH µlu/mL
T 3 ng/ml	r	0.542	-0.116
	P-value	0.00*	0.236
T 4 nmol/L	r		-0.033
	P-value		0.734
TSH µlu/mL	r		
	P-value		
	P-value		

*** = Correlation is highly significant = (P<0.01).**

Table 5: Correlation between serum thyroid hormone and hematological exam in visit patients.

Pearson Correlation		S Iron
T 3 ng/ml	r	-0.142
	P-value	0.149
T 4 nmol/l	r	-0.064
	P-value	0.520
TSH µIu/ml	r	0.038
	P-value	0.704

*** = Correlation is significant = (P<0.05).**
**** = Correlation is highly significant = (P<0.01).**

CONCLUSION & RECOMMENDATION

The data presented in this study enable us to conclude that the hypothyroidism is more common in females than males. The patients aged (31-50) years showed a high prevalence of hypothyroidism when compared with other age groups. It was recognized that TSH is a good marker for laboratory diagnosis and follow up of patients with hypothyroidism. Iron –deficiency anemia was present in hypothyroid patients. It is important to discover hypothyroid patients as early as possible. It is also important to test all hypothyroid patients for serum iron level.

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