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## Role of zinc, ferritin, oxidative stress, and uric acid levels in hypothyroidism patients

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### Abstract

The thyroid hormone is a major metabolic hormone that regulates intestinal motility to reproduction. It has two major issues: thyroid hormone abnormalities, ferritin, oxidative stress, zinc, and uric acid levels are changes in hypothyroidism patients.

**Materials and Methods:** The study was conducted over a period of six months among the subjects having hypothyroidism at Narayana Medical College & Hospital.

The study includes 70 hypothyroid who attended OPD or were admitted to the wards. The cases taken for study belonged to the age group of 35- 60 years including both female and male subjects. 30, healthy individuals working in Narayana Medical College in the age group of 35- 60 were included in the control group.

Blood samples were collected and serum was separated by centrifugation and used for the estimation of Thyroid profile, Ferritin, MDA, Uric acid, and Zinc.

**Conclusion:** Hypothyroidism, hyperthyroidism. Hypothyroidism is one of the major problems in humans. Lack of secretion of thyroid hormone from the thyroid gland as a biologically active form of T<sub>3</sub> and T<sub>4</sub> oxidative stress is also one of the factors for hypothyroidism. In hypothyroidism condition, the GFR is very low and leads to uric acid clearance is low. This results in hypothyroidism and hyperuricemia is observed. Iron absorption of the iron binding protein Fe<sup>2+</sup> and protein (Ferritin) levels is also very low in hypothyroidism condition. Zn<sup>2+</sup> is also a very important mineral in thyroid hormone metabolism and improves the anti-oxidant mechanism in humans. In hypothyroidism patients, lack of Zn<sup>2+</sup> absorption leads to low levels of Zn<sup>2+</sup> is observed in hypothyroidism patients.

**Keywords:** Fe<sup>2+</sup> - Ferritin, MDA – Malondialdehyde, Zn<sup>2+</sup> - Zinc, OPD- out patient department, OFR –Oxygen Free Radical

### Introduction

Hypothyroidism is a common condition in which the amount of thyroid hormones in the body is below normal. It affects approximately 2% of the population. Thyroid hormones are chemical messengers that help to regulate the metabolic rate. In addition to thyroid hormonal profiles, certain additional parameters directly or indirectly linked with thyroid hormone metabolism may help to assess the thyroid status. Many of the hypothyroid subjects are found to be anemic when compared to the general population <sup>[1]</sup>.

Iron plays a role in the activity of the enzyme thyroperoxidase which is involved in thyroid synthesis because the enzyme is hem-dependent. It catalyzes the oxidation of Iodide (I<sup>-</sup>) to Iodine (I<sup>+</sup>), and contributes to the iodination of tyrosine & coupling of Iodothyronine. Ferritin, the storage form of Iron, reflects the Iron status of the individual. In anemic subjects, low levels of ferritin are found in association with impaired activity of the enzyme Thyroid peroxidase <sup>[1]</sup>.

Hence ferritin may serve as an indirect indicator of thyroid status because of its role in thyroid production <sup>[2]</sup>. As thyroid hormone is associated with the regulation of the metabolism, low levels of thyroid are associated with low metabolic rate. Oxidative stress production of free radicals is directly proportionate to the metabolic rate. In hypothyroid subjects, due to lower metabolic rate, levels of free radicals will be low and so will the products of lipid peroxidation. Hence the levels of Malondialdehyde reflect the extent of peroxidation which is low in hypothyroid subjects.

Uric acid is said to have antioxidant activity because of its ability to remove singlet oxygen, hydroxyl, and peroxide radicals. By this property, uric acid is estimated as a parameter of antioxidant activity. Considering the above facts, among to thyroid profile, ferritin, Malondialdehyde, and uric acid estimation are included  $Zn^{+}$  in the current study [3, 4, 5, 21].

$Zn^{+}$  is also a very important mineral in thyroid hormone metabolism and improves the anti-oxidant mechanism in humans. In hypothyroidism patients lack of  $Zn^{+}$  absorption leads to low levels of  $Zn^{+}$  is observed in hypothyroidism patients. Several minerals and trace elements are essential for normal thyroid hormone metabolism Eg: iodine, iron, selenium, zinc. Co-existing deficiencies of these elements can impair thyroid function [6, 11].

Evidence from earlier studies showed a positive correlation between the serum levels of  $T_3$  /  $T_4$  and ferritin in individuals with thyroid abnormalities. Elevated serum ferritin levels were observed in hyperthyroid individuals, and levels decreased significantly after anti-thyroid treatment with normalization of  $T_3$  levels. Furthermore more administration of  $T_3$  to hypothyroid individuals produced a significant increase in the serum ferritin level [20].

Thyroid hormones stimulate erythropoiesis Severe oxidative stress produces ROS and induces uncontrolled lipid peroxidation. Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in every individual. Those peroxidation reactions are toxic to cells and cell membranes. However, they are normally controlled by anti-oxidant mechanisms. (22) Oxygen Species (ROS) and Reactive Nitrogen Species are effectively kept in check by various levels of antioxidant defence. However when it gets exposed to adverse physiochemical, Environmental, or pathological agents such as atmospheric pollutants cigarette smoking, UV rays, radiation, toxic chemicals, and advanced glycation end products. This delicately maintained balance is shifted in favour of Pro-oxidants resulting in "Oxidative stress ". It has been implicated in the aetiology of several (>100) human diseases and in the process of aging [18, 19].

Oxygen free radicals (OFR) Play a significant role in the pathogenesis of chronic renal disorders [20]. On the other hand, an improper, activation of the Oxidative process may be chronically present in pathological situations and contribute to cell and tissue injury. The mitochondrial respiratory chain represents the most powerful cellular source of oxidants in the body [7, 3].

Attack of OFR on polyunsaturated fatty acids initiates lipid peroxidation which leads to alterations in biological membranes and causes progressive renal injury [4, 8]. Antioxidants are substances that neutralize the free radicals or their actions. Nature has endowed each cell with an adequate protective mechanism against any harmful effects of free radicals [9]. Oxidation reactions can produce free radicals, which start a chain reaction that damages cells, then the antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidant reactions by being oxidized themselves [10, 11].

FRAP (Ferric Reducing Anti Oxidant Power) is used to assess the total antioxidant capacity and is considered to be a useful indicator of the system's ability to regulate damage due to ROS. Studies of total antioxidant capacity in chronic kidney disease show enhancement of lipid peroxidation and decrease of antioxidants [12].

To prevent the damage caused by free radicals, a variety of enzymatic and non- enzymatic antioxidants are present in human serum. Due to oxidative stress, there is an increased level of pro-oxidants and depletion of antioxidants leading to tissue damage. Thyroid hormones have a strong impact on oxidative stress. They increase reactive oxygen species, while activating metabolic systems of the body. Hypothyroidism led to changes in oxidant and anti-oxidant systems [13, 22]. Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. At physiological concentrations, urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin [14, 15].

Zinc is a principal mineral for the regulation of metabolic events in humans, the thyroid hormone plays an important role in the absorption of zinc by the synthesis of metallothionein in protein in the intestine. Hypothyroidism patients, have very low or lack synthesis of metallothionein protein poor absorption of zinc impairs the thyroid hormone metabolism and increases the oxidative stress because of impairment of activation of antioxidant enzymes [16, 17].

### Materials and Methods

This study was conducted over 6 months in the Department of Biochemistry and General Medicine at Narayana Medical College & Hospital both sexes were included and all of them were in the age group of 35 to 60 years' male and female the patients were identified and diagnosis of thyroid disorders especially in hypothyroidism in the department of endocrinology Narayana Medical College & Hospital, Nellore. We are enrolled as control from the normal individuals such as healthy both sexes.

To whom so ever affected by thyroid disorders or hypothyroidism the clinical parameters of  $T_3$ ,  $T_4$ , TSH, Zinc, Ferritin, MDA, and Uric acid as inclusion parameters and exclusion parameters are Iron and copper.

Informed consent was taken for all subject's sample collection.

### Sample collection

After isolation of the patients from the department of Endocrinology collection of a 5ml blood sample was allowed to clot for serum separation the serum was used for the estimation of  $T_3$ ,  $T_4$ , TSH, Zinc, Ferritin, MDA, and Uric acid.

### Estimation OF $T_3$

**Method:** Enzyme-linked immune sorbent Assay (ELISA) method.

### Principle [23, 24, 25, 26]

The  $T_3$  ELISA is based on the principle of competitive binding between  $T_3$  in a test specimen and  $T_3$ -peroxidase conjugate for a limited number of binding sites on the anti- $T_3$  (sheep) coated well. Thus the amount of  $T_3$  peroxidase conjugate bound to the well is inversely proportional to the concentration of  $T_3$  in the specimen.

After incubation of the specimen and  $T_3$ -peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. Substrate solution is added and a blue colour develops. The intensity of colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of  $T_3$  in the specimen.

The absorbance of the specimen is determined by using an ELISA reader, with the wavelength set at 450 nm. Specimen concentration is extrapolated from a dose-response curve generated by utilizing serum calibrators of known antigen concentrations.

### Specimen

Serum

Do not use highly lipemic or haemolysed specimens.

### Reagents

1. micro strips.
2. calibrators
3. enzyme-antigen conjugate ( $T_3$  –HRP conjugate)
4. wash solution (MOPS-buffered saline)
5. substrate reagent-A (3,3',5,5'-tetra methyl benzidine sodium acetate buffer).
6. substrate reagent-B (urea hydrogen peroxide sodium acetate buffer)
7. stop solution (sulphuric acid).

#### Procedure

| Reagents and specimens should be at room temperature before use             |           |
|-----------------------------------------------------------------------------|-----------|
| Step 1                                                                      | Well (μl) |
| Specimen                                                                    | 50        |
| Conjugate                                                                   | 100       |
| Mix and cover with adhesive strips                                          |           |
| Incubate for 60 min at 20-25 °C                                             |           |
| Wash three times as described below                                         |           |
| Wash                                                                        | 300       |
| Step 2                                                                      |           |
| Substrate                                                                   | 100       |
| Incubate for 15 min                                                         |           |
| Stop                                                                        | 50        |
| Mix carefully                                                               |           |
| Measure the absorbance at 450 nm with in 10 min after adding stop solution. |           |

### Wash procedure

Remove the adhesive strips aspirate off the contents, add wash solution, and aspirate off in 30 sec. repeat washing twice. After washing remove the remaining liquid by tapping the plate upside down on tissue paper.

### Calculation

1. Plot the absorbance for each duplicate versus the corresponding  $T_3$  concentration in pg/ml on linear graph paper.
2. To determine the concentration of  $T_3$  of an unknown sample, locate the average absorbance of duplicates on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration from the horizontal axis of the graph.

**Normal values:** 1.4 – 4.2 pg/ml.

### Estimation of $T_4$

**Method:** ELISA method.

**Principle:** [27, 28, 29, 30, 31, 32]

The  $T_4$  ELISA is based on the principle of competitive binding between  $T_4$  in a test specimen and  $T_4$ -peroxidase conjugate for a limited number of binding sites on the anti- $T_4$  (sheep) coated well. Thus the amount of  $T_4$  peroxidase

conjugate bound to the well is inversely proportional to the concentration of  $T_4$  in the specimen. After incubation of the specimen and  $T_4$ -peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. Substrate solution is added and a blue colour develops. The intensity of colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of  $T_4$  in the specimen.

The absorbance of the specimen is determined by using an ELISA reader, with the wavelength set at 450 nm. Specimen concentration is extrapolated from a dose-response curve generated by utilizing serum calibrators of known antigen concentrations.

**Specimen:** Serum- Do not use highly lipemic or hemolysed specimens.

### Reagents

1. Micro strips.
2. Calibrators
3. Enzyme-antigen conjugate ( $T_3$  –HRP conjugate)
4. Wash solution (MOPS-buffered saline)
5. Substrate reagent-A (3,3',5,5'-tetra methyl benzidine sodium acetate buffer).
6. Substrate reagent-B (urea hydrogen peroxide sodium acetate buffer) stop solution (sulphuric acid).

#### Procedure

| Reagents and specimens should be at room temperature before use             |           |
|-----------------------------------------------------------------------------|-----------|
| Step 1                                                                      | Well (μl) |
| Specimen                                                                    | 50        |
| Conjugate                                                                   | 100       |
| Mix and cover with adhesive strips                                          |           |
| Incubate for 60 min at 20-25 °C                                             |           |
| Wash three times as described below                                         |           |
| Wash                                                                        | 300       |
| Step 2                                                                      |           |
| Substrate                                                                   | 100       |
| Incubate for 15 min                                                         |           |
| Stop                                                                        | 50        |
| Mix carefully                                                               |           |
| Measure the absorbance at 450 nm with in 10 min after adding stop solution. |           |

### Wash procedure

Remove the adhesive strips aspirate off the contents, add wash solution, and aspirate off in 30 sec. repeat washing twice. After washing remove the remaining liquid by tapping the plate upside down on tissue paper.

### Calculation

1. Plot the absorbance for each duplicate versus the corresponding  $T_4$  concentration in ng/dl on linear graph paper.
2. To determine the concentration of  $T_4$  of an unknown sample, locate the average absorbance of duplicates on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration from the horizontal axis of the graph.

### Normal values

0.8-2 ng/dl.

### Estimation of TSH

**Method:** Classical sandwich ELISA technique.

**Principle:** [33, 34, 35]

The TSH ELISA, makes use of a highly specific monoclonal anti-TSH antibody coated on the surface of the micro wells. In the first incubation step, the specimen and enzyme conjugate (peroxidase-labelled anti-TSH) are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction with the immobilized antibody. At the end of the incubation excess enzyme conjugate is washed out. A substrate reagent is added and the resulting colour, which turns into yellow after stopping the reaction with the stop solution is measured photometry.

Absorbance increase is directly proportional to the TSH concentration in the sample. The concentration is evaluated by means of a calibration curve which is established from the calibrators supplied with the kit.

### Specimen

Serum, Do not use highly lipemic or hemolysed specimen

### Reagents

1. Micro.
2. Enzyme conjugate-anti-TSH (goat) HRP labelled.
3. Wash solution – Triss buffer( $\mu$ l), NaCl.
4. Substrate – 3, 3', 5, 5' tetra methyl benzidine hydrogen peroxide.
5. Stop – sulphuric acid.

### Procedure

| Reagents and specimens should be at room temperature before use            |                 |
|----------------------------------------------------------------------------|-----------------|
| Step 1                                                                     | Well ( $\mu$ l) |
| Specimen                                                                   | 50              |
| Conjugate                                                                  | 100             |
| Mix and cover with adhesive strips                                         |                 |
| Incubate for 60 min at 20-25 °C                                            |                 |
| Wash five times as described below                                         |                 |
| Wash                                                                       | 300             |
| Step 2                                                                     |                 |
| Substrate                                                                  | 100             |
| Incubate for 15 min                                                        |                 |
| Stop                                                                       | 100             |
| Mix carefully                                                              |                 |
| Measure the absorbance at 450 nm within 10 min after adding stop solution. |                 |

### Wash procedure

Remove the adhesive strips aspirate off the contents, add wash solution, and aspirate off in 30 sec. repeat washing four times. After washing remove the remaining liquid by tapping the plate upside down on tissue paper.

### Calculation

1. Plot the absorbance for each duplicate versus the corresponding TSH concentration in mlu/L on linear graph paper.
2. To determine the concentration of TSH of an unknown sample, locate the average absorbance of duplicates on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration from the horizontal axis of a graph.

**Normal values: 0.3-6.2 mlu/L**

**Estimation of Ferritin**

**Method:** Enzyme Linked Immunosorbant Assay [ELISA]

**Principle:** [36, 37, 38]

Anti-human –ferritin antibodies are bound to micro wells. Ferritin, if present in diluted serum or plasma, binds to the respective antibody. Washing of the micro wells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human ferritin immunologically detects the bound patient ferritin forming a conjugate/ferritin/antibody complex. Washing of the micro wells removes unbound conjugate.

An enzyme substrate in the presence of bound conjugate hydrolyze to form a blue colour. The addition of an acid stops the reaction forming a yellow end product. The intensity of this yellow colour is measured photometry at 450 nm. The amount of colour is directly proportional to the concentration of ferritin present in the original sample.

### Specimen

Serum, Do not use highly lipemic (or) hemolysed specimens.

### Reagents

1. Micro
2. Sample buffer – Triss, NaCl
3. Enzyme Conjugate- anti ferritin, labelled with HRP
4. TMB Substrate
5. Stop solution – 1M Hydrochloric acid
6. Wash solution – PBS, NANT

### Procedure

| Reagents and specimens should be at room temperature before use                 |              |
|---------------------------------------------------------------------------------|--------------|
| Step – 1                                                                        | Well $\mu$ l |
| Specimen                                                                        | 25 $\mu$ l   |
| Sample buffer                                                                   | 100 $\mu$ l  |
| Mix and cover with adhesive strips                                              |              |
| Incubate for 30 minutes at 20 – 28 °C                                           |              |
| Wash 3 times as described below                                                 |              |
| Wash                                                                            | 300          |
| Step – 2                                                                        |              |
| Conjugate                                                                       | 100 $\mu$ l  |
| Incubate for 15 minutes at room temperature                                     |              |
| Wash                                                                            | 300          |
| Substrate                                                                       | 100 $\mu$ l  |
| Incubate for 15 minutes                                                         |              |
| Stop                                                                            | 100 $\mu$ l  |
| Mix carefully                                                                   |              |
| Measure the absorbance at 450 mm with in 30 minutes after adding stop solution. |              |

### Calculation

1. Plot the absorbance for each duplicate versus the corresponding ferritin concentration in ng/ml on linear graph paper.
2. To determine the concentration of ferritin of an unknown sample, locate the average absorbance of duplicates on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration from the horizontal axis of a graph

### Normal Range of Ferritin

Female : 20-50 Years 20-112ng/ml

Male : 20-50 Years 35-310ng/ml

### Estimation of Lipid Peroxides (Malondialdehyde)

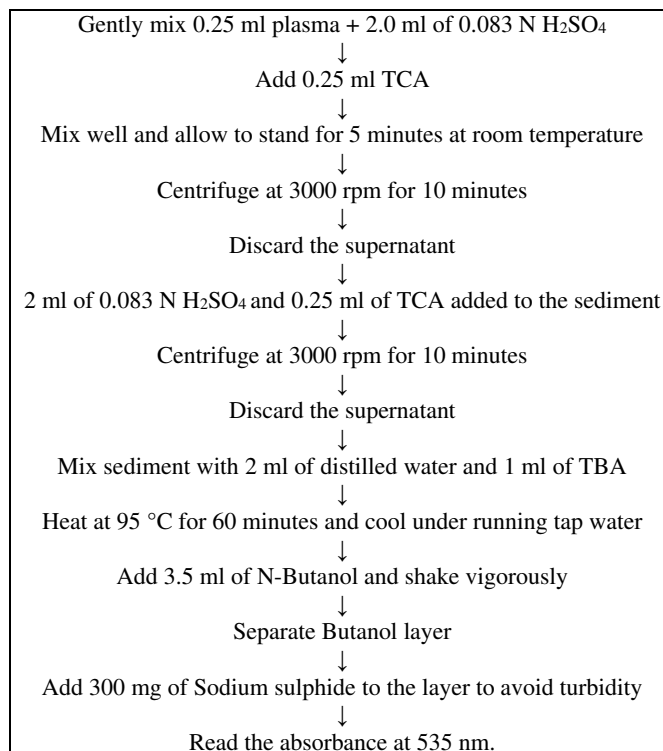
**Principle:** Phosphotungstic acid precipitates the protein present in plasma. Sulphuric acid hydrolyses the lipid peroxides from the protein to yield a malondialdehyde. This malondialdehyde reacts with thiobarbituric acid to produce MDA(TBA) adduct. It is extracted with butanol under boiling which gives a pink colour.

The absorbance is read at 535 nm, using a butanol blank.

## Reagents

- H<sub>2</sub>SO<sub>4</sub> N/12
- Phosphotungstic acid: 10% solution
- Thiobarbituric acid: A mixture of equal volume of 0.67% TBA aqueous solution and glacial acetic acid
- Butanol

## Procedure



## Calculation

$$\text{Lipid peroxides in terms of MDA} = \frac{A \times 3.5 \times 1000 \times 10^3}{1.56 \times 10^5 \times 1000 \times 0.025}$$

or

$$89.74 \times A \text{ mmol/L}$$

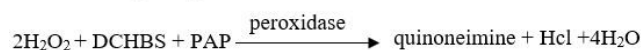
Where A is absorbance

## Estimation of URIC Acid

### Method: PAP METHOD.

Enzymatic colorimetric test for uric acid with lipid clearing factor (LCF).

### Principle [39, 40]



### Specimen

1. Serum.
2. Heparinized plasma or EDTA plasma.
3. Dilute urine 1 + 10 with DH<sub>2</sub>O.

### Procedure

| Working reagent | blank  | standard | test   |
|-----------------|--------|----------|--------|
|                 | 1.0 ml | 1.0 ml   | 1.0 ml |

|                    |   |         |         |
|--------------------|---|---------|---------|
| Uric acid standard | - | 0.02 ml | -       |
| Specimen           | - | -       | 0.02 ml |

Mix and incubate for 10min. at room temperature and read the absorbance at 520nm.

## Calculation

### Serum/plasma

$$C = 8 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ Std}} [\text{mg/dl}]$$

or

$$C = 476 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ Std}} [\mu\text{mol/L}]$$

### Urine

$$C = 88 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ Std}} [\text{mg/dl}]$$

or

$$C = 5235 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ Std}} [\mu\text{mol/L}]$$

Normal Value: 2.5 – 6.2mg/dl

### Estimation of Zinc [41, 42]

Estimation of Zinc Nitro-PAPS method (Phosphorus Adenosyl Phospho Sulfate) Zinc in an alkaline medium reacts with nitro-PAPS to form a purple-coloured complex.

The intensity of the complex formed is directly proportional to the amount of zinc present in the sample.  
Reference values Zinc = 66 – 106 mcg/dL

comparison of continuous variables was done. Using PEM subjects 't' test correlation and in case of Skeweeds Ranle correlation test was used p-value and t value.

### Statistical Analysis

The data was analyzed using the statistical software SPSS12. Version. Values are expressed as mean  $\pm$  SD

### Results

**Table 1:** Show the mean standard deviation and P value of all the investigations of the study done in both Hypothyroid subjects and control subjects.

| S. No | Parameter              | Mean  | S.D.  |
|-------|------------------------|-------|-------|
| 1.    | T <sub>3</sub> (pg/ml) | 2.07  | 0.85  |
| 2.    | T <sub>4</sub> (ng/dl) | 0.6   | 0.25  |
| 3.    | TSH (mIU/L)            | 25.4  | 8.14  |
| 4.    | Ferritin(ng/ml)        | 63.73 | 106.6 |
| 5.    | MDA (mmol/L)           | 4.11  | 2.53  |
| 6.    | Uric Acid(mg/dl)       | 5.45  | 1.09  |
| 7.    | Zinc                   | 42.3  | 11.3  |

Table 1 Showing study parameters in Hypothyroid patients

**Table 2:** Showing study parameters in Normal Control subjects

| S. No | Parameter              | Mean  | S.D.  |
|-------|------------------------|-------|-------|
| 1.    | T <sub>3</sub> (pg/ml) | 2.7   | 0.74  |
| 2.    | T <sub>4</sub> (ng/dl) | 1.15  | 0.24  |
| 3.    | TSH (mIU/L)            | 2.22  | 1.07  |
| 4.    | Ferritin (ng/ml)       | 92.54 | 46.55 |
| 5.    | MDA (mmol/L)           | 5.17  | 1.33  |
| 6.    | Uric Acid (mg/dl)      | 4.04  | 1.06  |
| 7.    | Zinc                   | 89.5  | 5.4   |

Table 2 Showing study parameters in hypothyroid controls

**Table 3:** Showing the comparable values of study parameters in hypothyroid patients and control subjects

| S. No | Parameter              | Patients |       | Controls |       | Z' value | P' value |
|-------|------------------------|----------|-------|----------|-------|----------|----------|
|       |                        | Mean     | S.D.  | Mean     | S.D.  |          |          |
| 1.    | T <sub>3</sub> (pg/ml) | 2.07     | 0.85  | 2.7      | 0.74  | -2.771   | <0.0001  |
| 2.    | T <sub>4</sub> (ng/dl) | 0.6      | 0.25  | 1.15     | 0.24  | -7.717   | <0.0001  |
| 3.    | TSH (mIU/L)            | 25.4     | 8.14  | 2.22     | 1.07  | 12.603   | <0.0001  |
| 4.    | Ferritin (ng/ml)       | 63.73    | 106.6 | 92.54    | 46.55 | -1.135   | <0.0001  |
| 5.    | MDA (mmol/L)           | 4.11     | 2.53  | 5.17     | 1.33  | -7.718   | <0.0001  |
| 6.    | Uric Acid (mg/dl)      | 5.45     | 1.09  | 4.04     | 1.06  | 4.513    | <0.0001  |
| 7.    | Zinc                   | 42.3     | 11.3  | 89.5     | 5.4   | 2.210    | <0.0001  |

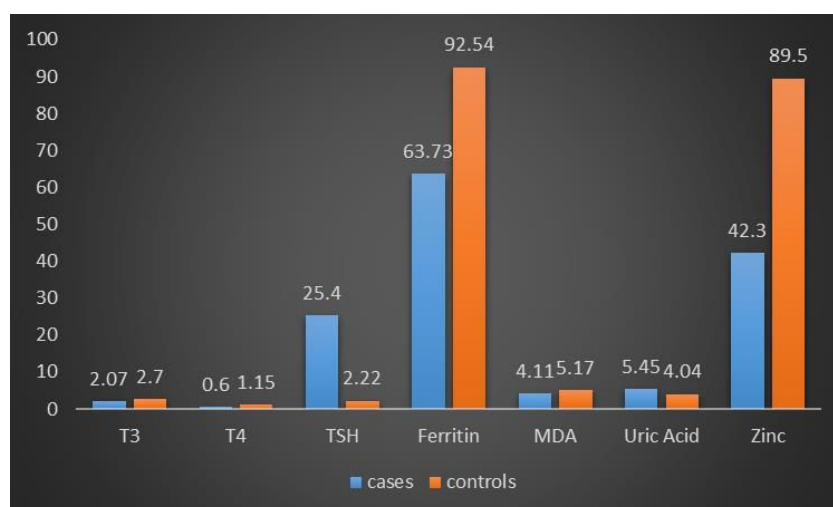


**Fig 1:** Bar diagram chart comparison of cases and controls of T<sub>3</sub>, T<sub>4</sub>, TSH, Ferritin, MDA, Uric acid and Zinc

T<sub>3</sub>, T<sub>4</sub>, TSH p-value, and statistical significance

The two-tailed p-value is less than 0.001 by conventional criteria, this difference is considered to be extremely statistically significant.

#### Confidence interval

The mean of hypothyroidism subjects minus controls equals intermediate values used in calculations  $T_3 Z = -2.771$ ,  $T_4 Z = -7.717$ ,  $TSH Z = 12.603$

#### Ferritin p value statistically significant

The two-tailed p-value is less than 0.001 by conventional criteria, this difference is considered to be extremely statistically significant.

#### Confidence interval

The mean of hypothyroidism subjects minus controls equals intermediate values used in calculations  $Ferritin Z = -1.135$

#### MDA p value statistically significant

The two-tailed p-value is less than 0.001 by conventional criteria, this difference is considered to be extremely statistically significant.

#### Confidence interval

The mean of hypothyroidism subjects minus controls equals intermediate values used in calculations  $MDA Z = -7.718$

#### Uric Acid p value statistically significant

The two-tailed p-value is less than 0.001 by conventional criteria, this difference is considered to be extremely statistically significant.

#### Confidence interval

The mean of hypothyroidism subjects minus controls equals intermediate values used in calculations  $Uric Acid Z = 4.513$

#### Zinc p value statistically significant

The two-tailed p-value is less than 0.001 by conventional criteria, this difference is considered to be extremely statistically significant.

#### Confidence interval

The mean of hypothyroidism subjects minus controls equals intermediate values used in calculations  $Zinc Z = 2.210$

### Discussion

#### Ferritin and Hypothyroidism

Hypothyroidism is a common disease state affecting adolescent and adult people, women are affected more relatively. It is not attributable to a single course. Metabolic, nutritional immunological, and genetic etiological factors were proposed. Many groups of scientists and physicians worked on this problem and proposed their observations and concepts based on experimental, Clinical, and epidemiological studies.

One of the observations is thyroid hormone status is associated with the ferritin status of serum and tissues and iron-deficient anemia may be a cause of hypothyroidism. This proposal stemmed from the fact that the thyroid peroxidase enzyme which oxidizes iodide to a higher valence state before it is incorporated to form thyroid hormones is a heme-containing enzyme and hence iron plays a role in thyroid hormone synthesis.

In view of the foregoing many scientific groups worked on it. Notable observations are as follows.

Shakir *et al.* (2007) <sup>[7]</sup> observed co-existence of iron deficiency anemia with hypothyroidism. Sanjay *et al.* (2002) <sup>[19]</sup> reported that iron deficiency impaired thyroid metabolism. Peter J. Leedman (2007) <sup>[11]</sup> *et al.* showed a positive correlation between thyroid hormone and ferritin in serum. Effterkhan MH *et al.* (2006) <sup>[14]</sup> observed iron deficiency affected thyroid status in adolescent girls.

Mahammed Hasan *et al.* (2008) <sup>[9]</sup> showed reduced thyroid peroxidase activity in thyroid-deficient rats. Liver ferritin synthesis rate was reduced by 36% in hypothyroid rats (Deshpande *et al.*, 1993) <sup>[17]</sup>. Zimmerman M *et al.* (2000) <sup>[13]</sup> showed an improved thyroid profile in iron-deficient adolescent girls after iron supplementation.

In view of the above available data, the present study was undertaken to evaluate the association of ferritin status with hypothyroidism. The studies were conducted in 30 hypothyroid patients. 20 age and sex matched controls were taken for study.

The mean value of serum ferritin hypothyroid patients is 63.73 ng/ml against 92.54 ng/ml in control subjects. It amounts to about 30% lower value. The P value was not statistically significant as the sample size was small. Low ferritin values were observed in about 70% of cases (17 out of 30 cases).

This observation indicates the correlation of iron metabolism with thyroid hormone synthesis. Considering the observations given by many groups it has to be searched whether it is due to nutritional deficiency of iron, or due to a defect in the synthesis of ferritin by the liver any genetic expression of ferritin synthesis is involved. A broader study with a greater number of hypothyroid subjects is required to confirm the association of iron with thyroid hormones.

#### Thyroid and Oxidative stress

Hypothyroidism brings down the metabolic rate and there will be low levels of electron transport chain activity. Iron deficiency may also contribute to it as the cytochromes, the main components of ETC are iron-containing heme compounds. Hence there is a likelihood of less release of superoxide and low lipid peroxidation. The observation was confirmed by many scientific groups.

Rasin Magukot *et al.* (2000) <sup>[24]</sup> demonstrated higher lipid peroxidation in hyperthyroid rats. Decreased free radical and lipid peroxidation was shown by Vendetti .P. *et al.* (1997) <sup>[25]</sup> and Veronica M. *et al.* (2005) <sup>[26]</sup>. Joe Chehad *et al.* (1999) <sup>[30]</sup> observed low MDA and MDA Proteins in hypothyroid rats. Chowdary. S. *et al.* <sup>[31]</sup> suggested based on a study that alteration in thyroid hormone levels affects the antioxidant defence system. In the present study, the mean value of MDA is 4.11 m mol/L in hypothyroid patients as against 5.17 m mol/L in control subjects. Though the values are not statistically significant, it necessitates a further study with broader sampling.

#### Hypothyroidism and uric acid

Uric acid is an end product of purine metabolism. In hypothyroidism, because of low metabolic profile ADP levels will be more as against ATP. Hence Adenine is oxidized through the Xanthine oxidase system and liberates more uric acid. Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. Chowdary G B *et al.* (1999) <sup>[31]</sup>, Bruce N. Aimes *et al.* (1981) <sup>[32]</sup>, and Yokogoshi *et al.* (1996) <sup>[33]</sup> observed elevated serum uric acid levels in hypothyroid subjects.

In the present study mean serum uric acid level is 5.45 mg/dl against 4.04 mg/dl in controls which is statistically significant. Hence studying serum uric acid levels in hypothyroid patients may be used as an associated biochemical parameter to follow the course of the disease. It may be also used to assess whether any myopathic changes are going on, as seen in advanced hypothyroidism.

Zn<sup>2+</sup> is also a very important mineral in thyroid hormone metabolism and improves the anti-oxidant mechanism in humans. In hypothyroidism patients lack of Zn<sup>2+</sup> absorption leads to low levels of Zn<sup>2+</sup> is observed in hypothyroidism patients

### Conclusion

1. Ferritin levels are low in hypothyroid patients suggesting the role of nutritional iron for thyroid hormone formation.
2. Malondialdehyde, an indicator of lipid peroxidation is also low in hypothyroidism which can be considered as one of the associated parameters to be considered for diagnosis and prognosis.
3. Uric acid levels within physiological limits are elevated in hypothyroid patients. It may help to observe the set-in of myopathic changes.
4. An extended study with broader sampling is required to strongly support the observations.
5. The dietary zinc is not observed from the intestine due to a lack of thyroid hormone-associated synthesis of metallothionein protein, the protein synthesis is also enhanced by GIT motility.
6. Due to hypothyroidism condition low GIT motility causes the impair synthesis of zinc absorption carrier protein. This results in acrodermatitis enteropathica is seen in hypothyroid patients. This leads to loss of appetite and impairment of antioxidant mechanism which results in MDA levels are elevated.

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