A study of oxidative stress, selected antioxidant enzymes, and total antioxidant activity in hypothyroid, hyperthyroid, and euthyroid subjects

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INTRODUCTION

Thyroid hormones are secreted from the thyroid gland and is one of the important hormones for the normal development of various body organs during infancy as well as for the mental and behavioral development during childhood and reproductive development during adolescence. There are various anti-oxidant systems in our body which under normal physiological and biological conditions governs the regulation of reactive oxygen species (ROS) by various protective mechanisms. Malondialdehyde (MDA), degradation metabolite of oxidation of PUFA by a major chain reaction & commonly used marker of oxidative stress.

Materials and Methods:

A total of 90 subjects were between the age groups of 25-50 years, both the IPD and the OPD patients who came for evaluation of thyroid hormone level. Estimation of serum T3, T4 & TSH was done by Chemiluminescence assay, Total Antioxidant Activity by D Koracevic et al, serum MDA by Quantichrome TM TBARS Assay Kit, Serum Superoxide Dismutase (SOD) by water soluble tetrazolium salt 1 (wst-1), Glutathione Peroxidase (GSH-pX) & Catalase (CAT) by colorimetric method. Statistical analysis: The data was expressed as Mean±SD, statistical analysis performed using one way ANOVA followed by two way considering p<0.001 as lowest limit of significance.

Results:

The concentration of MDA was significantly higher in cases of hyperthyroid subjects when compared with normal subjects. No change was observed in hypothyroid when compared with normal subjects. The serum levels of TAC & antioxidant enzymes are significantly decreased in both hyper and hypo activity of thyroid gland when compared with normal subjects.

Conclusion:

In hyper and hypothyroidism, the level of oxidative stress because of MDA might prove an important role in various systemic effects as well as also in the progression of other diseases.

KEY WORDS: Antioxidant enzymes, hyperthyroid, hypothyroid, LPO, TAC

INTRODUCTION

Thyroid hormones are secreted from the thyroid gland and are one of the important hormones for the normal development of various body organs during infancy as well as for the mental and behavioral development during childhood and reproductive development during adolescence. Throughout
the life span, thyroid gland controls the various metabolism of body which helps in maintaining homeostasis. Thus, thyroxin secreted from thyroid gland is an important hormone which is required for the normal growth and development. The hormone is essential for normal mental, reproductive, and also behavioral development during infancy as well as early childhood.\[3\]

Triiodothyronine (T\(_3\)) and tetraiodothyronine (T\(_4\)) are the two main thyroid hormones which are derived from amino acid tyrosine. Deficiency or decreased action of thyroxin is the cause of hypothyroidism. Hypothyroidism is one of the most common hormone deficiency disorders and it accounts for 15% severe form and mild in 2% among general population.\[4\]

Hyperthyroidism is a state of the thyroid gland where there is an increase in the secretion of thyroid hormone and is broadly classified into two types, namely, primary and secondary form. The hormones secreted from the thyroid gland are involved in maintaining various metabolism cycles of the body like lipid peroxidation (LPO) managing the oxidative stress of the body.\[5\]

Free radicals contain one or more unpaired set of electrons and are also capable of totally independent existence and these unpaired electrons increase its reactivity by making certain chemical changes. Some of the examples of reactive oxygen species (ROS) include superoxide and hydroxyl radicals, peroxyl and hydroperoxyl radicals, whereas hydrogen peroxide and hypochlorous acid are few examples of non-radical species. Free radical leads to lipid peroxidation and damage to macro- and micromolecules and also damage to the cellular structure of the organism.\[4\]

LPO is a normal process and occurs continuously in every individual at a very low level. These are harmful reactions and may cause damage to cells though these reactions are normally governed by various countervailing biological mechanisms of the body.\[5\]

Free radicals in various concentrations are formed in various pathological and biological conditions and there are various surrogate markers of oxidative stress which are reliable marker of oxidation process occurring in the body. Example of this is serum malondialdehyde (MDA) which is an organic compound and a degradation product of polyunsaturated fatty acid occurs by a major chain reaction and is one of the naturally occurring reactive species and is a reliable marker of oxidative stress.\[6\]

Antioxidants act as a reducing agents and these are the compounds which when added to lipids and/or lipid-containing foods retard the peroxidation of lipids and thereby help in increasing the shelf-life.\[7\] Excess of ROS formation or lowered antioxidants may lead to damage of biological structures thereby damaging macromolecules. this ultimately leads to inflammation followed by tissue injury and cell death.\[8\]

There are various antioxidant systems in our body which under normal physiological and biological conditions govern the regulation of ROS by various protective mechanisms and these, in turn, are regulated by various enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase are few examples of antioxidant enzymes.\[7\]

SOD is one of the metalloenzymes and the function of this enzyme is to catalyze the disintegration of superoxide anion to its molecular oxygen and hydrogen peroxide and this contributes as a main part of the antioxidant defense mechanism at cellular level. GPx is a tetrameric enzyme which has selenocysteine moiety as the active site and consists of four 22 KDa monomers. CAT is an enzyme which is mainly present in the peroxisomes of the mammalian cells and it contains an enzyme CAT and it acts by removing hydrogen peroxides (H\(_2\)O\(_2\)) by forming water and oxygen.

Although many studies have explained the several biochemical parameters in thyroid gland dysfunction, oxidative stress in thyroid hormones was not well documented. It is a well-known fact that thyroid hormones play an active role in metabolic and cellular pathway. However, there are lots of controversies regarding the results obtained from various studies in view of involvement of thyroxine in oxidative stress and influence on antioxidant enzyme. In many studies, the antioxidant status has been analyzed in terms of both enzymatic and non-enzymatic antioxidant levels, but there is lack of studies related to antioxidant status in terms of total antioxidant capacity at individual level. The present study has been undertaken to determine whether thyroid hormones in three clinical conditions, namely, hyperthyroid, hypothyroid, and euthyroid states have any bearing on total antioxidant capacity, antioxidant enzymes, and oxidative stress.

**MATERIALS AND METHODS**

The present study was carried out in the Department of Biochemistry, J.N. Medical College, Belgaum. A total of 90 subjects were between the age groups of 25 and 50 years, both the IPD and the OPD patients who came for evaluation of thyroid hormone level at KLE Prabhakar Kore Hospital, Belgaum, and who were hypothyroid or hyperthyroid and are not undergoing treatment were included in this study. Patients with the following were excluded from the study such as: Patients with habit of cigarette smoking and/or alcohol and/or history of chronic diseases such as hypertension, diabetes mellitus, coronary artery disease, rheumatoid arthritis, chronic liver and renal diseases, as well as those with known thyroid dysfunction with or without antithyroid medications were excluded from the study.

A written informed consent was obtained from the study participants. A total of 5 ml of fasting blood sample were taken and due emphasis was given on aseptic precautions. The serum was obtained by centrifuging the blood at the rate of 10,000 rotations per minute a period of 10 min and serum so obtained was divided and kept in two tubes. From one tube, an analysis was performed for serum T3, T4, and TSH levels was done and the subjects were then classified as normal, hyper, or hypothyroid patients.
and other samples in other tube were stored at 4°C for parameter other parameter analysis.

Estimation of serum T3, T4, and TSH was done by chemiluminescence assay, total antioxidant activity by Koracevic et al., serum MDA by QuantiChrom™ TBARS Assay Kit, serum SOD by water-soluble tetrazolium salt 1 (wst-1), GPx (GSH-pX), and CAT by colorimetric method.

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

Statistical Analysis

The data were expressed as mean ± SD, the analysis was done by SPSS 19.0.2 program. Unpaired t-test and one-way ANOVA were used and \( P < 0.05 \) was considered as lowest limit of significance.

RESULTS

These results showed a statistically significant decrease in the value of TAC \( (P < 0.001) \), whereas no change was found in the level of MDA when the same was compared to normal subjects, which were statistically significant.

Table also shows a statistically significant decrease in the value of TAC with \( P < 0.001 \) and a significant increase in the value of MDA with \( P < 0.001 \), when the same was compared to those with that of normal euthyroid subjects.

The results shown in the above table denote a significant decrease in the level of antioxidant enzymes with \( P < 0.001 \) in patients of both hyper and hypothyroid state when it was compared with subjects with euthyroid state.

These values obtained between the groups. When compared normal subjects to hypothyroid patients, the T4, TSH, TAC, CAT, SOD, and Gpx levels were significant. When compared normal subjects to hyperthyroid patients, the T3, T4, MDA, CAT, and Gpx levels were significant and when compared with hypothyroid patients to hyperthyroid patients, T3, T4, MDA, CAT, and Gpx levels were significant. These results show the wide variations in thyroid dysfunction [Table 1 and 2].

DISCUSSION

Thyroid hormone plays a variety of role in the metabolism of our body either directly or indirectly by regulating various antioxidant enzymes functions. They play a major role in LPO, leading to free radical production. Variety of antioxidant enzymes regulated and degraded under the influence of thyroid hormone is SOD, CAT, GPx, and glutathione reductase with many of non-enzymatic antioxidants. The redox balance in the body is influenced by changes in the enzymes and non-enzymatic substances and, in turn, regulates the function of thyroid gland by feedback mechanism. Increase in the level of ROS leads to increase in oxidative damage of the lipids present in the membrane by increasing the mitochondrial respiration and this is also under the influence of thyroid hormone.[9]

Hypersecretion state of thyroid gland results in increase in the basic metabolic rate of the body leading to a continuous formation of the reactive species. In the presence of decreased antioxidative defense mechanism in hyperthyroid patients, there is an associated increase in LPO, whereas in hypothyroid state of thyroid, since the basal metabolic rate is slow so the production and clearance of free radicals is also slow.[10]

Varieties of antioxidants are found in human blood that can protect circulating macro or micromolecules as well as cells from the stress of oxidative damage. Assessment of the status of the LPO requires the estimation of the oxidants markers as well as level of antioxidant enzyme. It is essential to determine the level of various enzymatic antioxidants along with total antioxidant activity assay in subjects with euthyroid as well as with thyroid gland dysfunction.[11]

In the present study, level of MDA and TAC showed a drastic change in both the groups with hypo and hyperthyroid patients compared to those with euthyroid state. Our study suggests a

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( T_3 ) (ng/ml)</th>
<th>( T_4 ) (µg/dl)</th>
<th>TSH (µIU/ml)</th>
<th>TAC (mmol/l)</th>
<th>MDA (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.16±0.28</td>
<td>7.78±2.45</td>
<td>2.46±1.37</td>
<td>1.87±0.22</td>
<td>20.48±6.58</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>0.49±0.21</td>
<td>2.50±1.60</td>
<td>29.93±50.12</td>
<td>0.67±0.36</td>
<td>15.27±3.61</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>5.55±3.12</td>
<td>19.45±5.52</td>
<td>0.12±0.11</td>
<td>0.78±0.47</td>
<td>78.52±29.87</td>
</tr>
</tbody>
</table>

**Table 1:** Distribution of mean ± SD of serum \( T_3 \) (ng/ml), \( T_4 \) (µg/dl), TSH (µIU/ml), TAC (mmol/l), and MDA (µm) levels among studied subjects (\( n=90 \))

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Euthyroid</th>
<th>Percentage</th>
<th>Hypothyroid</th>
<th>Percentage</th>
<th>Hyperthyroid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>40</td>
<td>21</td>
<td>70</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>60</td>
<td>09</td>
<td>30</td>
<td>05</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2:** Distribution gender among studied subjects (\( n=90 \))

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Euthyroid</th>
<th>Percentage</th>
<th>Hypothyroid</th>
<th>Percentage</th>
<th>Hyperthyroid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>40</td>
<td>21</td>
<td>70</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>60</td>
<td>09</td>
<td>30</td>
<td>05</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td>20</td>
<td>100</td>
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</tbody>
</table>
Oxidative stress and dysthyroid

Das et al. Oxidative stress and dysthyroid

International Journal of Advanced & Integrated Medical Sciences | Jan-Mar 2020

The study done by Duerak et al. showed an increase in the levels of MDA along with a decrease in the activity of enzymatic antioxidants in comparison with normal thyroid profile. Sedani and Nadkami have also reported that the increased generation of ROS is one of the factors responsible for an increased level of LPO in patients with hypo and hyperthyroid status. Mano et al. have also found that in thyroid dysfunction, there is an increase in the level of oxidative stress along with diminished antioxidant enzymes. They had also suggested that in thyroid dysfunction, there is an incomplete scavenging of lipid peroxides, and therefore, lipid peroxide has an important role in the cellular function of thyroid gland.

Cellular and tissue injury caused by an imbalance in antioxidant enzyme and total antioxidant activity systems. In hyperthyroidism, there is an observed change in the levels of the members of the enzymatic antioxidant systems which plays an important role against oxygen free radicals such as SOD, CAT, and GPX and the same has been observed by Kumosinska-Vassev et al. who conducted an observational study in hyperthyroidism.

There changes seen in the level of antioxidant enzymes among patients with thyroid dysfunctions are present in small number and are associated with certain controversial results. Wilson et al. found a decrease in the levels of SOD in patients with untreated hyperthyroid state when compared to those with euthyroid subjects, while some of the investigations showed an increased activity. The outcome of some other studies also gave conflicting information on the level of GPX activity in red blood cells among patients with hyperthyroid state as reported by Kumosinska-Vassev et al. Some studies have also reported an increase in the activity of level of GPX in patients with hyperthyroid Graves’ disease when they were compared with healthy individuals. In one of the recent studies done by Verca et al., where they concluded that after a treatment of 30 days with the oral dose of methimazole in patients with Graves’ disease, there is an increase in the concentration of GPX, but

Table 3: Distribution of mean serum T3 (ng/ml), T4 (µg/dl), TSH (µIU/ml), CAT (IU/g of Hb), SOD (IU/g of Hb), and GPx (IU/g of Hb) levels among studied subjects (n=90)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
<th>Catalase</th>
<th>SOD</th>
<th>GPx</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>8.49±2.27</td>
<td>845.32±84.67</td>
<td>23.15±1.82</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>5.05±1.87</td>
<td>492.58±76.98</td>
<td>15.16±0.88</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>2.58±30.62</td>
<td>402.93±109.36</td>
<td>12.54±1.53</td>
</tr>
</tbody>
</table>

Graph 1: Graph showing level of T3, T4, TSH, TAC, and MDA when the same was compared to normal subjects

Table 4: Distribution of P-value showing between all the groups (n=90)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal with hypothyroid</th>
<th>Normal with hyperthyroid</th>
<th>Hypothyroid 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=0.01</td>
<td>P=1</td>
<td>P=0.506</td>
</tr>
<tr>
<td>TAC</td>
<td>P&lt;0.01</td>
<td>P&lt;0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td>MDA</td>
<td>P=0.453</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>SOD</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.002</td>
</tr>
<tr>
<td>GPx</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
when it was followed for 60 days of treatment, the values did not change on subsequent measurement.\[18\]

Our study was also in concordance with and showed oxidative stress does not change in the hypothyroid condition. In hypothyroidism, there is an increase in LPO with an increase in the end product MDA which was measured by the thiobarbituric acid assay.\[19\] Sawant et al. found a significant increase in the value of mean serum MDA concentration when compared with control in both hyper and hypothyroid patients.\[20\]

The changes observed in the value of $T_3$, $T_4$, and TSH level in different thyroid dysfunctions clearly indicate an association between the levels of antioxidant enzymes and oxidative stress as shown by the present study. However, there is a controversy of effect of change in the activities of antioxidant enzymes and TAC in hyperthyroidism. There is an increase in level of MDA among hyperthyroid patients, whereas on the other hand, many studies show a decrease in the level of it in serum samples of some of the hyperthyroid patients. Mitochondrial respiration plays a major role which is influenced by thyroid hormone and causes increase by many complex changes in number as well as the activity of the chain components, but still the mechanism associating hypo and hyperthyroidism with LPO and antioxidants is not known and area of future research.\[14\]

However, the decreased antioxidant levels seen in hypo and hyperthyroidism are not yet proved even on the basis of pathological and physiological consequences. In cases of hypo and hyperthyroidism, this biochemical change is attributed to physiological adaptation. When seeing in collaboration with many other biochemical findings, the T3, T4, and TSH seem to be involved against the toxicity of lipid per oxidation both in animals and humans.

Our findings suggested that there is strong association between thyroid gland dysfunction and redox imbalance. However, oxidative metabolism as well as enzymatic antioxidants such as SOD CAT, GPx, and glutathione reductase and other non-enzymatic antioxidants can be regulated by thyroid hormones.

Some mechanism of explanation may be proposed that, in our body, the complex antioxidant enzyme defense system can cause alteration in the antioxidants effects on radical chain reactions as well as it also causes various other effects on oxidants which are subject to change. Oxidative stress markers can be further divided into broadly three categories which are also confirmed by many previous studies that: (1) Free radical reactions are formed by modified molecules, (2) antioxidant enzymes consumption, and (3) the transcription factors activators or inhibitors. With the dysfunction of the thyroid status, these categories of oxidative stress markers may change and are the area of future research.

**CONCLUSION**

Finally, we concluded that hyperthyroid subjects are more prone to oxidative stress, due to low antioxidant level and there are more requirements of antioxidants to improve the level of oxidative stress. Therefore, patients who are more exposed to oxidative stress have a tendency for an increased in the levels of oxidants along with a decrease in the levels of antioxidants and this may be proved in thyroid dysfunction. In hyper and hypothyroidism, the level of oxidative stress because of MDA might prove an important role in various systemic effects as well as also in the progression of other diseases. Further, a large population-based detailed study is the need for clarifying the role of oxidative stress in the thyroid disease and also to understand various other oxidative stress and antioxidant capacity.

**ACKNOWLEDGMENT**

I dedicate this work to my parents; their blessing will always remain with me.

I take this opportunity to express my deepest respect and gratitude, to my esteemed teacher and guide. I also thank all the other teaching staff of biochemistry department, Jawaharlal Nehru Medical College, Belgaum, for their kind support, encouragement, and helpful suggestions.

My sincere and special thanks to Medical Education Department, JNMC, Belgaum, for their valuable information and support.

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