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EFFECT OF UNBALANCED IN OXIDATIVE STRESS AND ANTIOXIDANT STATUS ON RBCS MEMBRANES IN THYROID DISORDERS PATIENTS

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Abstract

Many hypothyroid and hyperthyroid patients are anemic because the changes in the concentration of thyroid hormones result in the increased generation of reactive oxygen species (ROS) and oxidative stress leading to changes within the erythrocytes membrane; during this study, the osmotic fragility of the red blood cells and proteins of their membrane from non-treated hypo and hyperthyroid patients diagnosed on the idea of clinical examinations and paraclinical data were compared with a control group. The study included 140 samples collected from patients who suffer from thyroid disorders and 100 samples collected from healthy people as a control group. Ranged in age from patients and healthy individuals (17-79) years. Hormones, antioxidants, and malondialdehyde were measured by ELISA methods; whilethe osmotic fragility and erythrocyte membrane proteins were measured according to the standard method.Significant changes were observed in the level of hormones, TSH increased in hypothyroidism and decreased in hyperthyroidism while T3 and T4 increased in hyperthyroidism and decreased in hypothyroidism. CAT, SOD, and GR were changed significantly that increased in hyperthyroidism and decreased in hypothyroidism. No significant changes were observed in the osmotic fragility in hypothyroidism but showed significant changes in hyperthyroidism in the 0.45-0.9 NaCl; Whereas erythrocyte membrane proteins

decreased significantly in both cases. The present study confirmed increased oxidative stressand impairment of the antioxidant system in hypo and hyperthyroidism and its effect on the erythrocyte membrane by increasing osmotic fragility and decreasing membrane proteins.

Keywords: Antioxidant, Osmotic fragility, RBC Membrane, Hypothyroidism, Hyperthyroidism.

1. Introduction

The thyroid is a small endocrine located ahead of the trachea; It uses iodine to supply thyroid hormones, which are essential for normal growth, development, maturation, and regulation of metabolism when iodine deficiency occurs resulting in hormone deprivation (1). Thyroid hormones T3 and T4 act in many tissues to increase the basal rate, partly by regulating mitochondrial ATP synthesis(2). Therefore, because the actions of hormones are within the broad sense stimulatory, the manifestations of hyperthyroidism usually reflect the increased functioning of varied organ systems to satisfy the stress imposed by hyperthyroidism. (3)

Enzymatic antioxidants include catalase (CAT), which is that the first line of defense within the cell that removes peroxide (H_2O_2) formed during biological processes byconverting it into an aldehyde. There are three major families of Superoxide dismutase (SOD) enzymes: manganese SOD (Mn-SOD) within the mitochondria and peroxisomes, iron SOD (Fe-SOD) in prokaryote cells, and copper/zinc SOD(Cu-Zn SOD) within the cytoplasm ofeukaryote cells (4). Hence, changes within the metal co-factors can alter the effectiveness of SOD and should cause diseases as a result of oxidative stress (5). Glutathione reductase (GR) is additionally an enzymatic antioxidant that converts the oxidized glutathione to the reduced glutathione within the presence of NADPH(Nicotinamide Adenine Dinucleotide Phosphate), which is oxidized to NADP+(Nicotinamide Adenine Dinucleotide Phosphate)(6)

Mammalian red blood cells have a biconcave shape. If red blood cells are put in the isotonic solution, there is a small net osmotic movement of water, the shape of the cells stay the same. If RBCs are placed during the hypotonic solution than is found inside the cells, water moves into the RBCs by osmosis, causing the cells to swell. If the erythrocytes are placed inthe solution with a hypertonic solution, water moves out of the cell by osmosis, the shape ofthe cell becomes smaller (7;8).thyroid hormones can affect red cell volume by altering the Na+-K+-ATPase activity and can also influencethe fluidity and strength of the membrane within the RBCthrough changes within the composition of the plasma membrane, we attempted to analyze the osmotic fragilityof RBC from newly diagnosed hypo- and hyperthyroidpatients compared with of control(9).

The present study aimedto assessthe changes in oxidative stress, enzymatic antioxidant, and osmotic fragility in thyroid disorders.

2. Methods:

2.1 Blood Samples Collection:

Ten ml of venous blood were collected from a suitable vein. Tourniquet has applied about 4-5 finger width above the selected venipuncture site and was disinfected by 70% of Ethanol for 30 seconds and allowed to dry completely.

After sufficient blood has been collected, the tourniquet was released before withdrawing the needle. 3 ml of blood samples were transferred rapidly to a clean dry EDTA tube and shaken gently then used directly for osmotic fragility test. Anther 3 ml of blood samples put in other dry EDTA tubes and shaken gently then used to mitochondrial measurement and isolation of RBC membranes and estimation of their proteins. The residual part of the blood sample was transferred to a glass tube (free of anticoagulation) and let to coagulate for serum separation by using a centrifuge of 4000 rpm for 5 minutes. The isolated serum was collected in a sterile clean white tube and kept at -20 °C to be used for thyroid hormones and antioxidant studies.

2.2 Measurement of the Hormones levels

Accu-Bind ELISA microwell kit (form MonobindInC, USA) was used for the quantitative determination of Total Triiodothyronine (tT3), and total thyroxine (tT4) concentration in human serum by microplate Enzyme immunoassay. The quantitative immune enzymatic assay of TSH was based on the ELIFA technique by Mini VIDAS according to Bio Merieux company procedure (France).

2.3 Measurement of the Malondialdehyde and activity of enzymatic antioxidant

The antioxidant activities of MDA, CAT, SOD, and GR were measured using enzyme-linked immunosorbent assay kits purchased from Kamiya Biomedical Company (Seattle, US) and Cayman Chemical (Michigan, US), according to the manufacturer's instructions.

2.4 Measurement of the Erythrocytes osmotic fragility

Erythrocytes osmotic fragility measured according toAlvarez-Llamas et al.(10), cells were washed 3 times with 0.9% NaCl; for each washing, 1.5 ml of the blood sample was mixed with 8.5 ml of 0.9% NaCl and centrifuged for 5 min at 1000 rpm. After removing the supernatant of the last centrifugation, red cells were suspended in 1.5 ml of 0.9% NaCl and 50 µl of cell suspension was added in triplicate to the tubes containing 1 ml of different NaCl concentration (0-0.9%). Tubes were incubated for 30 min at 37 C in a water bath and then centrifuged for 10 min at 3000 rpm. The optical density (OD) of the supernatant was measured at 540 nm using a spectrophotometer.

2.5 Measurement of the Erythrocyte membrane proteins

The three-stage method was used to isolate erythrocyte membranes depending on the Clark & Switzer(11)method. After getting an RBC ghost, the membrane proteins were isolated by Ghwarsh et al, (12) method to get suspension representing RBCs membrane protein. the Folin-Lowry protein assay method was used to estimate RBCs membrane proteins in membrane protein extracts (13)

2.6 Statistical

The Statistical Analysis System- SAS (14)program was used to detect the effect of different factors in study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between the means.

3. Result

The present study showed (Table 1) a significant difference in the level of TSH in control and hypothyroidism and hyperthyroidism with p-value ≥ 0.01. The mean of the TSH level of hyperthyroidism was lower than that of hypothyroidism and control group. While the mean of TSH level of hypothyroidism was higher than that of control and hyperthyroidism; the means for three groups were 2.660 µIU/ml (control), 0.291 µIU/ml (hypothyroidism), and 7.271 µIU/ml (hyperthyroidism). Also, the present study showed a significant difference in the level of T3 and T4 in control, hypothyroidism, and hyperthyroidism (P≥0.01). The mean of the level of the hormonesinhypothyroidism was lower than that of hyperthyroidism and control group. While the mean of T3 level inhyperthyroidism was higher than that of control and hypothyroidism; the T3 means for three groups were 1.697 ng/ml, 0.464 ng/ml, and 3.956ng/ml; and for T4 5.933 µg/ml, 1.319 µg/ml, and 10.768 µg/ml respectively.

The results in Table 1show the means of enzymatic antioxidants activity in the serum of patients with thyroid disorders and control groups. The table showing a significant(P<0.01) decrease in the activity of CAT, SOD, and GR (55.869 IU, 1.641 U/ml, 1.036 IU) in hypothyroidism patients in comparison with the control group. Whereas the results of hyperthyroidism patients showed a significant (P≤0.01) increase in the activity of SOD (3.464 U/ml) and GR (3.871 IU); and a non-significant increase in the activity of CAT (75.387 IU) in compared with the control group. Also, the results in table 1 showed a significant (P≤0.01) decrease in MDA concentration in hypothyroidism patients (0.529 nmol/mg) compared with the control group (0.724 nmol/mg). While the result showed a significant increase (P≤0.01) in MDA concentration in hyperthyroidism (1.381 nmol/mg) compared with the control group.

The results for protein isolated from RBC membranes showed (Table 1) a significant decrease (P≤0.01) in protein concentration that isolated from RBC membranes in both hypothyroidism and hyperthyroidism patients (1.288, 0.973 µg/ml, respectively) compared with the control group (1.893 µg/ml).

The results of the statistical analysis in figure 1 for osmotic fragility of RBC in patients and control groups showed a significant decrease (P≤0.05) for RBC hemolysis (5.51) in hyperthyroidism patients of 0.9 NaCl concentration; also the results showed a significant decrease (P≤0.01) for RBC hemolysis in NaCl concentration 0.85 (5.34), 0.75 (5.41), 0.5(28.67) and, 0.45 (74.74) respectively compared with the control group. The results showed a non-significant decrease in RBC hemolysis in hypothyroidism patients in all NaCl concentrations.

4. Discussion

Hyperthyroidism is a clinical condition due to an excessive increase in thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4). The most common cause of hyperthyroidism is toxic goiter or Graves' disease (15). Under normal conditions, epithelial cells of the thyroid gland have a moderate production of reactive oxygen species (ROS) that are physiologically required for the formation of T3 and T4, ROS cause oxidative damage to the macromolecular structures of the cell and releasing large amounts of hormones (16).Hypothyroidism is that the most prevalent thyroid disorders in small ruminants and causes disorders that reduce the animal's ability to defend against infections and render it at risk of ketosis (17). American Thyroid Association, (18) Showed the role of iodine; iodine deficiency is the most common cause of hypothyroidism and too much iodine can also cause or worsen hypothyroidism. Marcocci et al. (19) suggest that increased ROS generation may contribute to generating some clinical manifestations of thyrotoxicosis and that antioxidant treatment may improve the clinical picture. The pituitary tells the thyroid how much hormone to form. If the pituitary is damaged by injury, a tumor, radiation, or surgery, it's going to not be ready to give the thyroid the right instructions, and therefore the thyroid may stop making enough hormone (20).

Oxidative stress is associated with both hyperthyroidism and hypothyroidism (21). However, the mechanisms by which oxidative stress is generated in these two clinical conditions are different: increased ROS production in hyperthyroidism and low availability of antioxidants in hypothyroidism. The increased turnover of mitochondrial proteins and mitoptosis also participate within the regulation of the oxidative status, by removing the mitochondria damaged by oxidative stress (22).

Within the hypothyroid state, there is a significant reduction in total antioxidant capacity and decreased total reduced glutathione content within RBC. These together could play a very important role in the development of oxidative stress and ultimately put the cell at risk of hemolysis (23). Also, the reduction in catalase activity has been reported in thyroid disorders that lead to the buildup of H_2O_2. Excess H_2O_2 could react with NO to get peroxynitrite and various other hydroxyl free radicals. These radicals could further react with and lead to damage to cellular structures which is understood as lipid peroxidation (24);(25).

Zahediasl et al. (26) clear mechanical hemolysis was found to be lower if thyroxine (T4) was included in RBC suspensions at concentrations near to the physiological levels. Toplan et al.,(27)and Evelyn et al.,(28) showed there was a significant reduction in hemolysis in erythrocytes treated with TSH in 0.4% NaCl, showing that TSH can protect lysis even during a very hostile media.

The effects of hyperthyroidism on the activity of antioxidant enzymes, includingSOD, CAT, and GPx, depend on the tissue investigated, with T3 and T4 having differentiated effects (29). In our study, MDA was found to be

significantly lower within the hypothyroid group in comparison to the healthy control group. Low MDA could also be due to the consumption of enzymatic antioxidants of the body including glutathione (GR) during inflammation(30).

The decrease in Rbc membrane protein caused by increasing ROS and decreasing of antioxidants; this cleared by Martínez et al., (31) suggest a dramatic change in spectral profile observed in the case of fisetin (antioxidant) that binding to the ghost membrane proteins induces changes in protein conformation, leading to maintain the protein from oxidative damages. Odunayo et al., (32) reported that stress increases osmotic fragility of RBC by lipid peroxidation and damage of erythrocyte membrane proteins.

5. Conclusion

The present work illustrates that changes in MDA as a marker of oxidative stress and changing in SOD, CAT, and GR as antioxidantsmaking imbalancein body hemostasis and effected the membrane of red blood cell leading to increased in erythrocyte osmotic fragility in hyperthyroidism; also made changing in RBC membrane proteins in by decreasingthese proteins in the patients with thyroid disorders.

Authors' Contributions

ASM, MMA, and NAM contributed to the study design and analyzed data. All authors contributed to the manuscript drafting and revising and approved the final submission.

Competing interests

The authors declare that they have no competing interests associated with this article.

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Mean ± SE								
Group	CAT (IU)	SOD (U/ml)	GR (IU)	MDA (nmol/mg)	T3 (ng/ml)	T4 (µg/ml)	TSH (µIU/m)	RBC membrane proteins (µg/dl)
Control	70.820 ± 0.871 a	2.933 ± 0.044 b	1.965 ± 0.006 b	0.724 ± 0.003 b	1.697 ± 0.071 b	5.933 ± 0.044 b	2.660 ± 0.223 b	1.893 ± 0.027 a
Hypothyroidism	55.869 ± 8.223 b	1.641 ± 0.035 c	1.036 ± 0.011 c	0.529 ± 0.006 c	0.464 ± 0.021 c	1.319 ± 0.058 c	7.271 ± 0.065 a	1.288 ± 0.011 b
Hyperthyroidism	75.387 ± 1.274 a	3.464 ± 0.038 a	3.871 ± 0.065 a	1.381 ± 0.007 a	3.956 ± 0.090 a	10.768 ± 0.038 a	0.291 ± 0.018 c	0.973 ± 0.020 c
Means having the different letters in the same column differed significantly. ** (P≤0.01).								

Table 1: Distribution of the Study Parameters in Thyroid Disorders Patients

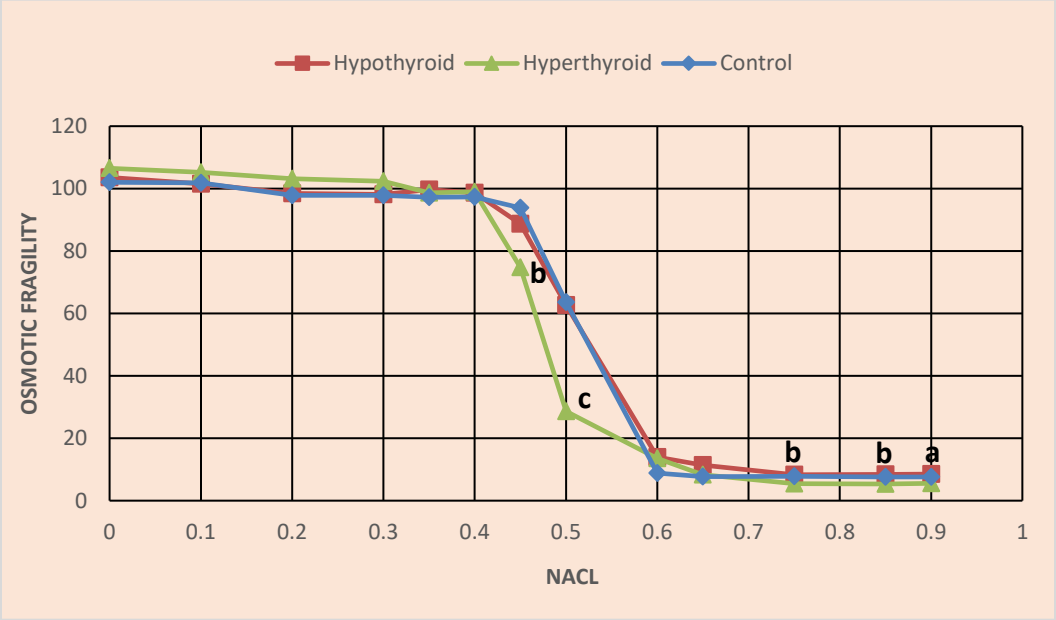

Figure (1): Percentage of Osmotic Fragility in deferent salt concentration.Different letters: means there is a significant difference at P < 0.01