A Study of Lipid Peroxidation and Total Antioxidant Capacity in Hyperthyroid & Hypothyroid Female Subjects

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ABSTRACT

**Introduction:** Thyroid hormones from the thyroid gland are necessary for the normal development of body organs. Variation in thyroid hormones (TH) is the most important factors involved in the regulation of the basal metabolic condition as well as in the oxidative metabolism. TH stimulates the production of free radicals and their disorders have pathogenic impact on human tissues. MDA, breakdown product of major chain reaction leads to definite oxidation of PUFA & serves as reliable marker of lipid per-oxidation (LPO). Total Antioxidant Capacity (TAC), instead of individual antioxidants, and LPO with changes in TH were seen in this study.

**Materials & Methods:** Serum T₃, T₄ & TSH by chemiluminescence assay, LPO by Okawa H et al (modified) and TAC by D Koracevic et al were estimated in 40 euthyroid, 20 hyperthyroids and 30 hypothyroid female subjects of age group of 25-50 years.

**Results:** The data was expressed as Mean±SD, statistical analysis performed using one-way ANOVA considering p<0.05 as lowest limit of significance. All these parameters differed significantly among the groups. The level of MDA was significantly higher in cases of hyperthyroid females when compared with euthyroid female subjects (p<0.01). No change was observed in hypothyroid females when compared with normal female subjects. The levels of TAC decreased significantly in both hyperthyroid and hypothyroid female patients when compared with euthyroid female subjects (p<0.01).

**Conclusion:** In conclusion, hyperthyroidism in females may cause LPO in tissues whereas hypothyroidism has no effects on LPO. The hyperthyroidism and hypothyroidism in females may cause changes in antioxidant defence system indicating increased tissue damage with alteration of thyroid hormones.

**Key words:** - Hyperthyroid, Hypothyroid, Lipid Peroxidation, Total Antioxidant Capacity.

INTRODUCTION

Thyroid hormones from the thyroid gland are necessary for the normal development of body organs. Thyroid is an important endocrine gland as it primarily governs the rate at which metabolism occurs in the individual cells. Thyroid hormone profoundly influences normal growth and development of the individual. They are essential for mental and psychological development in infancy and early childhood. (1)

The thyroid gland secretes two characteristic hormones - thyroxine and L-triiodothyronine, these two hormones abbreviated T₄ and T₃ respectively are the derivatives of tyrosine. (2) Hypothyroidism is defined as a deficiency in thyroid hormone secretion and action. It is a common disorder that occurs in mild and severe forms in 2% to 15% of the population. (3)

Hyperthyroidism occurs due to hyper secretion of thyroid hormone from thyroid gland or extra thyroidal tissues which may be broadly divided into primary and secondary varieties. Thyroid hormones are the most important factors involved in oxidative metabolism. (4) Free radicals are chemical species with unpaired electrons by the virtue of which they are unstable, reactive and have the capacity to attack other molecules to attain a stable electronic...
configuration. ROS include free radicals like: Superoxide (\(\bullet O_2\)), Hydroxyl radical (\(\bullet OH\)), Peroxyl radical (\(\bullet RO_2\)), Hydro Peroxyl radical (\(\bullet HRO_2\)). The non-radical species include: Hydrogen peroxide (H\(_2\)O\(_2\)), Hypochlorous acid (HOCl). Free radical may cause lipid peroxidation & damage macromolecules & cellular structure of the organism, endothelium & erythrocytes. Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in every individual. These peroxidation reactions are toxic to cells and cell membranes; however, they are normally controlled by countervailing biological mechanisms. Oxidative stress, characterized by an elevation in the steady-state concentration of reactive oxygen species (ROS), has been involved in a wide range of biological and pathological conditions.

Serum MDA is the breakdown product of the major chain reaction leading to definite oxidation of polyunsaturated fatty acid such as lenoleic & linolenic acid & thus serves as reliable marker of lipid per oxidation.

Under physiological conditions, ROS generation is controlled by a large number of anti-oxidant systems which act as protective mechanisms. These systems consist of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase as well as non-enzymatic antioxidants, among which the most important are vitamins C and E, carotenoids, and glutathione.

Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals. They are compounds that when added to lipids and lipid containing foods, can increase their shelf-life by retarding the process of lipid per oxidation. Any imbalance due to an excess oxidant formation or lowered antioxidants leads to an oxidative stress damaging lipids, proteins and nucleic acids which lead to inflammation followed by tissue injury and cell death.

TBARS (Thiobarbituric acid reactive substances) are the direct markers of oxidative stress. The antioxidant parameters that can be analyzed are the non-enzymatic ones like vitamin A, E and C, glutathione, apart from the enzymatic ones like catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx). To study all these parameters is tedious, time consuming and uneconomical. To replace the analysis of the various antioxidants with a single test, the total antioxidant capacity (TAC) assay is done in normal and pathological condition. The accurate assessment of oxidative stress in biological systems is a problem for all investigators working on the role of free radical damage in disease. Numerous assays have been described to measure various free radical damage products or antioxidant status, and the plethora of available techniques attests to the fact that no ideal method is available. The concept of a single test that might reflect total antioxidant capacity (TAC) is beneficial, and in this issue Koracevic et al describe one such test. Low total antioxidant capacity could be indicative of oxidative stress or increased susceptibility to oxidative damage.

Though many studies have explained the several biochemical parameters in thyroidism, oxidative stress in thyroidism was not well documented. The role of thyroid hormones in metabolic pathway is well known. However, their involvement in lipid per oxidation and antioxidant enzyme activity is not known and the results obtained by other studies are contradictory. In the previous studies the antioxidant status has been studied in terms of enzymatic and non-enzymatic antioxidant levels but antioxidant status in terms of total antioxidant capacity has not been extensively studied. It was also hypothesized that patients with hypothyroidism & hyperthyroidism have only poor anti-oxidative capacity, but there are little data to describe the relationship between the levels of thyroid hormones and oxidative stress in females. Therefore the present study is undertaken to determine whether thyroid hormones in females have
any effect on total antioxidant capacity and lipid per oxidation.

**MATERIALS AND METHODS**

The present study was carried out in the department of biochemistry, Jawahar Lal Nehru Medical College, Belgaum. 90 Females were between the age groups of 25-50 years, both the in-patient and the out-patient departments who came for thyroid function test at KLE Prabhakar Kore Hospital and medical research centre and who were hypothyroid or hyperthyroid and are not undergoing treatment were included in this study. Patients with Alcoholics, Smokers, Hypertensive and Diabetics, Coronary artery disease, Rheumatoid arthritis, Suffering from any other systemic disease (liver diseases, renal diseases etc.) and Individuals identified as hyperthyroid or hypothyroid and undergoing treatment for the same were excluded from the study. All patients were subjected to detailed history and thorough physical examination.

Then 5 ml of Fasting blood was collected from subjects by the vein puncture into the plain vial under aseptic condition and the serum was obtained by centrifuging the blood at 1500 g for 5 minutes and taken into two aliquot vials (or tubes). The serum T3, T4 & TSH levels were determined and the subjects were categorized as normal subjects and hyperthyroid, hypothyroid patients. The sample that remained was stored at 4°C for further analysis.

Ethical clearance was obtained from the Institutional Ethical Clearance Committee, J. N. Medical College, Belgaum.

**Methods of assay:** -

The following parameters were analysed from the serum sample
1. Estimation of serum T3, T4 & TSH by immunoassay.
2. Estimation of serum Total Antioxidant Activity by D Koraevic et al method.
3. Estimation of serum MDA by Quantichrome TM TBARS Assay Kit (DTBA-100).

**Statistical analysis:** -

The data was expressed as Mean±SD, statistical analysis performed using one way followed by two-way ANOVA considering p<0.05 as lowest limit of significance.

**RESULTS**

Of the 90 subjects studied, 40 were euthyroid, 20 hyperthyroid and 30 hypothyroid female subjects. Subjects having T4 values >12µg/dl, T3 > 1.50 ng/ml and TSH < 4.67 µIU/ml were classified as hyperthyroid, those having levels of T4 < 4.50 µg/dl, T3 < 0.75 ng/ml and TSH is > 4.67 µIU/ml as hypothyroid and subjects as normal had T4 as 4.50 to 12.0 µg/dl, T3 as 0.75 to 1.50 ng/dl and TSH as 0.49 to 4.67 µIU/ml respectively.

**Table 1. Distribution of Euthyroid, Hypothyroid and Hyperthyroid female subjects**

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
<th>Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Total Females</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 2. Mean Total Antioxidant Capacity and Malondialdehyde Levels in Euthyroid Subjects, Hyperthyroid and Hypothyroid female Patients**

<table>
<thead>
<tr>
<th></th>
<th>T₃(ng/ml)</th>
<th>T₄(µg/dl)</th>
<th>TSH(µIU/ml)</th>
<th>TAC(mmol/l)</th>
<th>MDA(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>1.15±0.29</td>
<td>7.77±2.46</td>
<td>2.45±1.38</td>
<td>1.86±0.21</td>
<td>20.44±6.57</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>0.48±0.20</td>
<td>2.49±1.59</td>
<td>29.91±50.09</td>
<td>0.65±0.35</td>
<td>15.26±3.60</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>5.54±3.11</td>
<td>19.44±5.50</td>
<td>0.11±0.12</td>
<td>0.77±0.46</td>
<td>78.42±29.88</td>
</tr>
</tbody>
</table>

The Mean±S.D of T3, T4, TSH, TAC and MDA group in euthyroid female subjects were 1.15±0.29, 7.77±2.46, 2.45±1.38, 1.86±0.21, and 20.44±6.57 respectively.

The Mean±S.D of T3, T4, TSH, TAC and MDA in hypothyroid female patients were 0.48±0.20, 2.49±1.59, 29.91±50.09, 0.65±0.35 and 15.26±3.60 respectively.
The results showed a significant decrease in TAC (p<0.001) as compared to normal female subjects while there was no change in MDA as compared to normal female subjects.

The Mean±S.D of T3, T4, TSH, TAC and MDA in hyperthyroid female patients were 5.54±3.11, 19.44±5.50, 0.11±0.12, 0.77±0.46, 78.42±29.88 respectively showing significant decrease in the levels of TAC (p<0.001) and highly increase in the levels of MDA(p<0.001) as compared to normal female subjects.

Graph: 1 - Mean Total Antioxidant Capacity and Malondialdehyde Levels in Normal Female Subjects, Hyperthyroid and Hypothyroid Female Patients

Graph showing the decrease levels of TAC in hypothyroid and hyperthyroid patients and increase levels of MDA in hyperthyroid female patients and no change seen in hypothyroid female patients as compared to euthyroid female subjects.

<table>
<thead>
<tr>
<th></th>
<th>Normal females with Hypothyroid</th>
<th>Normal females with Hyperthyroid</th>
<th>Hypothyroid females with Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>p=0.188</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>T4</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TSH</td>
<td>p=0.001</td>
<td>p=1</td>
<td>p=0.002</td>
</tr>
<tr>
<td>TAC</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.506</td>
</tr>
<tr>
<td>MDA</td>
<td>p=0.453</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

These values were obtained between the control & studied groups. When compared with normal female subjects to hypothyroid female patients the T4, TSH and TAC levels were significant. When compared with normal female subjects to hyperthyroid female patients the T3, T4, TAC and MDA levels were significant. And when compared with hypothyroid female patients to hyperthyroid female patients T3, T4 and MDA levels were significant.

**DISCUSSION**

Thyroid hormones are necessary for the normal development of body organs. When the thyroid becomes overactive and releases too much T3 and T4 into the blood, leading to thyrotoxicosis. In primary hypothyroidism happen when the thyroid itself fails. In secondary hypothyroidism the “hypothalamic-pituitary-thyroid-axis” works inadequately. Hypothyroidism can also be caused by a lack of iodine in the diet, which prevents the thyroid from
making enough hormones, or as a side effect of certain drugs, like lithium. (13)

Thyroid hormones are associated to the oxidative and antioxidative status of the human beings. Depression of metabolism due to hypothyroidism and hyperthyroidism has been reported to decrease oxidant production and thus protects tissues against oxidant damage. Oxidative stress, characterized by an elevation in the steady-state concentration of reactive oxygen species (ROS), has been involved in a wide range of biological and pathological conditions. (14) However, data on the oxidative status of hypothyroidism and hyperthyroidism are limited and controversial. (15,16)

The mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body. Thyroid hormones might be able to regulate the activities of antioxidant enzyme systems in the various organs. The role of thyroid hormones in metabolic pathways and antioxidant enzyme activities are also well known in many species such as rat and camel. The serum levels of thyroid hormones are mainly affected with general body metabolism. Recently, increasing experimental and clinical studies have shown that free radicals play a key role in the etiology of many diseases. Thyroid hormones cause oxidative stress as they increase ROS, while activating metabolic systems of the body in general.

Our data showed significant changes in levels of MDA (p<0.01) and TAC (p<0.001) in studied females of hypothyroid and hyperthyroid compared with normal female subjects. Our study clearly demonstrates the low levels of total antioxidant capacity in hyperthyroid and hypothyroid female patients (p<0.001) and levels of lipid peroxidation highly increase in hyperthyroid female (p<0.01) patients and no changes were seen in hypothyroid female patients which is in agreement with other studies that have been done in this field. (Table 1)

Previous studies suggested that the hypermetabolic state of hyperthyroidism is associated with an increase in free radical production, while the hypometabolic state of hypothyroidism symmetrically leads to a reduced free radical production. (17-19) Indeed, both hyperthyroidism and hypothyroidism are associated with enhanced oxidative stress involving enzymatic and non-enzymatic antioxidants. (20)

Data on hypothyroidism in women are conflicting. Baskol et al. showed in a group of 33 female patients with primary hypothyroidism elevated MDA and NO levels and low paraoxonase (PON1) activity. Interestingly, thyroid treatment decreased MDA and increased PON1, without reaching levels observed in controls. (21) They concluded that a pro-oxidant environment in hypothyroidism could play a role in the pathogenesis of atherosclerosis in these patients. Elevated MDA levels were also observed in subclinical hypothyroidism; an increased oxidative stress was attributed to lack of antioxidants, Total antioxidant status was similar in overt hypothyroidism, subclinical hypothyroidism and controls. (22)

In previous studies, different interpretations were given. Venditti et al. (1997) showed that in all tissues of hypothyroid rats, the Malondialdehyde (MDA) levels did not differ significantly from euthyroid values while in hyperthyroid rats it is highly increased. (23) Mano et al. (1995) found that the concentration of lipid peroxides, determined indirectly by the measurement of thiobarbituric acid reactants, did not change in hypothyroid rats when compared with euthyroid animals. (24) Gredilla et al. (2003) demonstrated that in vivo and in vitro lipid peroxidation did not change in the hypothyroid state. (25) which are in agreement with our study. Dumitriu et al. (1988) showed that the mean malondialdehyde level was significantly higher in both hyperthyroid and hypothyroid patients by comparison to the control group. (26)
Effects of thyroid hormones on lipid peroxidation have been subject of investigation in several laboratories but the results are rather contradictory. It was reported that hypermetabolic conditions in hyperthyroid females were associated with an increase in free radical formation and lipid peroxidation levels. In previous studies, there are conflicting results about oxidative stress in hyperthyroidism. In some studies, it was demonstrated that the products of lipid peroxidation were decreased. On the contrary, Fernandez et al and Dumitriu et al found high products of lipid peroxidation in female patients. Similarly, Iangalenko et al found that lipid peroxidation was increased in hyperthyroid female patients. Asayama et al showed that the damaging effect of lipid peroxidation was increased in liver, heart and some skeletal muscles of rats, diminishing antioxidant enzymes in experimental hyperthyroidism.

In previous studies different interpretations were given. It was demonstrated that thyroxin decreased the concentration of the products of lipid peroxidation in animal experiments. However, another study showed that the products of lipid peroxidation were increased in rats that were given triiodothyronine. Dumitriu L et al found high plasma MDA levels in hyperthyroid patients as opposed to the control group. Costantini F et al demonstrated that hyperthyroidism can stimulate lipid peroxidation. Venditti P et al investigated the effects of hyperthyroidism on lipid peroxidation in rats. Iangolenko V et al reported that hyperthyroidism increases the products of lipid peroxidation in several tissues. found that lipid peroxidation was increased in hyperthyroid patients.

Venditti et al have showed that antioxidants are not affected in the same manner in different tissues of hypothyroid rats; some of them increase, while several decrease or remain unchanged. The physiological state of the thyroid gland, the dose and the duration of treatment are also of a major influence on antioxidants enzymes.

There is no difference in antioxidant activity between hyperthyroid patients and controls or between hypothyroid patients and controls in the studies of both. Effects of thyroid hormones on antioxidant activity have been evaluated by others, but results are rather contradictory. The increase of antioxidant capacity has been shown in the blood of patients with hyperthyroidism. On the contrary, Erdamar et al. found decreased antioxidant activity in the blood samples of patients with hyperthyroidism.

CONCLUSION

Our results suggest that thyroid hormones in excess are accompanied by increased oxidative stress and impairment of the antioxidant system in females.

In conclusion we suggest that hyperthyroid females are more prone for lipid peroxidation that need more supplementation of antioxidants because of low antioxidant level to improve the level of lipid peroxidation as compared to hypothyroid females.

Antioxidant supplementation in hyperthyroid female could exert beneficial effects in favour of the diminution of thyroid hormone levels. Antioxidants treatments might be helpful in reducing the oxidative damage due to hyperthyroidism. Therefore, further studies have to be carried out on female patients, in order to evaluate its role on antioxidant mechanisms to defend the organism from oxidative stress. These findings indicate that thyroid hormones have a strong impact on oxidative stress and the antioxidant system.

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