Lipoxidative and Glycoxidative Modifications of Erythrocytes-Proteins in Relation to Thyroid Status

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Abstract:
Objective: Different mechanisms for lipid peroxidation activation in patients presenting with thyroid hormone abundance or deficiency have been analyzed. However, changes in metabolism associated with thyroid dysfunction through the glycoxidative reactions and its contribution to such enhancement of lipid preoxidation is assessed in this study.

Methods: The study included seventy-two patients with either hyperthyroidism, hypothyroidism or euthyroidism, in addition to 25 control subjects for the estimation in their erythrocytes:
1. Concentration of the end product of lipid peroxidation (malondialdehyde-MDA).
2. Susceptibility to oxidative challenge (H2O2-induced methaemoglobin-Met-Hb).

Results: The hyperthyroid patients group were presented with significantly elevated MDA levels (p < 0.001) among other thyroid disorders. Elevated Met-Hb levels were detected in different thyroid diseases. Moreover, significant modulation of glycated haemoglobin values was observed in both hyper- and hypothyroid patients (7.8 ± 1.8, 5.9 ± 1.7, respectively Vs control 5.1 ± 0.82).

Conclusion: The changes in lipoxidative and glycoxidative modification of proteins in patients with thyroid pathology may have some clinical and biological implications.

Key words: thyroid hormones, MDA, Glycated-Hb.

Introduction:
Thyroid hormones are physiological modulators of both tissue oxidative reactions [1] and protein degradation [2], probably by their effects on carbonyl compounds formation [3]. Thyroid hormones are able to modify the response of lipids to oxidative damage by affecting the cellular redox potential in various tissues [4], either by inducing the production of superoxide anion, in humans [5], or by depressing the antioxidant enzymes maturation (e.g. superoxide dismutase, catalase and glutathion peroxidase) [6,7]. Moreover, thyroid diseases are associated with altered compartmentalization of metal ions, that are essential for antioxidant enzymes activity (such as zinc, copper, manganese and selenium) [8,9,10].

Abnormal glucose metabolism has been documented in patients with thyroid diseases but its pathogenesis is not fully understood [11]. Generally, glycosylation reactions are enhanced by hyperglycaemia, the non-enzymatic glycosylation is so diverse that it involves all known proteins. Such modification of proteins could result in the impairment of various cellular function, [12]. Interestingly, the glycosylation process could be accelerated by oxidative stress, where the glucose can undergo autoxidation in the presence of transition metals. Meanwhile, glycosylation reactions progress with the generation of free radicals [13]. Hence, the oxidation and glycosylation profile for the biomembrane lipids and proteins in erythrocytes, under different thyroid pathology were examined in this study.

Subjects & Methods:
This study was performed on 20 hyperthyroid (having toxic goiter, or recurrent toxic goiter), 17 hypothyroid (having non-toxic goiter or carcinoma) and 35 euthyroid patients (have non-toxic goiter or non-toxic nodular goiter) and 25 healthy control subjects. The patients were selected from the out patient clinic by a senior physician at the Unit of Nuclear Medicine at Al- Yarmook hospital, for the period from June to November 2002. Other characteristics of the participants are illustrated in table –1-.
Heparinized blood samples were analyzed for hormonal parameters (tri iodo-thyronine –T3, thyroxine- T4, thyroid stimulating hormone- TSH) by Radioimmunoassay technique (RIA) [14,15,16] using Immunotech-Kits, (Czech). The measurement of erythrocytes- malondialdehyde (MAD) was based on the reaction with thiobarbituric acid (TBA) to form TBA2-MDA adduct according to the standard method of Stocks and Dormandy [17], which is modified by Gilbert et al [18]. H2O2- induced methaemoglobin (Met-Hb) to assess erythrocytes susceptibility to oxidative challenge determined was marked Betke et al [19] method. Glycated haemoglobin (GHb) was estimated by the colouremetric method of Winterhalter [20].

**Statistical Analysis:**

All data were expressed as mean ± SD. Results were analyzed by a commercial software (statistica) that used unpaired student’s t-test (2-tailed) for single comparisons. Statistical significance level was defined as p < 0.05.

**Table –1-Biochemical Characteristics of the Participants**

<table>
<thead>
<tr>
<th>Group</th>
<th>N.</th>
<th>Age (yr)</th>
<th>Sex (F/M)</th>
<th>T3 (n Mol/L)</th>
<th>T4 (n Mol/L)</th>
<th>TSH (m μ/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>31.52 ± 9.33</td>
<td>12/13</td>
<td>1.21 ± 0.10</td>
<td>105.68 ± 9.26</td>
<td>2.72 ± 1.0</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>35</td>
<td>31.97 ± 10.2</td>
<td>20/15</td>
<td>1.56 ± 0.44</td>
<td>116.23 ± 33.28</td>
<td>1.37 ± 0.82</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>20</td>
<td>33.5 ± 10.18</td>
<td>13/7</td>
<td>2.91 ± 1.09</td>
<td>190 ± 44.66</td>
<td>0.24 ± 0.17</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>17</td>
<td>43.7 ± 9.89</td>
<td>9/8</td>
<td>0.50 ± 0.31</td>
<td>39.99 ± 26.70</td>
<td>36.68 ± 18.11</td>
</tr>
</tbody>
</table>

F = female , M = male

**Results:**

The effects of different thyroid hormone levels on MDA production is summarized in figure—1-. The hyperthyroid patients were presented with more than 100% increase in erythrocyte- MAD content (P< 0.001). While the hypothyroid and euthyroid patients expressed non-significant alterations in their erythrocyte- MAD content.

The H2O2-induced Met-Hb production was much greater by the erythrocytes obtained from hyperthyroid patients than other thyroid disorders. However, all thyroid dysfunctional states (hyper and hypothyroid, euthyroid,) were associated with increased susceptibility to in vitro H2O2 challenge, to produce Met-Hb, indicating either defective antioxidant defenses or enhanced oxidants formation (Figure -2-). The modulation of glycosylation reactions for proteins (presented here by glycation of haemoglobin) by thyroid hormones is illustrated in Figure –3-. Both increased and decreased levels of thyroid hormones could significantly enhance glycated haemoglobin GHb formation.
Thyroid hormones, MDA, Glycated-Hb.

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Figure 1: Erythrocyte malondialdehyde in various groups* Significantly different from control (p < 0.01)

* Significantly different from control (p < 0.01)

Figure 2: H$_2$O$_2$. Induced met hemoglobin in control and thyroid disorder groups
Thyroid hormones, MDA, Glycated-Hb.

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* Significantly different from control (p <0.05)
* * Significantly different from control (p <0.01)

Discussion:
The involvement of thyroid hormones in the metabolic pathways have been extensively studied. However, their effects on lipid peroxidation and glycosylation is not well understood. [21]

The results of this study shows that hyperthyroidism is associated with large increment in lipid peroxidation, as indicated by high levels of MDA, as compared to other groups, whereas, hypothyroidism showed lowered MDA levels, which in agreement with previous reports [22]. Moreover, all thyroid disorders were associated with significant enhancement in Met-Hb production in response to in vitro challenge with hydrogen peroxide (Figure -2) indicating the modification of oxidants/antioxidant balance by thyroid hormones, through different mechanisms, by affecting either the rate of oxidants production (as in hyperthyroid state) [23] or antioxidant defenses activity (as in hypothyroid and euthyroid states) [24].

On the other hand, the modification of protein glycosylation by thyroid hormone excess or deficiency (Figure-3) might contribute to the increased susceptibility of erythrocytes proteins, as well as other tissues to in vitro oxidative challenge [25,26], manifested by a greater ability to form Met-Hb than in control subjects. Furthermore, the finding that the ratio of GHB/MDA in hyperthyroid state (0.44 ± 0.08) was significantly lowered compared to hypothyroid state (0.86 ± 0.09) [Table -2], reflecting an enhancement in lipid peroxidation by the accelerated glycosylation of biomolecules (i.e. the possible involvement of glycosylation reactions in MDA formation) in hyperthyroid patients. The greater production of MDA levels by hyperthyroid tissues could result from the cumulative contribution of the hormone excess on oxidants production [1] and by rendering the antioxidant defenses (enzymes) inactive by enhancing their glycosylation [25]. Whereas in the hypothyroid tissues thyroid hormones deficiency result in lowered oxidants production [28] but their need for full antioxidant enzymes development might contribute to enhanced susceptibility to oxidative challenge. The high ratio of GHB/ MDA in the hypothyroid patients indicating an increased rate of glycosylation although it is not significantly related to the rate of MDA formation, but might contribute to the increased susceptibility of biomolecules in erythrocytes to oxidative challenge (increased Met-Hb formation) because the glycosylation of some biomolecules had been reported to impair their function including some antioxidant enzymes [29].

A significant correlation between the estimated MDA levels and GHB in erythrocytes of the studied groups (r= 0.66, P< 0.005). The existence of a relationship between the two biochemical reactions (i.e. lipid peroxidation and glycosylation), had been proved by inhibiting MDA formation in response to in vitro challenge with H2O2 by the administration
of inhibitors of the non-enzymatic glycosylation of proteins such as aminoguanidine \[27\].

It's worthwhile to mention that both hypo- and hyperthyroidism could enhance modification of some biomolecules e.g. LDL-lipoprotein oxidation, hence both conditions are known to be associated with some cardiovascular complication (i.e. atherosclerosis), coronary heart disease, respectively \[30\]. Finally, the altered glycated-proteins and MDA-bond proteins profile in those patients may have some clinical implications, so that those patients may benefit from dietary supplement of antioxidants or inhibitors of glycosylation.

### Table –2:GHB/MDA ratio in Erythrocytes of various Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Euthyroid</th>
<th>Hyperthyroid</th>
<th>Hypothyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=25</td>
<td></td>
<td>N=35</td>
<td>N=20</td>
<td>N=17</td>
</tr>
<tr>
<td>0.65</td>
<td>0.63</td>
<td>0.44 *</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>± 0.02</td>
<td>± 0.02</td>
<td>± 0.08</td>
<td>± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

- Significantly different from the control group

### References:
29- Clement-Rs, Cohen-MP and Robinson-GW : Albumin glycation inhibition, a new treatment discovered to prevent diabetic eye disease. From the National Eye Institute, presented at the National Meeting of the ADA May/2000 [Enternet].

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