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AN EVALUATION OF MELATONIN AS ANTIOXIDANT IN IRAQI PATIENTS WITH HYPERTHYROIDISM

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ABSTRACT
Hyperthyroidism is associated with increase in concentration of thyroid hormones and increase the speed of basal metabolism, accompanied by an increase in the total consumption of oxygen. In untreated patients, hyperthyroidism increases the formation of reactive oxygen species (ROS) leading to oxidative damage to biomembrane lipids. To evaluate the antioxidant effect of melatonin to improve the clinical and biochemical parameters in Iraqi patients with hyperthyroidism, 60 patients with newly diagnosed and untreated hyperthyroidism were enrolled in this study. 20 volunteers were served as control with normal TSH. The patients were divided into two groups randomly first group treated with melatonin + carbimazole, the second group treated with carbimazole. Enzyme activity (SOD), (GPx), (CAT), and (MDA) were measured in plasma. A clinical score was established according to the most common symptoms and signs of hyperthyroidism**.** The result showed that hyperthyroid patients had increased (MDA) content significantly, (CAT) and (SOD) activity also increased but not significantly while Gpx3 decreased significantly compared to controls ($p<0.05$) . Patients who received extra supplementation with Melatonin (group A, n = 30) attained euthyroidism and improvement in antioxidative status faster than the patients treated only with carbimazole (group B, n = 30). Most signs and symptoms of hyperthyroidism might be related to an increase in free radicals. Melatonin as antioxidant could be an effective adjuvant therapeutic tool to improve the clinical manifestation of this illness.

KEYWORDS: Hyperthyroidism, reactive oxygen species, melatonin, carbimazole.

INTRODUCTION

Thyroid epithelial cells are constantly exposed to reactive oxygen species (ROS). ROS are physiologically necessary and even intimately associated with thyroid hormone synthesis [1]. On the other hand thyroid hormones have a strong effect on oxidative stress and antioxidant systems [2-4]. Hyperthyroidism is associated with increase in concentration of thyroid hormones and increase in speed of basal metabolism, accompanied by an increase in the total consumption of oxygen. In untreated patients, the hyperthyroidism increases the formation of reactive oxygen species (ROS) leading to oxidative damage to biomembrane lipids[5-8].The levels of lipid peroxidation products correlate with serum concentration of thyroid hormones in patients with symptomatic hyperthyroidism [9]. So it is crucial for thyrocytes to be efficiently protected against excessive ROS production; otherwise, it would not be possible for these cells to be kept alive and, obviously, to function properly. Thyrocytes have developed protective systems that limit the toxicity of endogenously and naturally produced ROS. The presence of the following antioxidative enzymes in the thyroid gland has been documented, these include Superoxide dismutases (SOD), Catalase (CAT), and Glutathione peroxidise (GPx)[10–13]. Based on findings of recent studies; it might be reasonable to consider antioxidant supplementation as a possible additional therapy in hyperthyroidism[14,15] . A number of studies have shown that melatonin is significantly better than the classic antioxidants in resisting free-radical–based molecular destruction. In these in vivo studies, melatonin was more effective than vitamin E [16, 17], β-carotene [11], vitamin C [12], and superior to garlic oil [18].

Beneficial antioxidant effects of melatonin have been recently shown in clinical settings for several chronic diseases, including patients with rheumatoid arthritis [19], elderly patients with primary essential hypertension [20], and females with infertility[21]. Several animal studies documented the effect Melatonin as antioxidant to manage lipid peroxidation and oxidative stress caused by hyperthyroidism[22-24]. However, there was no human study concerned with the effect of melatonin as antioxidant in hyperthyroidism.

METHODOLOGY

60 patients (49 female, and 11 male) with newly diagnosed and untreated with hyperthyroidism were enrolled in this study. 20 (16 female and 4 male) healthy volunteers were served as control with normal TSH. Demographic characteristics and biochemical parameters are explained in table 1

TABLE1: Demographic characteristics and biochemical parameters in normal healthy subjects and hyperthyroidism

Parameter	Control	Hyperthyroid	P value
Number of persons	20	60	
male	4(20%)	11(18.33%)	
female	16(80%)	49(81.67%)	
Age(years)	35.43-6.47	36.43-5.46^{NS}	0.076
Blood urea(mg/dl)	30.47-3.15	29.80-4.81 ^{NS}	0.61
Serum creatinine(µmol/l)	81.4-7.06	80.34-7.10 ^{NS}	0.29
Body mass index(kg/m^2)	24.75-2.44	25.01-2.60 ^{NS}	0.31
NS= not significant			

In this study we excluded smoking and alcohol drinking individuals, as well as individuals suffering from chronic or acute diseases or any condition that may affect oxidative stress. The sixty patients were divided into two groups randomly one group treated with Melatonin 3 mg + Carbimazole 20mg daily, the second group treated with Carbimazole 20mg daily. The treatments continued for eight weeks. A clinical score was established according to the most common symptoms and signs of hyperthyroidism: A) nervousness, insomnia; B) hotness, sweat; C) diarrhea; D) tachycardia, and E) tremor. One of the symptoms or signs present in each item was enough to score 1 point. The activity of SOD, GPx, CAT and MDA level were measured by using (ELIZA) KIT, reagents supported by Cusbio company (Cat No. CSB-E08554h, CSB-E09496h, CSB-E13635h and CSB-E08557h) respectively.

Sample collection

Venous blood was obtained from the forearm of each individual (control and patient) between 8.00-10.00 a.m. after overnight fasting, by vein puncture before the initiation of therapy, as a base line, after 4 weeks, and after 8 weeks for both groups. Each blood sample was placed in EDTA free tube and allow samples to clot for one hour at room temperature, to be centrifuged for 15 minutes at 3000rpm .Serum was then divided into several 1.5ml eppendrof tubes and stored at (-20ºC) until time for assay. Estimation of Malondialdehyde (MDA) and antioxidant enzymes; superoxide dismutase (SOD), glutathione peroxidase (GPx3), and catalase (CAT) in serum samples was as follows:

Malondialdehyde content, the end product of the free radicals initiating lipid peroxidation, was measured by using thiobarbituric acid reactivity as described by Uchiyama and Mihara[25]. SOD was measured by the method of S. Marklund and G. Marklund [26] as modified by Nandi and Chatterjee [27]. Superoxide anion radical is involved in the autooxidation of alkaline pyrogallol. Catalase activity was estimated by the method of Aebi [28]. Catalase degrades hydrogen peroxide, which can be determined by the decrease in absorbance at 240nm in a spectrophotometer. GPx activity was analyzed by the method of Hafeman et al., based on the consumption of GSH[29].Glutathione peroxidase degrades tertiary butyl hydroperoxide in the presence of GSH. fT4 & fT3 and TSH were determined by using commercially prepared enzyme linked immunosorbent assay (ELISA), reagents supported by cusbio company china (cat no. CSB. E05078h, CSB. E05075h and CSB-E05114h) respectively.

Statistics

Results are Means ± STD, statistical analysis was performed by one - way analysis of variance[30] .

RESULTS

A double-blind approach was carried out to perform the clinical parameters in this study. Neither the patient nor the observer knew about the patient treatment during the course of treatment. The same physician determined each patient score. As some signs and symptoms are difficult to quantify, we decided to evaluate only their presence or absence (table2).

TABLE 2: Effect of treatment on signs and symptoms of hyperthyroidism

Signs and symptom	Before treatment	After treatment			
	Hyperthyroidism (n=60)	Group A(n=30)		Group B(n=30)	
		4 week	8 week	4 week	8 week
Tremor	59 (98.33%)	0(0%)	0(0%)	16 (53.33%)	7 (23.33%)
Diarrhia	39 (65%)	1(3%)	0(0%)	17 (56.66%)	3 (10%)
Tachycardia	59 (98.33%)	1(3%)	1(3%)	23 (76.66%)	2 (6.66%)
Heat intolerance/ increased sweating	58 (96.7%)	1(3%)	0(0%)	26 (86.66%)	2 (6.66%)
Nervousness / insomnia	59 (98.33%)	0(0%)	0(0%)	27(90%)	9 (30%)

In (Table: 3) Patient in group B (Carbimazole) diminished the clinical score to (0-3) only after 8 weeks. While group A (Carbimazole + Melatonin) showed improvement in clinical score (0-2) after 4 weeks. The heart rate decreased significantly in both groups from 106.6 ±2.4 pulsation/min before treatment to 80 ± 6.8 pulsation/min at 8 weeks for group B and at 4 weeks for groups A (76.5 ±4.0 pulsation/min) ($P< 0.05$).

TABLE 3: Effect of treatment on the clinical score in hyperthyroidism

Group	Clinical score	
	4 week treatment	8week treatment
Group A	(0-2)	(0-1)
Group B	(3-5)	(0-3)

In table 4 all patients had high levels of T3 and T4, and low serum levels of TSH before treatment. After 4 weeks treatment of group B patients (Carbimazole), the thyroid hormones concentration in sera remained elevated. Only after 8 weeks of treatment did all patients in this group diminish their thyroid hormones concentration to normal levels. After 4 weeks of a combined treatment with from the beginning (group A), thyroid hormone levels in sera decreased to normal levels.

TABLE 4 : Effect of treatment on thyroid hormones levels in sera of hyperthyroid patients

Parameter	Control (n=20)	Before treatment (n=60)	Group A		Group B	
			4 week after treatment (n=30)	8 week after treatment (n=30)	4 week after treatment (n=30)	8 week after treatment (n=30)
fT3 (pmol/l)	4.60±0.69	25.52±9.23	4.35±0.58	4.31±0.53	20.06±7.26	4.57±0.84
fT4 (pmol/l)	14.88±2.4	61.83±10.94	14.15±2.22	13.87±2.1	54.08±9.57	14.769±1.88
TSH (UI/ml)	1.78±0.67	0.019±0.015	2.026±0.638	2.11±0.66	0.0583±0.048	1.687±0.59

In (table:5) there was very highly significant increase in MDA values in hyperthyroidism than in controls ($P < 0.001$) and decreased to values after Carbimazole administration for 8 weeks group B(by 38%), but still significantly higher than controls ($P < 0.05$), as compared with (group A), there are significant decrease in MDA values after treatment with Carbimazole + Melatonin after just 4 weeks ($P < 0.05$), where there is no significant difference compared with controls for all patients($P=0.38$) .SOD and CAT in hyperthyroid patients were higher than in controls by 20% and 15% respectively, but not significantly ($P = 0.2$ for SOD, and $P = 0.1$ for CAT).After Carbimazole treatment (8 weeks), SOD activity diminished 9% and CAT activity decreased 23.4%, comparing with group A, SOD activity diminished 10% and CAT activity decreased 28.6% , where both groups show no significant difference ($P = 0.11$ for SOD , $p=0.06$ for CAT)

there was significant decrease GPx activity in hyperthyroidism than in controls ($P < 0.05$) and there was significant improvement in the enzyme activity after Carbimazole administration (group B) for 8 weeks, as compared with (group A), the improvement attained in GPx activity significantly ($P <0.05$) after treatment with carbimazole + melatonin after just 4 weeks, where both groups show no significant difference ($P=0.09$).

TABLE 5: Effect of treatment on antioxidant levels and lipid peroxidation in sera of hyperthyroid patients

Parameter	Control (n=20)	Before treatment (n=60)	Group A		Group B	
			4 week after treatment (n=30)	8 week after treatment (n=30)	4 week after treatment (n=30)	8 week after treatment (n=30)
SOD (U/ml)	7.244±2.193	8.190±3.0619	7.440±2.675	7.19±2.35	7.997±2.724	7.510±2.672
GPX3(U/ml)	0.610±0.111	0.521±0.227	0.607±0.092	0.626±0.09	0.555±0.133	0.575±0.110
CAT(U/ml)	2.428±0.973	2.803±0.684	2.179±0.522	2.4±0.388	2.645±0.700	2.271±0.467
MDA(µmol/l)	1.526±0.312	2.511±0.755	1.547±0.247	1.521±0.256	2.254±.0.705	1.888±0.691

DISCUSSION

When hyperthyroid patients were treated with Carbimazole, they became euthyroids clinically and biochemically after approximately 8 weeks. Antioxidant enzymes normalized their values, and plasma (MDA) decreased but still higher than levels of euthyroid controls. Combined treatment Carbimazole and Melatonin shortened the period of normalisation of the clinical score as well as the normalisation of hormonal concentrations from approximately 8 to 4 weeks when compared with the administration of Carbimazole alone. According to these results, treatment of hyperthyroidism with melatonin as antioxidant potentiates the effects of the antithyroid Carbimazole on the synthesis of thyroid hormones. Similar result was obtained when Vrca *et al*., 2004 used a combination of antioxidants in graves disease [31] . Guerra, postulated that elevated thyroid hormones produce signs and symptoms of hyperthyroidism through an increase in free radicals. When this increase is neutralised with antioxidants, the patients improve clinically, although this treatment has no effect on the production of thyroid hormones[32]. The possible explanation about the increased effect of Carbimazole when administered together with Melatonin, that Melatonin could interfere with the action of the peroxidase in one or both reactions, iodide oxidation and/or coupling reaction. Carbimazole acts by inhibiting the peroxidase; in this way, a synergetic result could be obtained.

Contradictory results have been reported regarding the activity of the antioxidant defense system in hyperthyroid

patients. Most studies have shown an increase of antioxidant defense enzymes, which represent a homeostatic response to balance the hyperthyroidism-induced increased ROS generation[33,34]. Bednarek[35] measured antioxidant activities in plasma of hyperthyroid patients of short duration (1–2 months) , and found an increase compared with healthy controls of (SOD) and (CAT), but not of (GPx) and glutathione reductase, which was decreased. It was nearly similar to the results of our study . Conversely, other study have shown no evidence of increased antioxidant defence in patients with untreated hyperthyroidism[36]. More recently, Aslan *et al.* [37] reported a significant decrease compared with controls of serum total antioxidant capacity in hyperthyroid patients with an average duration of hyperthyroidism of 2.3 ± 1.5 months.

The discrepant data on antioxidant activity in patients with hyperthyroidism may be due, in addition to differences in assessment methods, to the duration of the hyperthyroid status at the time of evaluation. In our study 23.33% (7) patients had enzyme activity significantly higher than control ($p< 0.05$), where the enzyme activity in those patients increased 49%, 52%, and 70% for (GPx), (CAT) and (SOD) respectively. Indeed, in patients with short-lasting hyperthyroidism, we might expect an increment in the antioxidant defense mechanisms in order to balance the increased oxidative stress. On the other hand, in patients with a more prolonged duration of the hyperthyroid state, the exhaustion of the antioxidant defense system may account for the decreased serum antioxidant activities. Despite the above discrepancies, restoration of euthyroidsim with antithyroid drugs is associated with a reversal of the abnormalities of the intra- and extracellular antioxidant defence system [33-35]. These effects of antithyroid drugs are likely due to the restoration of the euthyroid state. A scavenging effect of antithyroid drugs may also contribute to these changes [38,39].

CONCLUSION

The faster control of hyperthyroidism in patients treated with Carbimazole and Melatonin suggests a synergistic mechanism at the level of thyroid hormone synthesis. Based on the latter findings it might be reasonable to consider melatonin as antioxidant supplementation in the early phase of antithyroid drug therapy in order to obtain a more rapid control of clinical manifestations and a faster achievement of euthyroidism, especially in those hyperthyroid patients with a need for rapid clinical improvement such as danger of thyrotoxic crisis, pregnancy, and cardiopathy. It might be also an indication before a therapeutic dose of I^{131} administration or surgery. Clearly, melatonin as antioxidant therapy does not replace conventional treatment of hyperthyroidism with antithyroid agents such as Carbimazole or I^{131}. Nevertheless, it appears as a new therapeutic tool to improve the clinical manifestation of this illness. Further studies on larger series of hyperthyroid patients are needed to confirm these results.

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