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Evaluation of Oxidative Stress Status and Adenosine Deaminase Activity in Hyperthyroid and Hypothyroid Patients

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Abstract

A few prospective studies evaluated the oxidative stress variables and adenosine deaminase activity in patients with hyperthyroidism and hypothyroidism. Thus; we aimed to investigate oxidative stress variables and adenosine deaminase activity in patients with hyperthyroidism and hypothyroidism. Study populations consisted of 20 patients with hyperthyroidism, 20 patients with hypothyroidism and 20 age matched healthy volunteers as control group. Plasma nitric oxide, malondialdehyde levels and glutathione, adenosine deaminase activities were measured in all subjects. plasma nitric oxide and adenosine deaminase levels were higher in patients with hyperthyroidism, but lower in patients with hypothyroidism compared to those of controls (P<0.05). Plasma nitric oxide levels were higher in patients hypothyroidism compared to the control group (P<0.05). Glutathione levels were lower in both patients with hypo and hyperthyroidism compared to the control group (P<0.05). Our results suggest that both patients with hyperthyroidism and hypothyroidism may have effect on oxidative stress status and adenosine deaminase activity.

Key words: Hyperthyroidism, hypothyroidism, oxidative stress, adenosine deaminase activity

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Introduction

Hyperthyroidism is a subgroup of thyrotoxicosis that associated with the acceleration of basal metabolism and sympathetic nervous system activation as a consequence of the increase in thyroid hormone production. In hypothyroidism, basal metabolism rate is decreased as a result of the deficiency of thyroid hormone or rarely its inadequacy [1,2]. Adenosine deaminase (ADA) is the aminohydrolase which deaminases inosine and deoxyinosine to adenosine and deoxydenosine irreversibly in purine nucleotide catabolism. It has been shown that ADA activity, which a marker of cellular immunity, increases in serum in some diseases during the mutagenic response process of lymphocyte, monocyte and macrophage cells [3].

Glutation (GSH)(γ-glutamyl cysteine glycine) is one of the most effective antioxidants that protecting cells from oxidative damage via reacting with free radicals and peroxides. It can be found in liver and many other tissues in high concentrations. GSH is an important intracellular antioxidant due to tiole group bound with cysteine in its structure and its high concentration. It forms a milieu with high redox potential in the cell and protects the cell from oxidative damage [4,5]. Malondialdehyde (MDA), which is dialdehyde with three carbons, is the end product of non-enzymatic oxidative lipid peroxidation. Increase in its plasma levels mirrors the degree of oxidative damage. MDA may causes damage via forming cross bridges with lipid and proteins. Thus, it changes intrinsic membrane characteristics such as ion transport, enzyme activity, and aggregation of the components of cellular surface [6]. Nitric oxide (NO) is a free radical gas that is known to contribute to apoptosis, cytotoxicity, mutagenesis, and cellular antioxidant consumption. Reactive nitrogen oxygen species (ROS), produced as a result of the acceleration of oxidation, give rise to lipid peroxidation and protein oxidation in membranes as strong oxidants and lead to the formation of nitrogen dioxide and hydroxyl radical [7,8].

In the present study, the aim was to evaluate the oxidative stress variables and adenosine deaminase activity in patients with hyperthyroidism and hypothyroidism.

Materials and Methods

Study populations were consisted of 20 patients with hyperthyroidism and 20 patients with hypothyroidism. 20 age-matched healthy volunteers were also included in study the as control group. Patients that smoking, consuming alcohol or using any drugs over at least the last 20

days were excluded from the study. The diagnosis of hyperthyroidism and hypothyroidism were established by thyroid hormone tests. 5 ml-heparinized blood was drawn from all subjects just before initiation of therapy for hyper or hyperthyroidism. After the blood samples were centrifuged at +4 0C and at 3000 rpm for 10 minutes, plasma part at the top was kept at -70 degree until assayed. Adenosine deaminase enzyme activity was determined by Ellis and Goldberg method based on spectrophotometric measurement of colored complex produced by Berthelot reaction ADA activity was calculated as µmol /L [9] . In the determination of glutathione, the method developed by Fairbanks and Klee, which is based upon the reaction of sulfhydryl groups with Elman marker, was used [10]. Sample absorbance were multiplied by the factor obtained from standard graphic and GSH activity was calculated as µmol /L. Plasma MDA levels were determined by the method developed by Uchimaya and Mihara, which is based on spectrophotometric measurement [11]. Sample absorbance was multiplied by dilution factors and the factor obtained from standard graphics and the amount of MDA was calculated as nmol /L. Plasma NO amount was determined by Cortas and Wakid method, which is based on the spectrophotometric measurement of the colored complex produced by the interaction of NO formed by NOS activity in the environment with Griess reactive [12]. Sample absorbance were multiplied with dilution factor and the factor obtained from standard curve of nitrate and calculated as µmol/L.

Statistical analysis

Data were given as means and standard errors. Normality test was carried out with Kolmogorov Smirnov Test. In statistical analyses, one-way analysis of variance (ANOVA), Independent Sample T Test, and Pearson Correlation analysis were used. For multiple comparisons, Bonferroni test was used and $p < 0.05$ value was considered statistically significant. SPSS 13.0 for Windows (SPSS In Chicago, USA) packet program was employed in statistical analysis.

Results

There were no significant differences between the patients with hyperthyroidism (Group 1), hypothyroidism (Group 2) and control group (Group 3) as regard to the age (42.45±3.81, 39.85±2.76 and 33.60±1.32 years respectively). As expected, there were significant differences between groups as regard to the TSH, T3, T4, FT3 and FT4 levels (Table 1). ADA, NO and MDA levels were higher in patients with hyperthyroidism compared to the

patients with hypothyroidism and control group. GSH levels were lower in patients with hyperthyroidism compared to the patients with hypothyroidism and control group (Table 2).

Mean ages of all subjects were positively correlated with MDA and NO levels, and were negatively correlated with ADA and GSH levels.

Table 1. Statistics for Age, TSH, T_3, T_4, FT_3 and FT_4 in Hyperthyroid, Hypothyroid and Control groups

	Group 1	**Group 2**	**Group 3**
	(n=20)	**(n=20)**	**(n=20)**
Age	42.45 ± 3.81	39.85 ± 2.76	33.60 ± 1.32
TSH	$0.28^{a,b}$ ± 0.19	34.65^{c} ± 8.94	1.94 ± 0.17
T_3	$260.13^{a,b}$± 26.71	126.14^{c} ± 9.16	130.40± 2.95
T_4	$14.19^{a,b}$± 0.95	4.84^{c} ± 0.66	7.65± 0.40
FT_3	$5.05^{a,b}$± 0.41	2.57^{c} ± 0.23	3.01± 0.11
FT_4	$2.37^{a,b}$ ± 0.21	0.69^{c} ± 0.09	1.96 ± 0.16

a=difference between Group 1 and Group 2 is statistically significant; (p= 0.001)
b = difference between Group 1 and Group 3 is statistically significant; (p= 0.001
c = difference between Group 2 and Group 3 is statistically significant (p= 0.001)

Table 2. Statistics for ADA, GSH, NO and MDA variables

	Group 1	**Group 2**	**Group 3**
	(n=20)	**(n=20)**	**(n=20)**
ADA (µmol/L)	$38.29^{a,b}$ ± 1.78	21.80 ± 0,86	22.87 ± 0.52
GSH (µmol/L)	$2.12^{a,b}$ ± 0.12	4.34^{c} ± 0.26	8.09 ± 0.44
NO (µmol/L)	$53.00^{a,b}$ ± 1.17	30.06^{c} ± 1.25	15.29 ± 0.64
MDA(nmol/L)	$80.72^{a,b}$ ± 3.75	35.12^{c} ± 1.84	10.92 ± 1.29

a=the difference between Group 1 and Group 2 is statistically significant; (p= 0.001)
b=the difference between Group 1 and Group3 is statistically significant; (p= 0.001)
c=the difference between Group 2 and Group 3 is statistically significant; (p= 0.001)

Discussion

Thyroid hormones are supposed to play an important part in the regulation of mitochondrial oxidative metabolism and antioxidant enzyme levels. Hypermetabolic state occurring in hyperthyroid patients increases the production of superoxide radical in mitochondrial electron transport region. Elevated superoxide radical levels set the stage for the formation of radical species playing role in the initiation of lipid peroxidation and may lead to increased formation of free radical in mitochondria. This leads to acceleration of oxidative metabolism while causing decrease in lipid and lipoprotein plasma levels. Decrease in thyroid hormone levels occurring in hypothyroidism causes a decrease of basal metabolism rate and result in the decrease of the formation of free radicals and may protect the tissues from lipid peroxidation [13-15].

Peroxynitrite, nitrogen dioxide (NO2), dinitrogen trioxide [N2O3] and nitroxile ion may lead to oxidative stress. Nitrogen oxide species may stimulate lipid peroxidation [16]. NO level in Basedow's disease is significantly higher than that in control group [17]. It has been shown that NO release is increased in rats with hyperthyroidism while it is decreased in rats with hypothyroidism [18]. In another study it was shown that plasma NO levels increase in rats in when the experimental hyperthyroidism was induced [19]. In the present study, it was established that plasma NO levels of patients with hyperthyroidism and hypothyroidism were significantly higher than those of control subjects (Table 2, $p<0.05$). Cells may be exposed to excessive amounts of oxidants as a result of the increase in lipid peroxidation products in relation to the severe presentation of hyperthyroidism. However, in the group with hypothyroidism, a fall has not been observed unlike Huffman et al. This may stem from the fact that experimental hypothyroidism induced in rats is still at its early stage. The severity of oxidant stress reactions is determined by the plasma concentration of MDA, which is another oxidant and end product of lipid peroxidation. Plasma MDA levels with hyperthyroidism prior to treatment to be significantly higher than control group, with the levels decreasing after treatment [20]. Consistent with other studies [21,22], in our study MDA levels were higher in patients with hyperthyroidisms compared to control group (Table 2, $p<0.05$). Increase in MDA levels can be explained by lipid peroxidation chain reactions triggered by the increase in basal metabolism rate. In a study on rats with experimental hypothyroidism showed that MDA levels were higher in liver and thymus compared to control group while decreased in

heart, hepatic and brain tissue [23,24]. It is known that lipid peroxidation and generation of ROS leads to oxidative stress and damage.

The detoxification of these products takes place through the transformation of reduced form of GSH to its oxidized form mediated by glutathione peroxidase enzyme. When cells are exposed to excessive amounts of oxygen radicals, the generation of oxidized dimer form of glutathione exceeds metabolic limits and oxidative stress occurs. Oxidants, which cannot be detoxified, have an adverse effect on proteins, membrane lipids, DNA, carbohydrates and enzymes [4,25]. Adalı et al found that in patients with hyperthyroidism, GSH activity was significantly lower compared to the control group, and GSH activity increased significantly following treatment [20]. In a study in which experimental hyperthyroidism model in rats, serum GSH levels were significantly lower than those in control group [26].

In other studies on testis tissue of adult rat with experimental hypothyroidism [27] and on red blood cells of hypothyroidism patients [28], GSH activity was found to be lower than controls. In the present study, significantly lower plasma GSH activity was found in patients with hyperthyroidism and hypothyroidism compared to control group, which is consistent with the findings in the literature [Table 2, $p<0.05$]. Decrease in the GSH activity in patient groups suggests that it may be an indicator of cell damage caused by ROS and that response to oxidative stress was reduced.

There is a strong relation between ADA activity and differentiation, maturation and proliferation of T lymphocytes. Therefore, it is thought that ADA is an indicator of cellular immunity. Yet, there are a limited number of studies on ADA activity in hyperthyroid patients [2,29,30-32].

Karbownik et al. established that in patients with Graves and Hashimoto thyroidits, leukocyte ADA activity increased significantly compared to controls [29]. Reported that in homegenats of heart tissue of rats with experimental hyperthyroidism, ADA activity decreased by 15% in comparison to controls. According to our results, ADA activity increased significantly in the plasma of patients with hyperthyroidism, which is compatible with the findings in the literature [Table 2, $p<0.05$] [30]. As ADA is a purine catabolizing enzyme, it is thought that the increase in basal metabolism rate in hyperthyroidism may lead to the increase in ADA activity. This suggests that breakdown of protein is higher than its production in hyperthyroidism. It is known that the number of neutrophils increase in myocardial infarction,

burns, carcinoma, and metabolic diseases such as diabetic acidosis and thyrotoxicosis. Since ADA activity is the marker of activated neutrophil functions such as chemotaxia, phagocytosis and oxygen production supporting the formation of probable ROS, it may have increased significantly in the plasma of patients with hyperthyroidism [33]. The activation of neutrophil function by proinflammatory cytokines and their contribution to NOS production through upregulation of endothelial cells may have contributed to the increase in the plasma NO levels of patients with hyperthyroidism [34]. This may be explained as the formation of negative azote balance in hyperthyroidism in view of the literature [1]. Saggerson et al reported that in the adipocytes of rats with experimental hypothyroidism, ADA activity was low compared to controls [31]. However, created temporary hypothyroidism in psoriasis patients by administering PTU and thyroxin and established that tissue and plasma ADA activity decreased compared to pretreatment levels while erythrocyte ADA activity increased [32]. In the present study, it was established that plasma ADA activities in hypothyroidism group was significantly lower than controls [Table 2, $p<0.05$]. The decrease in plasma ADA activity in hypothyroidism may be explained by the rise in oxidative stress. In the one by one comparisons made between TSH, T3, T4, FT3 and FT4 variables and hyperthyroidism, hypothyroidism and control group [Table 2, $p<0.005$] can be explained with the fact that TSH, T3, T4, FT4 and FT3 can vary independent of age. In a previous study, it was found that TSH values were higher in rats which are 60 days old than those which are 30 days old [35]. This study supports the influence of age thyroid hormone levels.

It is known that main reactive species which are produced by the oxidation of nitric oxide and are influential in oxidative stress are nitrogen dioxide [NO2], peroxynitrite [ONOO], dinitrogen trioxide [NO2O3] and nitoxyl ion [NO.] [16]. The ability of NO2 and ONOO reactive species to initaite lipid peroxidation may be accounted for but the positive correlation between NO and MDA.

Higher plasma MDA and NO levels in hyperthyroidism group than in hypothyroidism group suggests that oxidative stress produced in hyperthyroidism is more severe and bring about more damage. In conclusion, it may be thought that in addition to increasing antioxidant [GSH] consumption, rise in both NO level and malondialdehide levels, which is an end product of lipid peroxidation, is an indicator of the severity of present oxidant stress.

Conclusion

Free radical damage and decrease in antioxidant activity both in hyperthyroidism and hypothyroidism groups suggests that response to oxidative stress may be decreased. Alteration in ADA activity in hyperthyroidism and hypothyroidism patients suggests that this enzyme may be used as an indicator of the poutlook and treatment of the disease besides present thyroid parameters. Based upon our findings, we propose that NO, MDA, ADA and GSH variables should be evaluated before and after treatment in patients under 30 using larger patient groups and that new studies should be planned to compare the same variables in patients who smoke and drink alcohol. We believe that present study elucidates the need for the investigation of oxidative stress in thyroid diseases such as hyperthyroidism and hypothyroidism with larger clinical and experimental studies.

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