EVALUATION THE CORRELATION BETWEEN TBARS AND SEPP1 LEVELS WITH RBCS INDICES IN IRAQI HYPOTHYROID PATIENTS.

Ezzate H. Ajeena1, Sami R.Alkatib2, Maysaa A. Hadi3

1 Department of Biology, College of Science, University of Kufa/Iraq.
2 Department of medical physiology, College of Medicine, University of Kufa/Iraq.
3 Department of Biology, College of Science, University of Babylon/Iraq.

1 Corresponding Author: E-mail: Ezath.abdulkarim@uokufa.edu.iq

ABSTRACT

Background and Objective: Hypothyroidism is a highly widespread condition of worldwide health which can significantly damage the well-being of sufferers. Hypothyroidism-induced respiratory chain malfunction in mitochondria leads to the increased generation of free radicals that subsequently induce oxidative stress. Due to the adverse effects of oxidative stress on the general health of hypothyroid patients especially erythrocytes and their indices, this study an attempt was made to explain these effects in Iraqi patients.

Material and methods: A total of 90 subjects were included in this study their age is between 20 and 70 years, divided into three groups: control (n=30), untreated hypothyroid patients (n=20) and treated hypothyroid patients (n=40). Blood sample was drawn from each participants to assess the levels of thyroid profile (TSH, T3 & T4), oxidative stress (TBARSwere increased significantly in patients versus to control. With regard to hematological parameters that recorded a decreasing except MCV which have elevated in patients versus to control. Sex-differences among patients were obvious in females with regard to thyroid profile, oxidative stress and antioxidant markers.

Conclusion: Hypothyroidism has a higher prevalence in women than men, the patients have imbalances in redox state reflected by increasing in oxidative stress and decreasing in antioxidant parameters. It appears that patients have a type of anemia by decreasing red blood cells indices.

Key words: Hypothyroidism, oxidative stress, antioxidants, Red blood cell indices.

I. INTRODUCTION

Hypothyroidism is a very common global issue in health which can significantly influence patient welfare (1). It is a long-term condition caused by a lack of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) (2). The environmental deficit of iodine worldwide and autoimmune thyroid syndrome in the areas of iodine sufficiency are consider the most prevalent sources of hypothyroidism (3). The prevalence of this disorder varies from 1% to 2%, increasing to 7% in people between 85 years of age and 89 years in countries that are iodine-satisfactory. An ageing population may contribute in the higher incidence of hypothyroidism in the absence of age-specific TSH reference levels (1). When a diagnosis of chronic thyroid hormone deficiency is confirmed, lifelong thyroid hormone treatment (replacement therapy) is required (4). An imbalance between the creation of free radicals, reactive metabolites(also known as oxidants or reactive oxygen species (ROS)) and their removal by protective mechanisms, such as antioxidants is described as oxidative stress. This disproportion will result in damages significant biomolecules and cells, potentially affecting the entire organism(5). The hypothyroidism-induced disruption of the mitochondria respiratory chain contributes to the acceleration of the formation of free radicals (i.e. superoxide anion, hydrogen peroxide and lipid peroxides), leading to oxidative stress (6). Hypothyroidism-related oxidative stress is the result of both elevated free radical generation and reduced antioxidant production (7).

Autoimmune-based hypothyroidism metabolic dysfunction and excess thyroid stimulating hormone (TSH) may lead to increased oxidative stress (8) (9). In hypothyroid patients, particularly women, this research aims to assess the oxidative stress and its impact on certain blood parameters.
II. MATERIAL AND METHODS

There were 60 hypothyroid patients who attended the Endocrinology and Diabetes Center in Al-Sadr Medical city of Al- Najaf Governorate's were included in this study, aged between 20 and 70 years. They include 20 non-treated patients who were recently diagnosed in the above-mentioned clinic by the doctor specialized in thyroid disorders and 40 diseased persons were taking a therapy (Levothyroxin). The patients were compared with each other and among 30 healthy persons without thyroid disorders, cardiac illnesses and non-smokers of both sexes. The research was completed between November 2019 and July 2020. Five milliliters of blood had been collected from both patients and healthy people. Two milliliters were placed into anticoagulant tube for hematological analysis while 3 ml of the blood have been put in a gel tube for further isolation and getting the serum for tests of thyroid hormone, oxidative stress and antioxidants biomarkers. Red blood cells indices were calculated by autoanalyzer hematology, Mindary while thyroid profile, oxidative stress and antioxidant markers were estimated using immunoassay methods (ELIZA kit, CALBIOTECH and BT-science) respectively.

Statistical analysis

Using the SPSS edition 23, statistical analysis is carried out for the acquisition of median and interquartile (IQR), mean, standard deviation and one way ANOVA. Correlation is accomplished among studied biomarkers by the Pearson coefficient. The results were significant at P<0.05.

III. RESULTS

In this study hypothyroidism was appeared to be increased at interval age (50≥) by recording 20 patients followed by (40-49) by having 10 patients as illustrated in figure(1) which reflect the ages of both control and patients (without and with treatment) groups.

With regard to gender there were an increasing in the incidence of hypothyroidism in females which were 25(83.3%), 18(90%) and 34(85%) in control, hypothyroid patients without treatment and with treatment groups respectively than males 5(16.7%), 2(10%) and 6(15%) in the former groups respectively as explained in figure (2).
The results of table (1) elucidated that the level of thyroid stimulating hormone (TSH) was increased significantly in hypothyroid patients of (without and with treatment) groups (10.90, 7.18) sequentially in compared to control group (0.89). Thyroxin (T4) was decreased significantly in patients of with treatment group (4.07) when compared to those didn’t received any treatment in patients group (3.47).

There was significant increasing in the oxidative stress representing by the levels of Thiobarbituric Acid Reactive Substances (TBARS) in patients with hypothyroidism whose didn’t received treatment (9.09) in comparison to subjects in control group (7.90) whereas after the patients treated with levothyroxine the level of TBARS slightly decreased (8.69) according to table (2). The same table also clarify that the level of selenoprotein P1 (SepP1) as a bio marker of antioxidation was decreased slightly in patients of without treatment group (0.45) in comparison to control (0.47).

Table (1) Comparison of serum TSH and thyroid hormones levels between control and patients groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Control group</th>
<th>Patients group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>0.89 (0.53-1.21)</td>
<td>10.90 (6.77-15.36)</td>
<td>7.18 (4.99-15.65)</td>
</tr>
<tr>
<td>T3 (pg/ml)</td>
<td>0.64 (0.36-1.72)</td>
<td>0.57 (0.13-0.86)</td>
<td>0.69 (0.42-1.20)</td>
</tr>
<tr>
<td>T4 (mg/dl)</td>
<td>4.27 (3.58-4.58)</td>
<td>3.47 (2.40-3.95)</td>
<td>4.07 (3.31-4.33)</td>
</tr>
</tbody>
</table>

(IQR) = Interquartile range. a= Significant differences between control and patient without treatment b= Significant differences between control and patients with treatment c= Significant differences between patients without and with treatment.

Table (2) Comparison of serum TBARS and SepP1 levels between control and patients groups.

<table>
<thead>
<tr>
<th>Control group</th>
<th>Patients group</th>
<th>P</th>
</tr>
</thead>
</table>
Studied groups oxidative stress & antioxidant markers

<table>
<thead>
<tr>
<th>Variable</th>
<th>without treatment</th>
<th>with treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/ml)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>7.90 (7.43-8.73)</td>
<td>9.09 (8.30-9.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.69 (7.59-9.80)</td>
</tr>
<tr>
<td>SepP1 (mg/L)</td>
<td>0.47 (0.33-1.27)</td>
<td>0.45 (0.30-0.53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.48 (0.41-0.80)</td>
</tr>
</tbody>
</table>

(IQR)= Interquartile range. a= Significant differences between control and patient without treatment

The median of red blood cells indices which include RBCs count, hemoglobin (Hb), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were explained in table (3). Some of above hematological parameters were decreased in patients of without treatments groups versus to that of control except MCV which show an increasing in patients compared to control.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Without treatment</th>
<th>With treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs count /10⁶</td>
<td>Median</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>4.59 (4.28-4.70)</td>
<td>4.57 (4.31-4.73)</td>
<td>4.39 (4.14-4.77)</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>12.40 (12.05-13.37)</td>
<td>12.00 (10.12-12.65)</td>
<td>12.15 (11.55-13.00)</td>
</tr>
<tr>
<td>HCT(%)</td>
<td>40.55 (38.77-42.00)</td>
<td>38.55 (34.82-40.45)</td>
<td>39.45 (37.00-41.40)</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>81.80 (75.62-86.92)</td>
<td>86.00 (77.35-92.52)</td>
<td>87.70 (80.17-92.00)</td>
</tr>
<tr>
<td>MCH(pg/cell)</td>
<td>28.20 (26.02-29.47)</td>
<td>26.35 (23.97-28.00)</td>
<td>26.25 (22.82-28.50)</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.15 (32.57-33.82)</td>
<td>30.35 (27.65-32.95)</td>
<td>30.20 (28.80-31.07)</td>
</tr>
</tbody>
</table>

(IQR)= Interquartile range. a= Significant differences between control and patient without treatment b= Significant differences between control and patients with treatment

Among studied groups, sex-specific differences between male and females with respect to thyroid profile were confirmed in table (4). There was a non-significant increasing in TSH level in the females of control, without and with treatment groups (1.20±0.88, 12.58±7.29, 9.69±6.31) respectively when compared to males in the same groups (0.75±0.53, 9.73±3.84, 6.96±6.45) respectively. The levels of T3 decreased significantly in the females of without treatment group (0.51±0.31) versus males of the same group (1.6±0.39). On the other hand the comparison among females of studied groups was revealed a significant increasing in the levels of TSH both patients group (12.58±7.29, 9.69±6.31) compared to control (1.20±0.88).

<table>
<thead>
<tr>
<th>Thyroid hormone</th>
<th>Studied groups</th>
<th>Gender</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table (4) Mean differences of thyroid profile according to gender among studied groups.
### Table (5) Mean differences of TBARS and SepP1 according to gender among studied groups.

<table>
<thead>
<tr>
<th>Oxidative stress and antioxidant variable</th>
<th>Studied groups</th>
<th>Gender</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>Control</td>
<td>7.97±1.23</td>
<td>8.29±1.37</td>
</tr>
<tr>
<td></td>
<td>Without treatment</td>
<td>7.98±0.78</td>
<td>9.34±1.49</td>
</tr>
<tr>
<td></td>
<td>With treatment</td>
<td>6.98±0.79</td>
<td>9.05±1.56</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>1.000</td>
<td>0.053**</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.37±0.10</td>
<td>1.13±0.86</td>
</tr>
</tbody>
</table>

* Significant difference P ≤ 0.05 between males and females each studied groups ** Significant difference P ≤ 0.05 among studied groups with respect to gender.

The levels of TBARS recorded significant increasing in females of with treatment group (9.05±1.56) in comparison to its level in the males of the same group (6.98±0.79). The results of SepP1 as an anti-oxidant marker recorded significant increasing in females of without treatment groups (0.54±0.23) versus to males of the former group (0.21±0.02) as explained in table (5).
The effect of oxidative stress on the numbers of RBCs in the patients of with treatment group was explained in figure (3) that reflect a negative significant correlation between TBARS and RBCs. (P value 0.048, r= -0.314).

There was a significant negative correlation (P value 0.011, r= -0.396) between thyroid hormone (T3) and one of red blood cells indices which was mean corpuscular hemoglobin MCH in hypothyroid patients after received a treatment as showed in figure (4).

IV. DISCUSSION
Hypothyroidism is a highly widespread condition of worldwide health which can significantly damage the well-being of sufferers. The results of the current study clarified that hypothyroidism incidencerises in the forties and...
fifties of age, which is consistent with the findings of Shao et al. (10) who found increased overt and subclinical hypothyroidism at the ages of (46-55) and (56-65). The most preceding studies were showed that the prevalence of this disease has risen at forties of age (11,12). Bremner and his research team proposed that an alteration of the TSH secretion fixed point by ageing leading to elevate serum TSH levels as a result of reduced thyrotropic exposure to harmful thyroid hormones feedback(13). There are clinical and economic effects concerning age-specific cut-points for thyroid dysfunction (14). In this study there was a predominance of hypothyroidism in females by (90%) and (85%) of without and with treatment patients groups respectively while the males were (10%) and (15%) of the above patients groups. This is nearly as a result of an investigation for an unselected population in Mid-Norway stated that the prevalence of hypothyroidism was 4.8% and 0.9% for female and male, respectively (15). Diab and his colleagues in 2019 reported women had a higher incidence of hypothyroidism compared to males (16). The higher prevalence of subclinical hypothyroidism in women is unknown, it may be attributed to estrogen, autoimmune thyroid disorders, and higher levels of thyroperoxidase and thyroglobulin antibodies in females (17). The present study assessed the levels of TSH, T3 and T4 hormones in patients of both groups and control. The result revealed significant increase in TSH levels in patients compared to control on other hand the levels of thyroid hormones have significant decreased between patients group. This results is identical to (18) who recorded high levels of TSH and low levels of T3 and T4 in hypothyroidism patients of both sexes compared to euthyroid healthy control. TSH levels are reduced in the patients of current study after treatment with levothyroxine, while thyroid hormone levels appear to be raised. This finding is consistent with (19) who reported a decreasing in thyrotropin levels and an increasing in FT4 and TT3 levels when levothyroxine was taken at bedtime. There was an increasing in the oxidative stress reflect by elevated the concentration of TBARS in the patients of this study versus to control. On other hand there was a slight decreasing after patients received the treatment. This results is confirm by (20),(21) who came to a conclusion that TBARS were significantly higher in patients with subclinical hypothyroidism than in healthy control.Marchiori et al. (22) reported in their study a reduction in the TBARS by 12 months of levothyroxine replacement therapy (LRT). Baskol and his colleagues confirmed the findings of the current study by documenting an increase in ROS before treatment and a decrease after treatment, but they used MDA instead of TBARS, and both consider reactive oxygen species (ROS) products result from lipid peroxidation (23). Thyroid dysfunction, especially hypothyroidism, may increase oxidative stress, lipid peroxidation (LPO) reactions and reactive oxygen species (ROS)(24) (25). Lipid peroxidation is an autocatalytic mechanism that causes oxidative membrane destruction. Cell death and the development of toxic and reactive aldehyde metabolites known as free radicals, the most common of which is malondialdehyde (MDA), can result from such destruction. ROS are known to cause oxidative damage to biological macromolecules such as lipids, proteins, and DNA (26). Free fatty acids are most likely the primary source of energy in the cells of thyroid glands, and free radicals dripping from thyroid cells can react with them to cause lipid peroxidation, which alters the functional features of cellular membranes, altering their permeability and initiating inactivation of enzymes and membrane-bound receptors (27). The results of table (2) demonstrated a decrease in the levels of selenoprotein P1 (SepP1) in patients of without treatment group when compared to control. This finding is going with the observation of (28)(29) who recorded a decreasing in SepP1 in patients with hypothyroidism and Graves diseases compared to healthy persons. Selenoprotein P1 is one of selenoproteins that belongs to the hepatokine family. Hepatokines are proteins that contain the amino acid selenocysteine in their polypeptide chain and can be present in all life forms (30). They are made in the liver and play a role in oxidative stress protection by participating in oxidation-reduction reactions that neutralize reactive oxygen species (ROS) (31). Elevated ROS levels in hypothyroidism can lead to a pro-oxidation environment, which can cause lower antioxidant SepP1 activity (24). Gerenova et al (2007) found a lack of cellular anti-oxidative defense in Hashimoto's thyroiditis patients at all stages of the disease (32). Among the patients groups there was a decreasing in the hematocrit, content of hemoglobin per red blood cell (MCH) and the amount of hemoglobin per unit volume (MCHC) in comparison to control group. This is quite similar to Iranian study conducted by (33) who reported a significant decreasing in the above red blood cell indices in hypothyroidism patients versus control and hyperthyroidism patients. Thyroid hormone deficiency was linked to lower total blood counts, including RBC count, Hb, MCH, MCHC, and BFU-E clonogenic potential, according to a report published in 2010 (34). Bashir et al. (35) were proposed that anemia is commonly correlated with thyroid deficiency in both subclinical and primary hypothyroidism. In this analysis, the mean cell volume (MCV) was found to be higher in the patient groups compared to control. This finding is consistent with the results of (34) who establish that the average RBC volume (MCV) in patients with thyroid disorders (hypo and hyperthyroidism) was greater than the reference values in healthy subjects, indicating macrocytosis. Thyroid hormones have a major influence on erythropoiesis. They increase secretion of erythropoietin (EPO) by inducing erythropoietin gene expression and promote erythropoiesis by hyper proliferation of immature erythroid
progenitors (36). Thyroid hormone, which works via the TR receptor, is needed for terminal human erythropoiesis (37). The fact that patients with hypothyroidism have a lower red blood cell count indicates that the bone marrow is depressed and that thyroid hormones play a significant part in the modulation of human hematopoiesis in the bone marrow (38). The levels of T3 reflected a significant difference between male and female as clarified table (4). This finding is virtually identical to a study published in 2018 by (17), who found that the mean of thyroid hormones in females with hypothyroidism were marginally higher than in males, but the difference was statistically insignificant. The current study's observations of a higher prevalence of subclinical hypothyroidism in females are consistent with those of a study performed in Northern Europe (39) and an Indian study by (40). There was a study conducted in 2000 by(41) explained that women over 60 years of age had the highest age and sex-specific rates of hypothyroidism occurrence. This disorder was distributed among men over the age of 74 years by (16%) ,however, the percentage was larger (21%) in women of the similar age (44).

Higher estrogen levels have been linked to elevated TSH and lower FT4 levels, resulting in hypothyroid symptoms, according to several reports (42). The females with hypothyroidism in this study have a higher level of TBARS than males with the same disease. For decades, the thyroid gland has been known to be a target of estrogen (43). Estradiol administration improves thyroid peroxidase (TPO) function in both intact and ovariectomized rats, meaning that estrogen induces not only thyroid iodide absorption but also iodide organification (44). Using rats as a model, researchers discovered that there is a higher H2O2 activity and NOX4 (an enzyme that plays a key role in the production of hydrogen peroxidase H2O2 in thyrocytes) expression in adult female rats' thyroid contrast to their male counterparts (45). Fortunato et al.(46) were speculated that the sex variations in thyroid dysfunction prevalence may be due to a redox imbalance induced by estrogen. This study reveals an increase in hypothyroid patients' oxidative stress and this substantial association between TBARS and red blood cells appears to elucidate the effects on erythrocytes as seen in the figure (3). Erythrocytes in the blood of individuals with thyroid problems may be subjected to excessive oxidative stress, which can cause damage and lead to suicide death or eryptosis. Oxidative stress stimulate Ca2+ input and subsequent scrambling of the cell membrane leading to phosphatidylserina exposure and the activation of Ca2+-Sensitive K+ channels that lead to K+ output, hyperpolarization, Cl output and finally cellular shrinking due to KCl and osmotic water loss (47). Figure (4) shows the relationship of thyroid hormones with red cell indices, explaining that the drop in T3 was combined with a decrease in MCH. Previous investigations have shown that thyroid dysfunction was linked to aberrant red blood cell indexes (48). Studies have shown that red cell abnormalities and a decreased proliferative ability of hematopoietic progenitor cells are associated with the effects of thyroid dysfunction in patients with hypothyroidism (34).

V. CONCLUSION

Hypothyroidism has a higher prevalence in women than in men, the patients have imbalances in redox state, reflected by increasing oxidative and decreasing in antioxidant parameters. Reduced thyroid hormones cause declines in the index of red blood cells (RBC counts, Hb, HCT, MCH and MCHC), while MCV was elevated in the patients which reflects a type of anemia should be taken into consideration in the treatment of them. There is a type of sex-disparities among patients with regard to oxidative stress and thyroid profile.

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