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Research article

Measurement of oxidative stress and total antioxidant capacity in hyperthyroid patients following treatment with carbimazole and antioxidant

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HIGHLIGHTS

- Antioxidant boosting with conventional treatment enhanced of thyroid function in hyperthyroidism.
- Treating antioxidants and carbimazole enhanced thyroid function more than carbimazole alone.
- MDA level was normalized along with improved TAC by treating antioxidants with the antithyroid drug.
- Serum T4 significantly reduces and TSH significantly improves boosting antioxidant with carbimazole.
- Antioxidant adding helps to improve thyroid hormone and oxidative damage of hyperthyroidism.

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ABSTRACT

Hyperthyroidism is a common endocrine disorder in which the thyroid produces too many hormones, resulting in the metabolism speed up. The present study was designed to measure oxidative stress and total antioxidant capacity in hyperthyroid patients following treatment with carbimazole and antioxidants supplements. This randomized clinical trial study was conducted to compare Malondialdehyde (MDA) and total antioxidant capacity (TAC) among 25 newly diagnosed hyperthyroid patients (Group A), 25 hyperthyroid patients treated with carbimazole (Group B) and 25 hyperthyroid patients treated with carbimazole and antioxidants supplement (Group C) of both sexes. In this study, the mean serum malondialdehyde (MDA) of the three groups were 4.60 ± 1.08 μmol/L (Group A), 2.79 ± 0.58 μmol/L (Group B), and 1.57 ± 0.29 μmol/L (Group C). We found the mean MDA level was significantly higher in Group A than Group B and Group C. This study found the MDA level was significantly higher in hyperthyroid patients treated with carbimazole alone (Group B, 2.79 ± 0.58 umol/L) than hyperthyroid patients treated with carbimazole and antioxidant combined (Group C, 1.57 ± 0.29 umol/L) among the study groups ($p < 0.001$). The results showed that the mean serum TAC was significantly lower in newly diagnosed hyperthyroid (Group A, 527.8 ± 78.44 umol/L] patients compared to carbimazole treated alone (Group B, 951.80 ± 99.67 umol/L) and combination with the antithyroid drug (carbimazole) and antioxidant treated (Group C, 1113.56 ± 121.69 umol/L). There was more improvement found in the treatment combined with the antithyroid drug (carbimazole) and antioxidant (Group C).

Conventional treatment of hyperthyroid patients significantly reduced oxidative stress and elevated serum TAC but not up to normal level. Therefore, the supplementation of antioxidants could be utilized to improve thyroid function in hyperthyroid patients by boosting antioxidants and restoring oxidant-antioxidant balance. However, further studies are required to determine the optimal dosage, route of administration, and timing of antioxidant therapy needed before this supplementation could be officially recommended as adjuvant therapy.

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1. Introduction

Hyperthyroidism is an excessive concentration of thyroid hormones in tissues caused by increased synthesis of thyroid hormones, excessive release of preformed thyroid hormones, or an endogenous or exogenous extrathyroidal source (Vinay et al. "Robbins &Cotran Pathologic Basis of Disease", 9th Edition), (Dina et al., 2018)). Hyperthyroidism caused by the overproduction of thyroid hormones can be treated with antithyroid medications (carbimazole, methimazole, propylthiouracil, etc.), radioactive iodine ablation of the thyroid gland, or surgical thyroidectomy Braun et al. (2007) and Breuhaus (2011).

The thyroid hormones (THs) are important regulators of gene expression. Through interaction with thyroid hormone receptors (TRs), THs regulate cell development, homeostasis, differentiation, growth and metabolism (Chi H et al., 2012). Thyroid hormones are necessary for the normal growth of our body and one of the most important involved hormonal factors in the regulation of the basic metabolic rate of target organs such as the liver, heart, kidney and brain (Guerrero A et al., 1999). The oxygen-free radicals (ROS) are produced from regular metabolic reactions after several steps. Generation of reactive oxygen species (ROS) occurs as a consequence of the oxidative cell metabolisms (Valko M et al., 2007; Aruoma, 1998)).

ROS are highly reactive molecules due to presence of unpaired electron(s) that include partially reduced forms of oxygen, such as hydrogen peroxide, hydroxyl radicals and superoxide anions and lipid peroxides. Lipid peroxidation can be described generally as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids Ayala et al. (2014). ROS performance as oxidizing agents, which may agitate intracellular reactions and damage cell structures, including membranes and cellular proteins, lipids and nucleic acids (Figure 1). An antioxidant system defends the cells from ROS-induced damage, under physiological conditions (Irshad and Chaudhuri, 2002). This system includes enzymes, such as superoxide dismutase (SOD), catalase, glutathione per-oxidase and glutathione reductase, as well as small nonenzymatic molecules, such as glutathione (GSH) and vitamins (ascorbic acid, carotenoid and tocopherol). The increased rate of ROS production or decrease in their scavenging ability will interrupt the oxidative stability of the cell, resulting in oxidative stress (Halliwell and Gutteridge, 2007; Halliwell and Gutteridge, 1984).

Thyroid hormones are worked as a modulators of basal metabolic state, protein degradation and oxidative metabolism (Katzung et al. "Basic and Clinical Pharmacology", 13th edition). The oxidative stress is produce due to increase the production of reactive oxygen species (ROS) as a result of hypersecretion of thyroid hormone (Abalovich M et al., 2003). Higher ROS generation is responsible for the development of many diseases such as cardiovascular, neurodegenerative, neoplastic, endocrine and the consequent effect is oxidative damage (Halliwell B and Gutteridge, 2007).

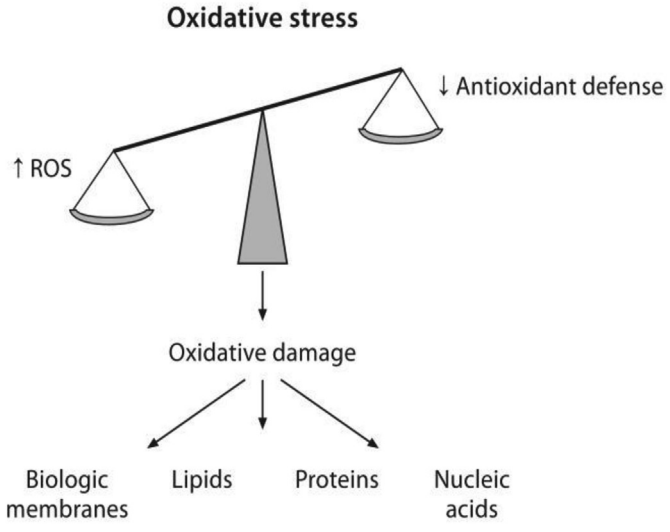

Figure 1. Mechanism of oxidative damage of biological membranes and molecules, such as lipids, proteins and nucleic acids (Claudio et al., 2012).

The excessive oxidative stress is responsible for the control of the immune system and pathogenesis of autoimmune diseases (i.e. increased inflammation, proapoptotic effect and breaking down the immunological tolerance). The role of oxidative processes is to create graves' diseases such as hyperthyroidism and tempted damage such as thyrotoxic myopathy and cardiomyopathy and graves' orbitopathy (Zarkovic M, 2012).

The most frequently used indicator of lipid peroxidation is Malondialdehyde (MDA) (Nielsen et al., 1997). The estimation of damage by reactive oxygen species is measured by MDA. A major reactive aldehyde is MDA is produced by the peroxidation of biological membranes polyunsaturated fatty acids (Esterbauer et al., 1991) and (Gawel et al., 2004). The spectrophotometric method developed by Placer et al.,1966 is used to count of serum MDA. The MDA level in increased due to increase oxidative stress (Mehmet and Niyet, 2011; Mirela et al., 2012).

The antioxidants are low molecular weight compounds in plasma such as Vitamin C, Vitamin E, bilirubin and uric acid (Krzysztof and Piotr, 2011). Decreasing strength of all these plasma constituents with both hydrophilic and hydrophobic character estimated all together is the Total Antioxidant Capacity (TAC) (Pisoschi and Negulescu, 2011). Oxidative stress is developed when ROS generation exceeds the antioxidant capacity of cells. The metabolic oxidation is increased by the modification of body's self-regulating process as a result of hyperthyroidism. This metabolic oxidation results increase oxidative stress and decrease antioxidant capacity. The signs and symptoms of hyperthyroidism many produce by increasing of oxidative stress (Manisha et al., 2017).). The serum concentration of thyroid hormones did not affect the antioxidant treatment alone but it might help reduce the oxidative damage due to hyperthyroidism (Irshad et al., 2002). Combined treatment with antioxidants and carbimazole reduced the time period to normalize thyroid hormones. It may improve clinical manifestation by reducing oxidative stress (Claudio et al.,2012).

Therefore, it could be assumed that antioxidant supplementation might be of benefit in combination with carbimazole in the management of hyperthyroidism.

1.1. Research hypothesis

Combined antithyroid drug and antioxidant supplement can reduce oxidative stress and improve total antioxidant capacity (TAC) faster than antithyroid drug does alone.

2. Objectives

A. General Objective:

To measure oxidative stress and total antioxidant capacity in hyperthyroid patients following treatment with carbimazole and antioxidants.

B. Specific Objectives:

1. To measure the level of Malondialdehyde (MDA) and total antioxidant capacity (TAC) in newly diagnosed hyperthyroid patients.
2. To measure the level of Malondialdehyde (MDA) and total antioxidant capacity (TAC) with the antithyroid drug (carbimazole) in hyperthyroid patients.
3. To measure the level of Malondialdehyde (MDA) and total antioxidant capacity (TAC) after treatment with an antithyroid drug (carbimazole) and antioxidant in hyperthyroid patients.
4. To compare Malondialdehyde (MDA) level and total antioxidant capacity (TAC) between groups.

3. Materials and methods

3.1. Study design

Randomized clinical trial.

3.2. Sampling technique

A convenient and purposive sampling technique will be employed to include the required number of patients.

3.3. Inclusion criteria

1. Carbimazole treated newly diagnosed hyperthyroid patients in the age group 16–50 years.
2. Combination with carbimazole and antioxidant (at least two months) in newly diagnosed hyperthyroid patients in the age group 16–50 years.
3. Both genders.

3.4. Exclusion criteria

1. Patients with serious comorbid diseases (DM, MI, HTN, dyslipidemia, kidney disease etc.)
2. History of using drugs such as glucocorticoids, oral contraceptives, or vitamin supplements.
3. Female patients with pregnancy and taking oral contraceptive pills.

3.5. Sample size determination

The same size will be determined by using the formula (i) for hypothesis testing for the difference between two means (Grzegorz and Tomasz, 2007).where,

$Z_\alpha = Z$ Value (two tail) at a definite level of significance.
1.96 at 95% confidence level or 5% level of significance.
$Z_\beta = Z$- Value (one tail) at definite power.
0.85 at 90% power when β is 0.2.
μ_1 = Mean of one group = 2.41.
σ_1 = SD of this group = 1.58.
μ_2 = Mean of other groups = 1.09.
σ_2 = SD of this group = 0.77.
So,

$$n = \frac{(Z_\alpha + Z_\beta)^2 X\{(\sigma_1)^2 + (\sigma_2)^2\}}{(\mu_1 - \mu_2)^2} = \frac{(1.96 + 0.85)^2 X\{(1.58)^2 + (0.77)^2\}}{(2.14 - 1.09)^2} = \frac{7.89X3.08}{1.05} = 23.14 = 25 \quad \text{(i)}$$

A total of 75 subjects will be sufficient for the authentic purpose of hyperthyroidism patients. Out of them, 25 newly diagnosed patients, 25 patients with the antithyroid drug (carbimazole) and 25 patients combined with the antithyroid drug (carbimazole) and antioxidant.

3.6. Sampling size

Seventy-five hyperthyroidism patients. This was a single-center study within a short period.

3.7. Procedure

After taking informed written consent, complete history taking and physical examination will be done and recorded in a performed datasheet. Then 4 ml venous blood will be taken from each subject in a test tube containing anticoagulant EDTA (Ethylene diamine tetraacetic acid). The serum will be connected after centrifuging for 15 min at 3000 rpm. Then serum MDA level and total antioxidant capacity will be measured.

According to Kei (1978) serum MDA level is determined by the following method with Eq. (ii):

Reagents	Specimen	Standard	Blank	Serum
	0.5 ml	-		-
20% TCA	2.5 ml	-		-
Stand for 10 min after string centrifuge at 35000 rpm and decent supernatant				
0.1 NH_2SO_4	2.5 ml	-		-
Centrifuge for 10 min at 35000 rpm and decent supernatant				
MDA standard solute	-	2.5 ml		-
0.1 NH_2SO_4	2.5 ml	2.0 ml		2.5 ml
Resuspend precipitate with glass stick				
0.2% TBA in 2M Na_2SO_4	3.0 ml	3.0 ml		3.0 ml
Stir and heat in the boiling water bath for 30 min then rapid cooling				
N-butanol	4.0 ml	4.0 ml		4.0 ml

3.8. Measurement of malondialdehyde (MDA)

Malondialdehyde (MDA) will be measured by thiobarbiturica acid–reactive substances (TBARS) (Al-Fawaeir et al., 2011).

3.9. Principle

MDA has frequently been measured in serum thiobarbituric acid – reactive substances (TBAARS) assay. Here TBA reacts with MDA to form pink 2:1, maximally at 532 nm. This colored complex was measured by a spectrophotometer.

3.10. Thiobarbituric acid method by spectrophotometry

Shake vigorously for 30 s then centrifuge for 10 min at 3000 rpm then colorimetry of butanol phase at 530 nm

$$\text{Serum MDA value} = \frac{0.D\ of\ unknown}{0.D\ of\ standard} \times 10\ \mu mol/l \quad \text{(ii)}$$

3.11. The measure of total antioxidant capacity (TAC)

Total serum antioxidant will be measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996) and Boligon et al., (2014).

3.12. Principle

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduce oxidant, Fe(III). Reduction of a ferric tripyridyltriazine complex to ferrous- (2,4,6-tripyridyl-s-triazine)$_2$ i.e. Ferric (III) (colorless) to Ferrous (II) (blue) can be monitored by measuring absorbance at 593 nm. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the test compound. Hence, the FRAP assay can rank the reducing power and the antioxidant potential of a wide range of test compounds.

3.13. Procedure

100μL serum will be mixed with 900 μL distilled water and 2 mL of FRAP working reagent and absorbance at 593nm will be measured after 30 min against FRAP reagent blank. Standard (ascorbic acid 1000 μM) will proceed in the same manner. The result will be expressed as μM/L of ferrous equivalent in Eq. (iii).

3.14. Calculation

$$\text{FRAP value of sample } (\mu M/L) \frac{Abs(sample)}{Abs(std.)} X \text{ FRAP value of std. } (\mu M) \quad \text{(iii)}$$

3.15. Addressing ethical issues

Helsinki Declaration for Medical Research Involving Human Subject 1964, last amended in 2013, will be compiled during the research processes as follows:

1. Helsinki Declaration on ethical guidelines will be strictly followed.
2. Consent will be obtained after briefing the study in Bengali to all respondents.
3. It will be made clear to them that they are free to take part or refuse at any part of the study. Refusal and withdrawal from the study at any point for any reason whatsoever will not hamper their usual treatment.
4. All personal information related to patients will be kept confidential.

4. Results and observations

The present study was attempted to determine the level of serum malondialdehyde (MDA) and total antioxidant capacity (TAC) in hyperthyroid patients and to find out their correlation among groups treated with carbimazole alone and carbimazole with antioxidant supplement. The study included 25 newly diagnosed patients, 25 patients with the antithyroid drug (carbimazole) and 25 patients with the antithyroid drug (carbimazole) in combination with antioxidant. The age distribution was from 16 to 50 years. The results were presented in the form of tables and figures with the necessary interpretation.

4.1. Demographic characteristics

Demographic characteristics of the study subjects are given in Table 1.

Table 1, shows the mean age of the study subjects was around 35 years. The female subjects were predominant. The mean BMI of Group A, Group B and Group C were 21.60 kg/m^2, 20.36 kg/m^2 and 19.64 kg/m^2 accordingly.

4.2. Comparison of physiological parameters among three groups

Table 2 shows the mean pulse of the study subjects was found around 111.66 bpm and the difference among the three groups were significant. Systolic blood pressure in Group B and Group C was found in the normal range than Group A. The mean diastolic blood pressure was found significantly lower in Group B and Group C than in Group A. The mean diastolic pressure of Group C was significantly lower in comparison to Group B.

Table 1. Comparison of demographic characteristics of the study groups.

Variables	Groups		
	Group A (newly diagnosed hyperthyroid patient) (n = 25)	Group B (hyperthyroidism with carbimazole alone) (n = 25)	Group C (hyperthyroidism treated with carbimazole and antioxidant) (n = 25)
Age* (years)	38.56 ± 9.30	34.88 ± 10.48	3.00 ± 9.67
Sex# Male	7 (9.3%)	8 (10.7%)	10 (13.3%)
Female	18 (24.0%)	17 (22.7%)	15 (20.0%)
BMI* (kg/m^2)	21.60 ± 1.60	20.36 ± 1.35	19.64 ± 1.03

* Data were presented as mean ± SD.

Table 2. Status of pulse rate and blood pressure among study groups.

Variables	Groups			p-value
	Group A (untreated hyperthyroid patient) (n = 25)	Group B (hyperthyroidism with carbimazole) (n = 25)	Group C (hyperthyroidism treated with carbimazole and antioxidant) (n = 25)	
Pulse in bpm	130.92 ± 4.72	120.32 ± 3.62	85.08 ± 3.77	<0.001
Systolic blood pressure in mm of Hg	144.44 ± 10.05	128.84 ± 10.81	119.60 ± 7.76	<0.001
Diastolic blood pressure in mm of Hg	102.28 ± 6.86	88.84 ± 2.88	79.00 ± 4.08	<0.001

*Data were presented as mean ± SD and test of significance done by ANOVA Test.

4.3. Serum level of MDA, TAC, T_4, TSH among study groups

Table 3 shows serum levels of MDA, TAC, T_4, TSH. The mean MDA was significantly lower in group B and group C than in group A. On the other hand, the MDA of group C found comparatively better improvement than group B. The mean of TAC was significantly higher in group B and group C than in group A, but group C was better improved than group B. The mean T_4 of group C was a better improvement than group B. The mean TSH of group C was a better improvement compared to group B.

4.4. Correlation of serum MDA, TAC, TSH and T_4

Table 4 shows a significant inverse correlation between MDA with TAC, MDA with TSH and TAC with T_4. A significant positive correlation was found between MDA with T_4 and TAC with TSH in hyperthyroid patients.

4.5. P-value comparison

In the Table 5 shows, there were significant differences between groups.

Table 3. Serum level of MDA, TAC, T4, TSH among study groups.

Variables	Groups			p-value
	Group A (newly diagnosed hyperthyroid patient) (n = 25)	Group B (hyperthyroidism with carbimazole) (n = 25)	Group C (hyperthyroidism treated with carbimazole and antioxidant) (n = 25)	
MDA (μmol/L)	4.60 ± 1.08	2.79 ± 0.58	1.57 ± 0.29	<0.001
TAC (μmol/L)	527.8 ± 78.44	951.80 ± 99.67	1113.56 ± 121.69	<0.001
T4 (fmol/ml) 8.56–25.6 fmol/ml	114.64 ± 48.21	17.20 ± 7.18	14.33 ± 4.32	<0.001
TSH (μIU/ml) 0.3–5 μIU/ml	0.27 ± 0.26	0.68 ± 0.84	0.73 ± 0.81	<0.001

*Data were presented as mean ± SD and test of significance done by ANOVA Test.

Table 4. Correlation of serum MDA, TAC, TSH & T4 in hyperthyroid patients.

Variables	Number	Correlation coefficient (r)	p-value
MDA with TAC	75	-0.657	<0.001
MDA with T4	75	0.473	<0.001
MDA with TSH	75	-0.775	<0.001
TAC with T4	75	-0.599	<0.001
TAC with TSH	75	0.685	<0.001

*Data were analyzed using Pearson's correlation coefficient test.

Table 5. p-value between different groups.

Variables	MDA p-value	TAC p-value	T4 p-value	TSH p-value
Group A with Group B	<0.001	<0.001	<0.001	<0.001
Group A with Group C	<0.001	<0.001	<0.001	<0.001
Group B with Group C	<0.001	<0.001	<0.001	<0.001

*Data were presented of significance done by ANOVA Post Hoc Test.

4.6. Correlation of serum MDA with serum TAC

Correlation of serum MDA with serum TAC in patients of the hyperthyroid patient shown in Table 6 & Figure 2.

Table 6 shows a strong negative correlation ($r = -0.658$, $p < 0.001$) between serum MDA and serum TAC in patients of hyperthyroid patients.

4.7. Correlation between MDA and TAC in hyperthyroid patients

The scatter diagram (Figure 2) shows an inverse correlation between serum MDA and serum TAC in hyperthyroid patients. The serum MDA and TAC exhibits a significantly negative correlation ($r = -0.658$, $p < 0.001$).

Table 6. Correlation of serum MDA with serum TAC in hyperthyroid patients.

Variables	Correlation coefficient (r)	p-value
MDA with TAC	-0.658	<0.001

*Data were analyzed using Pearson's correlation coefficient test.

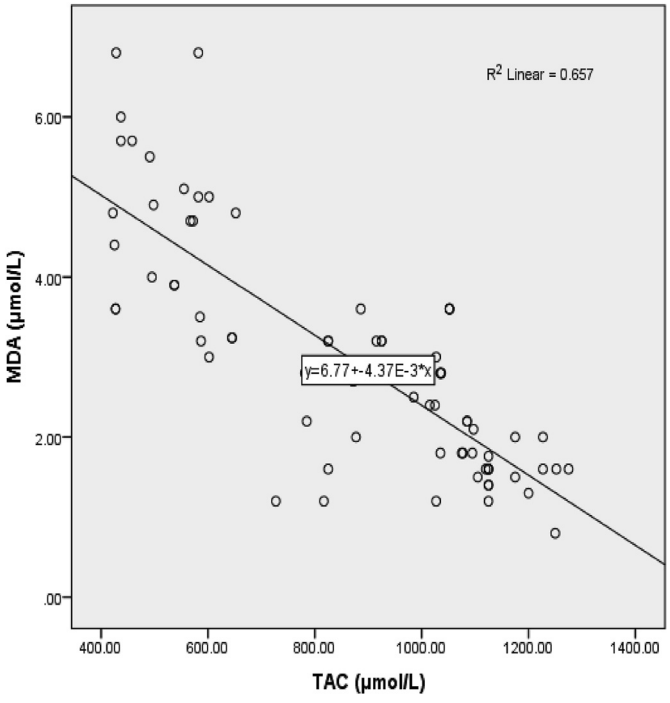

Figure 2. Correlation between serum MDA and serum TAC among Group A, Group B and Group C.

4.8. Correlation between MDA and T_4 in hyperthyroid patients

The scatter diagram (Figure 3) shows a positive correlation between serum MDA and serum T_4 in hyperthyroid patients. The serum MDA and T_4 exhibits a significantly linear correlation ($r = 0.473$, $p < 0.001$). This indicates that increasing T_4 along with increasing MDA.

4.9. Correlation between MDA and TSH in hyperthyroid patients

The scatter diagram (Figure 4) shows an inverse correlation between serum MDA and serum TSH in hyperthyroid patients that indicate higher serum MDA level at the lower level of serum TSH ($r = -0.775$, $p < 0.001$).

4.10. Correlation between TAC and T_4 in hyperthyroid patients

The scatter diagram (Figure 5) shows an inverse correlation between serum TAC and serum T_4 in hyperthyroid patients. The serum TAC and T_4 exhibit a significantly negative correlation ($r = -0.599$, $p < 0.001$) that indicates the lower value of T_4 with an increasing level of TAC.

4.11. Correlation between TAC and TSH in hyperthyroid patients

The scatter diagram (Figure 6) shows a linear correlation between serum TAC and serum TSH in hyperthyroid patients. The serum TAC and TSH exhibit a significant linear correlation ($r = 0.685$, $p < 0.001$) that's means TSH increased along with increasing the level of TAC.

5. Discussion

In the present study, 75 diagnosed hyperthyroid patients were included. Out of them, 25 patients were newly diagnosed hyperthyroid patients (Group A), 25 patients were treated with the antithyroid drug (carbimazole) alone for 2 months (Group B), and 25 patients were treated with the antithyroid drug (carbimazole) and antioxidants for 2 months (Group C). The mean serum malondialdehyde (MDA) of Group A, Group B and Group C were 4.60 ± 1.08 µmol/L, 2.79 ± 0.58 µmol/L and 1.57 ± 0.29 µmol/L respectively. The results showed that the mean serum MDA level was significantly higher in newly diagnosed hyperthyroidism patients compared to Group B and Group C (Table 3). Group C was more reduced than Group B. The mean serum total antioxidant capacity (TAC) of Group A, Group B and Group C were 527.8 ± 78.44 µmol/L, 951.80 ± 99.67 µmol/L and 1113.56 ± 121.69 µmol/L respectively. The results showed that the mean serum TAC was significantly lower in Group A patients compared to Group B and Group C. The patients of Group C were more improved than the patients of group B (Table 3).

Similar findings were noted by Guerra et al. (2001), they found decreased MDA levels and improved antioxidant enzyme activities in hyperthyroid patients when treated with antioxidants alone. They also found rapid improvement of MDA values and antioxidant enzyme activities when hyperthyroid patients were treated with a combination of antithyroid drugs and antioxidants (Guerra et al., 2005). Vrca et al. (2005) and Claudio et al. (2012) also noted a beneficial effect of antioxidant supplementation with the antithyroid drug. Aslan et al. (2011) stated that serum total antioxidant capacity (TAC) was significantly lower in patients with hyperthyroidism than in healthy controls. Oxidants are increased and antioxidants are decreased in patients with hyperthyroidism. Increased oxidative stress may play a role in the pathogenesis of hyperthyroidism and they concluded that supplementation of antioxidants may be helpful for hyperthyroid patients. In our study, we treated Group C by combination with carbimazole and antioxidants. Our finding was decreased level of MDA and increased level of TAC significantly by treating combined with antioxidants and carbimazole compare to carbimazole alone.

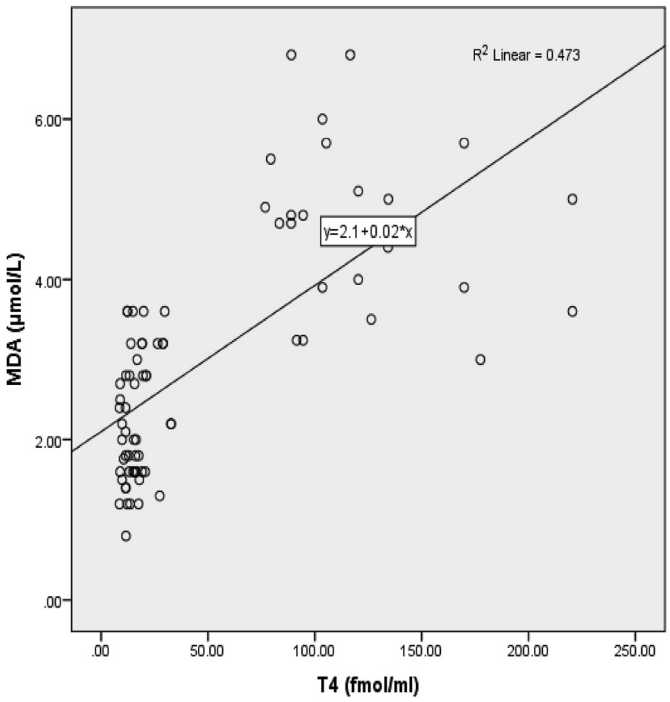

Figure 3. Correlation between serum MDA and serum T4 among Group A, Group B and Group C.

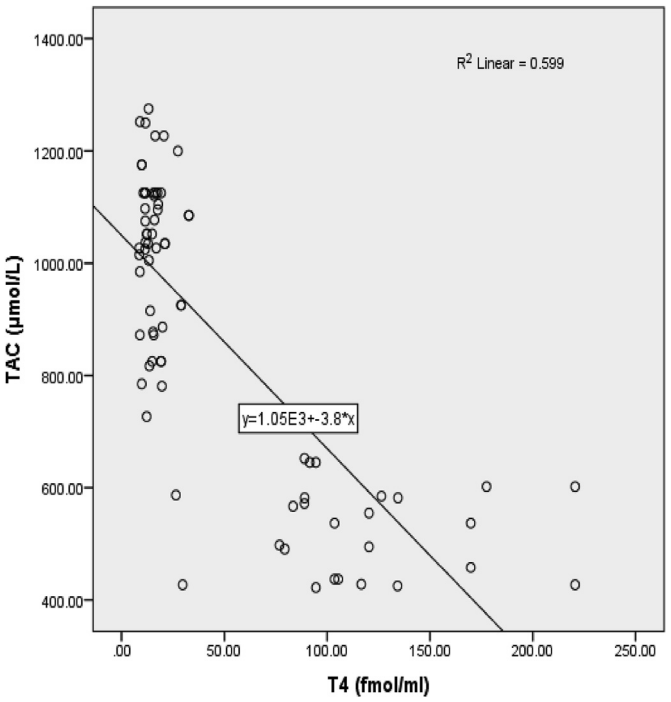

Figure 5. Correlation between serum TAC and serum T4 among Group A, Group B and Group C.

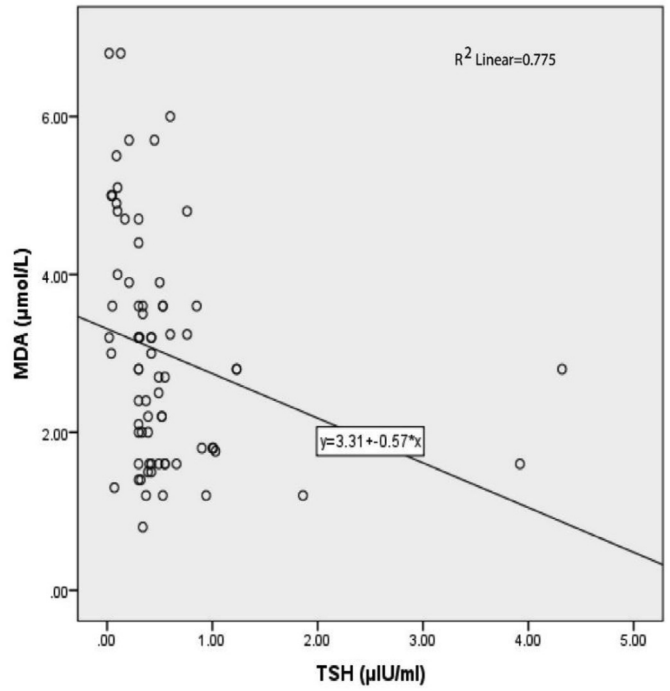

Figure 4. Correlation between serum MDA and serum TSH among Group A, Group B and Group C.

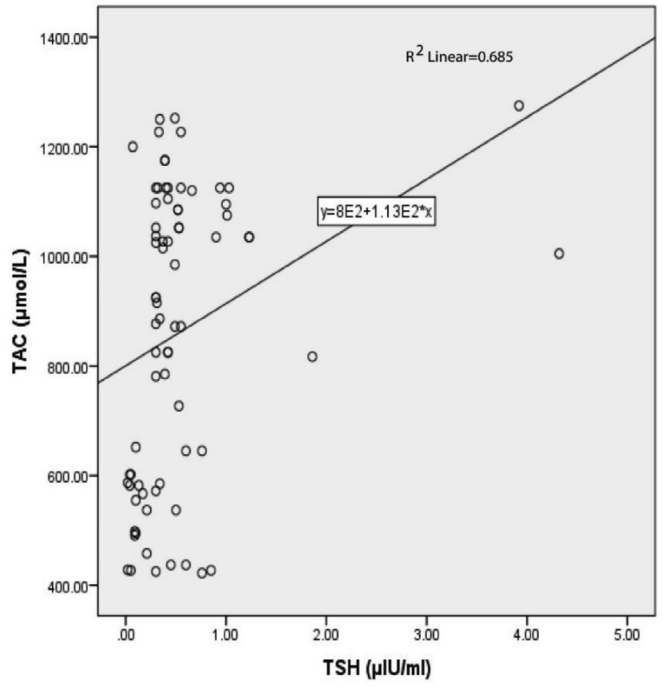

Figure 6. Correlation between serum TAC and serum TSH among Group A, Group B and Group C.

The present study found a strong inverse correlation between serum MDA and TAC in patients of hyperthyroid patients ($r = -0.658$, $p < 0.001$). The result showed that progressively increased oxidative stress was associated with progressively decreased total antioxidant capacity in hyperthyroid patients (Table 6, Figure 2). No previous study was found to correlate between MDA and TAC in hyperthyroidism patients, but some researchers attempted to correlate MDA level with several antioxidants. Olio et al. (2019), in their, demonstrated that total antioxidant capacity in patients with hyperthyroidism was reduced and increased malondialdehyde compared to the healthy control groups. These findings indicate that excessive thyroid hormone plays an important role in the production of oxidative stress.

In our study, the mean serum T4 (fmol/ml) of Group A, Group B and Group C were 114.64 ± 48.21 fmol/ml, 17.20 ± 7.18 fmol/ml, and 14.33 ± 4.32 fmol/ml respectively; baseline T4 (fmol/ml) 8.56–25.6 fmol/ml. The results showed that the mean serum T4 was significantly higher in Group A patients compared to Group B and Group C but more improved results were found in Group C than Group B (Table 3). The mean serum TSH of Group A, Group B and Group C were 0.27 ± 0.26 μIU/ml, 0.68 ± 0.84 μIU/ml and 0.73 ± 0.81 μIU/ml respectively; baseline TSH (μIU/ml) 0.3–5 μIU/ml. The results showed that the mean serum TSH was significantly lower in Group A patients compared to Group B and Group C. The highest TSH value was found in Group C (Table 3).

A similar study "Oxidative stress and antioxidant status in hypo-and hyperthyroidism patients" was conducted by Petrulea et al. (Petrulea et al., 2011). Their findings indicate that excessive thyroid hormones have a strong impact on oxidative stress and the antioxidant system. Antioxidant supplementation in hyperthyroid patients could exert a beneficial effect in favor of the diminution of thyroid hormone levels. Another study "Oxidative stress in Grave disease" was done by Claudio et al., (2012). They suggested that the combined treatment shorten the time required to normalize hormone and clinical manifestations.

Khdheir et al. (2014) studied "an evaluation of melatonin as an antioxidant in Iraqi patients with hyperthyroidism," within two groups randomly, the first group treated with antioxidant melatonin plus carbimazole and the second group treated with carbimazole alone. The result showed that patients who received extra supplementation with antioxidants attained euthyroidism and improvement in oxidative status faster than the patients treated only carbimazole. It's also recommended that antioxidants could be an effective adjuvant therapeutic tool to improve the clinical manifestation of this illness and improve thyroid hormones.

In our study, we found a remarkable correlation with thyroid hormones in hyperthyroid patients. The serum MDA showed a strong inverse correlation with TAC & TSH (Table 4, Figures 2 and 4) and a positive correlation with T4 (Table 4, Figure 5). The serum TAC showed a strong negative correlation with T4 (Table 4, Figure 5) and a positive correlation with TSH (Table 4, Figure 6). These findings suggest that increased oxidative stress was associated with the worsening of thyroid hormones in hyperthyroid patients.

The observations in the present study have clinical implications. The clinical symptom was established according to the most common symptoms and signs of hyperthyroidism such as nervousness, insomnia, hotness, sweat, weight loss, diarrhea, tachycardia, exophthalmos and tremor. The inclusion of patients in different treatment groups was at random.

The patients of group A were newly diagnosed with hyperthyroidism clinically identified. Meanwhile, groups B (carbimazole) and C (carbimazole and antioxidant) showed a significant improvement ($P < 0.001$) after 2 months' treatment. In group C found more improvement. The heart rate decreased significantly in the groups from 130.92 ± 4.72 bpm before treatment for group A and after treatment at 2 months for groups B (120.32 ± 3.62 bpm) and C (85.08 ± 3.77 bpm) ($P < 0.001$). (Table 2).

Patients with exophthalmos and goiter showed no differences from the rest of the patients, regarding the parameters measured.

Oxidative stress indicators significantly correlate with thyroid hormones in hyperthyroid patients, these markers may be used to assess the prognosis of the disease. The inflammatory process in the thyroid gland results in the excess generation of reactive oxygen species and free radicals that suppress the antioxidants leading to oxidant-antioxidant imbalance (Lien et al., 2008)). The oxidant-antioxidant balance is an essential factor for the normal function of the thyroids, augmentation of antioxidant defenses to restore the balance through therapeutic interventions might be beneficial in the management of hyperthyroid patients. Conventional treatment of hyperthyroid patients significantly reduced oxidative stress and elevated serum TAC but not up to normal level. Therefore, the supplementation of antioxidants with antithyroid drugs could be utilized to improve thyroid function and restore oxidant-antioxidant balance in hyperthyroid patients.

6. Conclusion

In this study, oxidative stress and antioxidant capacity were observed in patients with hyperthyroidism by measuring serum MDA and TAC. The results showed that the mean serum MDA level was significantly higher in newly diagnosed hyperthyroidism patients (Group A) compared to those treated with Carbimazole alone (Group B) and carbimazole with antioxidants (Group C). Among the study groups, Group C was showed a significant reduction of MDA level to and improvement of TAC level. The study revealed that the increased oxidative stress and decreased antioxidant capacity in serum were associated with worsening of thyroid function in hyperthyroid patients and the serum MDA showed a strong inverse correlation with TAC. The serum MDA level was normalized along with improved serum TAC by treating antioxidants with the antithyroid drug. These findings indicate that excessive thyroid hormones have a strong impact on oxidative stress and the antioxidant system. Antioxidant supplementation in hyperthyroidism could exert beneficial effects in favor of the diminution of thyroid hormone levels and might help to reduce the oxidative damage due to hyperthyroidism.

7. Recommendations

The following recommendations are suggested based on the findings of this study:

1. Antioxidant therapy could be practiced in the management of hyperthyroid patients along with conventional treatment.
2. The choice of antioxidant should be evaluated in future clinical trials with sufficient sample examination.
3. A multicenter study with a long duration of follow-up to recommend so that long-term safety of drugs could be studied.
4. Further studies have to be carried out on patients, to evaluate its role in the antioxidant mechanism to defend against oxidative stress.

8. Limitation of the study

1. This was a randomized clinical trial study and the sample size may not sufficient to generate strong findings within a short period.
2. This was a single-center study and a purposive sampling technique was employed. So the findings cannot be generalized confidently.

Declarations

Author contribution statement

Sultana, Dr. Razia; Ara, Dr. Shahin; Haque, Md. Jawadul: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data included in article/supplementary material/referenced in article.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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