Total Superoxide Dismutase, Cu/Zn Superoxide Dismutase and Glutathione Peroxidase in Untreated Hyperthyroidism and Hypothyroidism

Bharat N. Dave, Nutan M. Paradkar

Abstract
The aim of our present study was to determine the activity of erythrocyte glutathione peroxidase, plasma and erythrocyte total superoxide dismutase and Cu/Zn-superoxide dismutase in hyperthyroid and hypothyroid patients. We investigated twenty five hyperthyroid and twenty five hypothyroid patients prior to treatment. The results were compared to fifteen age and sex matched healthy controls. The duration of hyperthyroidism and hypothyroidism prior to treatment was one to six months in both the groups. We measured the activity of erythrocyte glutathione peroxidase, plasma and erythrocyte total superoxide dismutase and Cu/Zn-superoxide dismutase in these patients. The erythrocyte glutathione peroxidase activity was lowered significantly in the hyperthyroidism as compared to the control group. There was significant increase in the total superoxide dismutase (p<0.001) and Cu/Zn-superoxide dismutase (p<0.001) in plasma and erythrocyte total superoxide dismutase (p<0.001) and erythrocyte Cu/Zn-superoxide dismutase (p<0.01) of the hyperthyroid group. In hypothyroidism, erythrocyte glutathione peroxidase levels were higher, plasma total superoxide dismutase and Cu/Zn-superoxide dismutase levels were higher, erythrocyte total superoxide dismutase and Cu/Zn-superoxide dismutase levels were lower than in the control group but these changes were not statistically significant. These findings suggest that hyperthyroidism is accompanied by statistically significant changes in the total SOD and Cu/Zn-SOD enzyme levels while there was no significant difference between hypothyroid and control group in the studied antioxidant enzyme levels.

Keywords
Erythrocyte, Hyperthyroidism, Hypothyroidism, Plasma, Antioxidant

Introduction
Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide by glutathione (GSH). In erythrocytes, it protects hemoglobin from oxidation to methemoglobin by hydrogen peroxide. Red cells are known to produce hydrogen peroxide by various mechanisms such as the reaction between ascorbic acid and oxyhemoglobin and the decomposition of oxygen anion by superoxide dismutase (SOD) (1). The enzyme superoxide dismutase catalyzes the reaction between two superoxide radicals to yield one molecule each of oxygen and hydrogen peroxide (2). Two superoxide dismutase isoenzymes have so far been described in vertebrates i.e Cu/Zn-SOD (Copper Zinc Superoxide dismutase) and Mn-SOD (Manganese Superoxide dismutase). Cu/Zn-SOD has been demonstrated in the cytoplasm and also in the intermembrane space in mitochondria. Mn-SOD is found in the matrix space in mitochondria (3) and, in primates, apparently also in the cytoplasm. These enzymes...
Several studies (1-6) have been performed to assess the levels of antioxidant enzymes in specific organs of experimental animals with hyperthyroidism and hypothyroidism. The data regarding the blood levels of antioxidant enzymes in untreated hyperthyroidism and hypothyroidism is limited and contrasting in the literature. Therefore, the present study was undertaken to measure the activities of erythrocyte GPx, plasma and erythrocyte total SOD and Cu/Zn-SOD in hyperthyroid and hypothyroid patients.

Materials and Methods

Blood samples were obtained from 58 hyperthyroid (43 Graves' disease, 14 Toxic multinodular goiter (TMG), 1 Toxic adenoma) (aged 43 ± 4.12 years; 49 females, 9 males) and 56 hypothyroid (aged 45 ± 5.69 years; 49 females, 7 males) patients who attended the outpatient department of the Endocrinology department at KEM Hospital & Seth GS Medical College, Mumbai. These patients were not on any anti-thyroid treatment and were newly diagnosed on the basis of clinical symptoms and signs, serum hormone levels of T3, T4, TSH and ultrasonography. Hyperthyroidism or hypothyroidism had been present for 1 to 6 months prior to treatment in these subjects. 34 age and sex-matched healthy voluntary subjects (aged 41 ± 7.25 years; 29 females, 5 males) were used as controls. Informed consent was obtained from all subjects.

Excluding criteria for all individuals who were smokers, alcohol drinkers, diabetes mellitus, pregnancy, liver or kidney disorders, severe vascular diseases, other endocrine, immunological or inflammatory disorders, regular drug ingestion or antioxidant use.

Venous blood samples were collected in heparinized vials after overnight fasting. Blood samples were centrifuged at 1500g for 5 minutes; the plasma was separated and stored at - 20º C until biochemical tests. The remaining erythrocytes were washed three times with 0.9% NaCl, and lysed in 1:1 (v/v) of double-deionised water. Erythrocyte lysates were used for determination of glutathione peroxidase and superoxide dismutase.

Serum thyroid hormones (T3 and T4) and TSH levels were determined by chemiluminescence assay. Erythrocyte glutathione peroxidase activity was measured using a kit (RANSEL) from Randox Laboratories. The
method is based on that of Paglia and Valentine (11). GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340nm was measured on a semi automated analyzer (Star 21, Rapid Diagnostics).

Superoxide dismutase activity was measured using a kit (RANSOD) from Randox Laboratories. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye (12). The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T. The absorbance was measured on a semi automated analyzer (Star 21, Rapid Diagnostics) at 505nm at 37ºC. Cu/Zn-SOD activity was measured using chloroform-ethanol reagent which inhibits the activity of Mn-SOD.

Statistical Analysis

Results obtained are expressed as mean ± SD. Student’s t-test was used to assess the significance between the values of patients and controls. Statistical significance was set at p<0.05.

Results

In the hyperthyroid group erythrocyte GPx decreased significantly (p<0.001) in comparison to control group (Table I). Increase in the levels of plasma and erythrocyte, total SOD as well as Cu/Zn-SOD in hyperthyroidism was statistically significant (p<0.001) (Table I). Erythrocyte GPx activity in the hypothyroid group was higher as compared to the control group, but this difference was not statistically significant (Table I). There was no statistically significant difference detected in plasma and erythrocyte levels of total SOD and Cu/Zn-SOD in the hypothyroid group. Serum T₃ and T₄ were significantly higher (p< 0.001) and TSH level was significantly lower (p< 0.001) in hyperthyroid patients compared to control subjects (Table II). While in hypothyroid patients serum and T₄ were significantly lower (p< 0.001) and TSH level was significantly higher (p< 0.001) compared to control subjects (Table II).

Discussion

Thyroid hormones maintain the oxidant/antioxidant equilibrium to protect the cell. High levels of thyroid hormones are known to accelerate metabolic reactions, increase oxygen consumption and owing to oxidative reactions, free radical production is increased as well. Thyroid hormones while leading to increase in free radicals also activate the antioxidant enzymes. As energy needs increases in hyperthyroidism, it is observed that oxidants accumulate in the cell. Therefore, thyroid hormones constitute a risk of oxidant stress for cells (13).

In this study the plasma and erythrocyte, total as well as Cu/Zn-SOD showed statistically significant increase activity in the hyperthyroid patients. One study (6) have shown increase in erythrocyte SOD in patients with Graves’ disease while Janusz B et al. (14) reported increase in plasma SOD of Graves’ and TMG patients. Aliciguzel et al. (15) and Sebanat et al. (9) showed increase in erythrocyte Cu/Zn-SOD in TMG patients. Janusz B et al. (14) in their study showed that plasma and erythrocyte, total SOD as well as Cu/Zn-SOD activity was evidently higher in hyperthyroid group, presumably secondary to increased ROS generation.

Table I - Plasma and Erythrocyte Antioxidant Enzyme Levels in Hyperthyroid, Hypothyroid Patients and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n=34)</th>
<th>Hyperthyroid (n=58)</th>
<th>Hypothyroid (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Plasma SOD (U/ml)</td>
<td>3.48 ±1.36</td>
<td>6.76 ± 1.33*</td>
<td>3.86 ± 0.65</td>
</tr>
<tr>
<td>Plasma Cu/Zn-SOD (U/ml)</td>
<td>2.84 ± 0.90</td>
<td>5.67 ± 0.81*</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Erythrocyte Total SOD (U/mg Hb)</td>
<td>729.9 ± 160</td>
<td>1165.6 ± 81.84*</td>
<td>669.2 ±140.7</td>
</tr>
<tr>
<td>Erythrocyte Cu/Zn-SOD (U/mg Hb)</td>
<td>573.5 ± 111</td>
<td>675.9 ± 119.4*</td>
<td>526.1 ±132</td>
</tr>
<tr>
<td>Erythrocyte GPx (U/mg Hb)</td>
<td>28.52 ± 9.1</td>
<td>18.21 ± 4.2*</td>
<td>30.08 ± 8.02</td>
</tr>
</tbody>
</table>

All results are expressed as mean ± SD. *p<0.001

Table II - Thyroid Hormones & TSH Levels in Hyperthyroid Hypothyroid Patients & Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n=34)</th>
<th>Hyperthyroid (n=58)</th>
<th>Hypothyroid (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT₃ (ng%)</td>
<td>126.9±56.01</td>
<td>402.5±198.06*</td>
<td>32.07±13.27*</td>
</tr>
<tr>
<td>TT₄ (μg/dL)</td>
<td>8.07±2.65</td>
<td>18.6±2.36*</td>
<td>1.13±0.46*</td>
</tr>
<tr>
<td>TSH (μU/mL)</td>
<td>2.85±1.06</td>
<td>0.04±0.5*</td>
<td>40.09±13.5*</td>
</tr>
</tbody>
</table>

All results are expressed as mean ± SD.
Similarly, SOD activity was reported to increase in serum (16) and erythrocytes (6, 17, 18) of Graves’ disease patients, as well as in erythrocytes of individuals with toxic multinodular goiter (9, 15). Experimental investigations of ROS-scavenging enzymes in erythrocytes of several species showed SOD may be constitutively present only at low levels but highly inducible under oxidative stress while GPx is normally abundant and less inducible (19).

Similar observations were made for erythrocyte enzymes in Graves’ disease patients (17) revealing significant induction of SOD activity, whereas GPx activity was not significantly different from controls. Various authors (1,15,20) have shown that erythrocyte GPx decreased while others (9,6,15) showed that it increases in hyperthyroidism significantly. In our study in the hypothyroid group, erythrocyte GPx showed increased activity which was not significant. Erythrocyte GPx decreased significantly in hyperthyroid patients.

GPx is a selenoenzyme like D1 (5'-deiodinase I), which is involved in T4 transformation into active T3. As the enhanced hormone production is very pronounced in hyperthyroidism, deiodination of T4 is also increased. Since the body stores of selenium are limited, deiodination is given preference over GPx in selenium supply (21). In 1994, Kohrle described GPx as a sort of selenium store easily available for D1 activity (22). Other selenoproteins such as selenoprotein P mediate the transfer of selenium between the two enzymes. Human erythrocyte GPx, a selenium-dependent peroxidase, does not show enzymatic activity in selenium deficiency (23). Campbell et al. (24) reported that there was a correlation between erythrocyte selenium level and the levels of erythrocyte GPx and plasma selenium in healthy subjects. Thus, selenium deficit might be one of the causes of reduced GPx activity (25).

Thyroid hormones are known not only to induce the synthesis of certain proteins such as mitochondrial enzymes but they also enhance the protein degradation (5). It is reported that GPx activity was decreased in the muscle, heart, and some lymphoid organs in the hyperthyroid rats (26, 5).

The decreased activity of erythrocyte GPx in the present study might be explained by the increase degradation of this enzyme in the liver and muscles due to increased thyroid hormone levels. Our results on GPx and some literature data (13, 21) on the issue have shown that selenium, which plays a vital role in thyroid hormone metabolism, should be present in the preparation of antioxidant combination for hyperthyroid patients.

**Conclusion**

According to the results of the present study the significant increase in the activity of total SOD and Cu/Zn-SOD reflect the oxidative stress due to the hypermetabolic effects of the increased levels of thyroid hormones in hyperthyroidism. There was no significant difference between hypothyroid and control group in the studied antioxidant enzyme levels. So these findings reflect that in hypothyroidism oxidative stress may not have any significant role to play.

**Acknowledgements**

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**References**


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