Estimation of Oxidative Stress and Serum Mineral (Ca, Mg, P) Status in Hashimoto's Thyroiditis Patients

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ABSTRACT

Background: Thyroid hormones have a crucial physiological role in maintaining the balance of the body’s metabolism. These hormones also play an important role in the metabolism of the bone system. On the other hand, oxidative stress has been implicated in the pathogenesis of several inflammatory and immune-mediated disorders including Hashimoto’s thyroiditis. Therefore, the present study has aimed to find the changes in the serum calcium, phosphorous, and magnesium levels, and to evaluate the effect of HT on the body’s antioxidant status. Methods: The studied people consisted of 86 subjects, who were divided into two groups: 43 individuals with Hashimoto’s thyroiditis (HT) and 43 age-matched healthy individuals. This research checked the amounts of total triiodothyronine (T3), total thyroxine (T4), thyroid stimulating hormone (TSH), and also the mineral status and some other antioxidant status parameters. Results: It was observed that the mean TSH and SOD levels were increased significantly in HT patients (1.56 ± 0.73), compared to the control group (1.09 ± 0.62). On the other hand, the levels of T4, Ca, and Mg were meaningfully lower in HT patients, compared to the control group (P < 0.05). However, there was no significant difference in the mean of T3, P, and PON-1, between the hypothyroidism and control groups (P < 0.05). Conclusion: The obtained outcomes established this hypothesis that people with HT have an elevated oxidative stress and a decreased mineral level. Therefore, the importance of monitoring the levels of those antioxidant capabilities and the mineral status in HT patients before treatment became more evident.

Keywords: Hashimoto’s thyroiditis, Ca, P, Mg, SOD, PON-1.

INTRODUCTION

Hypothyroidism is one of the general types of thyroid disorders resulting from the insufficiency of thyroid hormones or their reduced activity¹. Hashimoto’s thyroiditis (HT) is a typical autoimmune disease that contributes to hypothyroidism. HT is normally seen in families and influences on the women and men of every age group, even though it has been frequently observed in middle-aged women²³. HT is identified by diffuse lymphocytic infiltration of the thyroid gland, the raised levels of serum anti-thyroid antibodies, the presence of goitres or atrophic gland, and the general thyroid disorders in various levels⁴⁵. The main biochemical feature of this disease is the presence of thyroid autoantibodies (TAb) in the patients’ sera against two main thyroid antigens; i.e., thyroid peroxidase (TPO) and thyroglobulin (Tg). TPO antigen, found at the apical membrane of the thyrocyte, is necessary for the thyroid hormone synthesis, catalysis of iodine oxidation, iodination of tyrosine residues in Tg, and the coupling of the iodothyrosines into thyroxine (T₄) and triiodothyronine (T₃). In individuals with Hashimoto’s disease, the concentration of free thyroxine (FT₄) and free triiodothyronine (FT₃) is lower⁶. Thyroid hormones present a wide range of metabolic actions, such as the regulation of lipids, carbohydrates, proteins, electrolytes, and mineral metabolism⁷. In thyroid dysfunctions, mineral metabolism, similar to calcium, magnesium and phosphorous, is often disrupted. Thyroid disorders are typically related to the disturbances of calcium and phosphorous homeostasis⁸. Several studies have demonstrated the normal serum calcium and phosphorous ranges⁹¹⁰, whereas some others have shown the reduced levels in hypothyroidism¹¹¹². Although the adjustments in calcium and magnesium might be minor in thyroid diseases, these disturbances, in the long term, will be vital for patients¹². In addition, it was perceived that the adjustments in the oxidative status and the antioxidant defence are needed in the experimental and clinical hypothyroidism¹³. The variety of differences in the amounts of thyroid hormones might be one of the major physiological modulators of in vivo cellular oxidative stress, as a result of their known acts on mitochondrial respiration. Exclusively, it is often suggested that the

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enhancers in reactive oxygen species, identified by a deficiency of thyroid hormones, may result in an oxidative stress occasion in the liver and the heart, and also, in some skeletal muscle tissues with a consequent lipid peroxidative response. The metabolic disorder of autoimmune-based hypothyroidism may further improve the oxidative stress. The hypothyroidism-induced dysfunction of the respiratory chain in mitochondria may result in an advanced generation of free radicals that will consequently lead to oxidative stress. On the other hand, in, the depression of metabolism as a result of hypothyroidism was reported, which decreases oxidant production and protects tissues against oxidant injury. There are two investigations that demonstrated the raised oxidative in HT, as assessed by the elevated lipid peroxidation and/or the reduced antioxidant status; however, the data related to the iodine status, glutathione levels, and autoantibodies are inadequate. Furthermore, it has been documented that the oxidative stress is moderately (but significantly) increased in hypothyroid individuals with positive antithyroperoxidase antibody (TPO-AB), in comparison with negative TPO-AB matched controls. In this context, the present study has aimed to find the changes in the serum calcium, phosphorous and magnesium levels and to evaluate the effect of HT on the body’s antioxidant status.

**MATERIAL AND METHOD**

**Study of population**

The study population consisted of 86 adults (aged 19-42 years) divided into two groups: Hashimoto’s thyroiditis patients who were not on thyroxin or antithyroid drugs at the time of sample collection (n=43) and healthy control subjects (n=43). All the patients and controls were recruited from Isfahan Imam Hussein hospital during April to November of 2014. General healthy characteristics such as age, sex, history of disease and disorders, smoking status, alcohol consumption, and dietary habits were investigated by a self-administered questionnaire. Then subjects with a history of Cardiovascular disorders, diabetes, hypertension, metabolic disorders, chronic liver or kidney disease, Smokers, antioxidants dietary and other endocrine disorders were omitted from the study. The ethics committee of the Yazd shahid sadoughi University of Medical Sciences approved the study and informed consent was attained from all patients after explaining the aims and also protocol of the study.

**Blood Collection**

Venous Blood samples were collected by venous puncture, and EDTA-plasma and sera were obtained by centrifugation and stored at -70°C until they were analyzed.

**Hormonal analyses**

The levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxin (T4) were measured by using enzyme-linked immunosorbent assay (ELISA) methods (according by kits from PishtazTeb Co, Tehran, Iran).

**Assay of superoxide dismutase**

SOD was assayed utilizing the technique of Kakkar et al. based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazinemethosulfate and amino blue tetrazoliumformazan. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitrobluetetrazolium) reduction min/ mg/Hb

**Assay of paraoxonase activity**

Serum PON1 activity was measured according to a method described elsewhere. We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25°C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl2 in 0.05 M glycine buffer pH 10.5. One unit (IU) of paraoxonase activity is defined as 1 mol of p-nitrophenol formed per min, and activity was expressed as U / L of serum.

**Auto-antibodies assays**

Anti-TPO titers were measured by chemiluminescence methodology in the serum of 43 subjects, using the Liaison Anti-TPO kit (DiaSorin, Italy) for anti-TPO assay with normal values ranging from 0–10 unit/ml.

**Biochemical analyses**

Serum calcium, magnesium and phosphorous was estimated on semiautoanalyzer (Diruei) using commercially available kits.

**Statistical analysis**

The results are expressed as means ± standard deviation (SD), of three repetitions. All data were subjected to Analysis of Variance (ANOVA) and significant differences (p<0.05) between the results were identified using Independent T –Test. SPSS version 16.0 was used for data analysis.

**RESULTS**

The mean age of Hashimoto patients was 35.18±6.63 years and of control subject 30.71 ±7.20 years (P<0.05). The levels of TSH of HT patients show significant increase (P<0.05) in a comparison with healthy control. HT patients also had significantly lower levels of T4 (P<0.05). Moreover, there was no significant difference in the mean of T3 between hypothyroidism and controls (Table 1). For studying the deleterious consequence of Hashimoto on antioxidant status, SOD and PON-1 were measured. Results show significant difference in the mean of between HT and controls, but PON-1 didn’t significant change between two groups (Table 2). For studying the deleterious consequence of Hashimoto on Mineral status, serum calcium, magnesium and phosphorus were measured. Results show significant difference in the mean of calcium and magnesium between hypothyroidism and controls (Table 2). As shown in Fig. 1 & 2, No significant correlations were seen between Ca, P, Mg and PON 1 with either thyroid hormones or TSH. But a week association was also seen between TSH and T4levels and SOD compare to other parameters.

**DISCUSSION**

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Hashimoto’s thyroiditis (HT) can be characterised by the chronic immunological injury of thyrocytes that contributes to hypothyroidism\textsuperscript{23}. HT can be also described by the presence of very high serum thyroid antibody levels (TG and/or TPO), followed by hypothyroidism or goitre\textsuperscript{24,25}. Thyroid hormone is critical for standard development, regulation of mineral, and maturation of the skeleton. Thyroid hormone is the main regulator of the body’s hemodynamics, thermoregulation, and metabolism. Thus, it has an imperative effect on renal hemodynamics, glomerular filtration, and electrolyte handling\textsuperscript{23}. Thyroid hormone influences on the glomerular filtration rate and also, the flow of blood, and has an immediate impact on Ca, P, and Mg resorption\textsuperscript{26}. On the other hand, previous studies have shown an enhanced formation of reactive oxygen species in Hashimoto patients\textsuperscript{13,19,27}. Therefore, the present study was undertaken to assess the levels of serum calcium, phosphorous, magnesium and also, the oxidative stress status in patients with HT.

In hypothyroidism, there is certainly a depressed turnover, as a result of damaged mobilization of calcium into bones, which leads to reduce the blood calcium rate. In addition, in hypothyroidism, there is an improved generation of thyroid calcitonin, which promotes the tubular reabsorption of phosphate and also, favours the tubular excretion of calcium, which contributes to hypocalcemia and hyperphosphatemia. In hypothyroidism, there is hypomagnesaemia due to the urinary output and the fractional excretion of magnesium via urine\textsuperscript{28}. This paper revealed a significant decrease in the serum calcium and magnesium levels in HT (8.57 ± 0.63 and 2.24 ± 0.46, respectively), compared to

Table 1: Demographic and hormone features of patient and control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control subjects</th>
<th>Hypothyroid patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.71 ± 7.20</td>
<td>35.18 ± 6.63*</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>22.85 ± 4.37</td>
<td>24.28 ± 4.38</td>
</tr>
<tr>
<td>T3 (nmol/L)</td>
<td>1.34 ± 0.32</td>
<td>1.26 ± 0.17</td>
</tr>
<tr>
<td>T4 (nmol/L)</td>
<td>81.68 ± 18.39</td>
<td>59.82 ± 15.68*</td>
</tr>
<tr>
<td>TSH (μmol/L)</td>
<td>2.57 ± 1.07</td>
<td>10.64 ± 6.61*</td>
</tr>
</tbody>
</table>

-Values are given as mean±SD

-Hypothyroid patients compared with control subjects (*P<0.05)
Table 2: SOD and PON-1 activates in patients and Control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control subjects</th>
<th>Hypothyroid patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide Dismutase (SOD) (U/dl)</td>
<td>1.23 ± 0.56</td>
<td>4.23 ± 1.08*</td>
</tr>
<tr>
<td>PON-1 (U/L)</td>
<td>14.59 ± 11.1</td>
<td>17.60 ± 15.1</td>
</tr>
</tbody>
</table>

-Values are given as mean±SD
-Hypothyroid patients compared with control subjects (*P<0.05)

- euthyroidism (9.22 ± 0.51 and 2.67 ± 0.29, respectively),
but the levels of phosphorous in hypothyroid patients did not show a significant change, compared to healthy levels (P > 0.05) (4.35 ± 0.24 and 4.32 ± 0.32, respectively). It is worth mentioning that while a number of studies have presented normal serum calcium and phosphorous ranges8,9, others have shown the reduced amounts in hypothyroidism10,11. Even though the adjustments in the calcium and magnesium levels might be minor in thyroid diseases, these disruptions, in the long term, will be crucial for patients12. In general, thyroxine regulates the blood calcium concentration by releasing calcium from cells. In hypothyroidism, there is much lesser thyroxine in the bloodstream; thus, less thyroxine will enter the cells and less calcium will be released29. Moreover, in hypothyroidism, the sensitivity of bone and kidney to parathormone (PTH) is decreased30. Thus, the enhanced production of active vitamin D and PTH in hypothyroidism does not induce hypercalcemia, because of the fall in tissue sensitivity30,31. Suneel B et al. investigated the mineral status in individuals with thyroid diseases and discovered the dropped calcium level and the raised phosphorous amount in hypothyroidism, mostly due to the impact of PTH and calcitonin. The magnesium level was decreased, attributable to the effects on GFR and also, the decreased clearance. In hypothyroidism, there is certainly an increased renal blood circulation, which results in the high clearance of magnesium from the kidneys. Therefore, the minimal amounts of magnesium will cause hypomagnesemia32.

The oxidative stress (free radicals, homocysteine, methylglyoxal, etc.) is a critical factor in the pathogenesis of many diseases, such as cardiovascular diseases33, clotting disorders34,35, and thyroid disorders36. This study showed an increase in the levels of superoxide dismutase (SOD) and paraoxonase 1 (PON1); however, PON1 was not different from the control groups. On the other hand, Baskol et al.37 showed an increased MDA and a low
paraoxonase (PON1) activity, in a group of individuals with primary hypothyroidism; but, the SOD was not different from the control groups. This finding is in contrast to the current results. Furthermore, Azizi et al. noticed a significant lowering in PON-1 activity in both hyper- and hypothyroid patients. Raisazdeh and his colleagues disclosed a decreased PON-1 activity in the patients with hyperthyroidism, who turns into normal, right after euthyroidism. The serum PON-1 activity was discovered to be negatively related to the free T4 levels, as proven in earlier studies. Also, Yavuz et al. revealed that PON-1 activity is negatively correlated with serum TT4 and TT3 concentrations, and positively correlated with insulin sensitivity.

Free radical-scavenging enzymes (like SOD) are the initial line of cellular defence against oxidative damage, breaking down, and H2O2, prior to the reaction for forming an extra reactive hydroxyl radical (OH). These enzymes preserve the red cells against O2 and H2O2-mediated lipid peroxidation. In the study of Carmeli and his co-workers, the SOD activity was augmented in hypo- and hyperthyroidism. This finding is consistent with the results of Dave et al. about the improvement in SOD activity in hypo- and hyperthyroidism.

CONCLUSION
The present study exhibited that HT patients display lower serum calcium and magnesium concentrations, compared to the normal control subjects. Therefore, it was comprehended that HT individuals must be frequently examined for serum calcium, phosphorous, and magnesium levels. An early identification and also, therapy may help avoid additional bone problems. On the other hand, this study suggested a high generation of ROS and also, oxidative stress in patients with HT, with an increased lipid peroxidation and a concomitant malfunction of the antioxidant defence system. In addition, it was perceived that the physical symptoms and signs in individuals with hypothyroidism are much less reliable and there is a need for serum testing in order to realize the suitable dosage of replacement thyroid hormones. Overall, the objective of this study was accomplished, which was to provide an evidence for the blood analysis of HT patient’s antioxidant mechanism, in order to monitor the progression of pathology and accelerate the consideration of medical care.

AUTHORS’ CONTRIBUTION
Whole authors were in the same.

Table 3: Parameters of iron status in hashimoto patients and Control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control subjects</th>
<th>Hashimoto patients</th>
</tr>
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<tbody>
<tr>
<td>Ca (mg/dL)</td>
<td>9.22 ± 0.51</td>
<td>8.57 ± 0.63</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>4.32 ± 0.32</td>
<td>4.35 ± 0.24</td>
</tr>
<tr>
<td>Mg(mg/dL)</td>
<td>2.67 ± 0.29</td>
<td>2.24 ± 0.46</td>
</tr>
</tbody>
</table>

-Values are given as mean±SD
-Hypothyroid patients compared with control subjects (*P<0.05)

CONFLICT OF INTEREST
There is no conflict of interest.

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