THE EFFECT OF ANTITHYROID DRUGS ON OSTEOPONTIN AND OXIDATIVE STRESS IN GRAVES' DISEASE

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Abstract

Objective. The aim of the present study is to evaluate the effects of methimazole (MTZ) and propylthiouracil (PTU) treatments on osteopontin (OPN) and oxidative stress in Graves' disease (GD).

Material and Methods. The study included 60 cases with GD in hyperthyroid state and taking no antithyroid treatment, and 30 healthy volunteers. GD patients were randomly separated into two groups; 30 of them took PTU, and the other 30 took MTZ treatments. Blood samples were taken from the patients with GD before the treatment, and three months after the treatment was begun, when they were in the euthyroid state; blood samples of the healthy control subjects were also taken at these times.

Results. TAS and OSI levels before treatment were significantly higher in the GD group, when compared to the control group ($p < 0.001$, for each). GD subjects taking PTU treatment had significantly higher TAS levels ($p = 0.001$), and significantly lower TOS and OSI levels ($p = 0.008$ and $p = 0.001$, respectively). TAS levels significantly decreased in the patients taking MTZ treatment ($p = 0.029$), but TOS and OSI levels did not change significantly ($p > 0.05$). Pretreatment OPN levels were significantly higher in GD patients, when compared to the control group ($p = 0.014$). OPN level significantly decreased in the GD group taking PTU treatment; however OPN levels in the group taking MTZ treatment did not change significantly when compared to the pretreatment value.

Conclusion. PTU treatment is more effective in decreasing OPN and oxidative stress in GD patients, when compared to the MTZ treatment.

Key words: Graves' disease, oxidative stress, osteopontin, methimazole, propylthiouracil.

considered to play a role in the pathogenesis of various autoimmune diseases by stimulating the immune cells' chemotaxis, activation, and their release of inflammatory cytokines (1, 2). OPN increase has been indicated in various autoimmune diseases (3, 4). OPN level and number of OPN receptors have been demonstrated to increase in Graves' disease (GD). In addition, positive correlations have been determined between OPN levels and diagnostic parameters of GD (5, 6).

Increased oxidative stress in autoimmune thyroid diseases leads to impairment of immune tolerance, proapoptotic effect and thyroid gland inflammation, which are considered to contribute to the pathogenesis of autoimmune thyroid diseases (3, 7-9). Increased oxidative stress in GD is compensated by the use of antithyroid drugs. Oxidative stress increases again during the relapse state (10). Antioxidant treatment administration in association with the antithyroid drugs in hyperthyroidism causes a more rapid improvement of clinical and laboratory signs (8, 11).

Methimazole (MTZ) and propylthiouracil (PTU) used in GD treatment cause immunoregulation, decrease antibody levels and decrease thyroid tissue inflammation; these actions facilitate the remission of the disease (12). We could not reach a study in the literature that compares the effects of these two drugs on oxidative stress and inflammation. The aim of the present study is to compare the effects of MTZ and PTU treatments in GD on osteopontin, which is an inflammatory indicator, and on the indicators of oxidative stress.

INTRODUCTION

Osteopontin (OPN) is a protein that can be synthesized in various tissues and cells, which are primarily T lymphocytes, dendritic cells and macrophages. OPN is

MATERIALS AND METHODS

The local ethic committee approved the study. Informed consent was obtained from all participants prior to the study.

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The study included 60 cases with GD in hyperthyroid state and taking no antithyroid treatment (mean age= 29.60±13.02 years; 40 females, 20 males), and 30 healthy volunteers (mean age, 34.93±13.68; 18 females, 12 males).

Healthy control group was randomly selected among volunteers who applied to polyclinic for health screening and did not have any disease.

Thyroid were evaluated by ultrasonography (Logiq p6, GE healthcare, USA) by the same physician.

GD was diagnosed according using function tests (thyroid stimulating hormone (TSH) levels), free thyroxine (T4), serum free triiodothyronine (T3), and anti-thyroid peroxidase antibody (anti-TPO), thyroid stimulating hormone receptor antibody (anti-TRAB), anti-thyroglobulin antibody (anti-TG), thyroid scintigraphy and thyroid ultrasound.

The patients having infectious or liver disease, inflammatory diseases, malignancies, cardiovascular or neurological diseases, metabolic bone disease, metabolic diseases (metabolic syndrome, hypertension, diabetes mellitus, obesity), renal failure, smokers and those receiving antioxidants were excluded from the study.

GD patients were randomly separated into two groups; 30 of them took PTU (mean age, 41.13±12.71y; 21 females, 9 males), and the other 30 took MTZ treatments (mean age, 38.06±13.35y; 19 females, 11 males).

In patients with GD, serum samples were taken just before the onset of treatment then later the treatment was begun. On the third month of treatment, blood samples were taken again and stored at -80°C. Serum samples of the healthy control subjects were also taken.

A novel automated measurement was used to determine TAS and TOS levels (13, 14). Oxidative

stress index (OSI; Arbitrary Unit) = [TOS (mmol H₂O₂ Equiv./L)/TAS (mmol Trolox Equiv./L)]X100 (15, 16).

Sandwich ELISA was used to determine OPN levels (eBioscience, USA).

Statistical analysis

Mann-Whitney U and Student's t-tests were used to compare 2 independent groups. Kruskal Wallis and one-way analysis of variance (ANOVA) tests were used to compare more than 2 independent groups. DUNN test was used for post-hoc comparisons. The differences between the measurement before and after the treatment were analyzed by Student t test for paired samples in normal distribution and the Wilcoxon test for two dependent samples, when distribution was not normal. The relation between the variables and OPN were determined by Spearman correlation. Data were analyzed using SPSS version 15.

RESULTS

There were no differences in terms of age and gender distribution (p=0.141 and p=0.715).

While fT3 and fT4 levels were high, TSH levels were low before the treatment in patients with GD. There was not any difference in terms of thyroid functional tests between the GD groups that would take MTZ or PTU before and after the treatment by Wilcoxon test analysis.

TAS and OSI levels were higher in the pretreatment GD group compared to the healthy control group by Mann-Whitney U test (p<0.001; Table 1). Pretreatment OSI levels did not differ significantly between the GD groups that would take MTZ or PTU (p=0.756). On the third month of treatment in GD subjects taking PTU treatment, TAS levels significantly

Table 1. Demographic and laboratory findings of the groups

Parameters	GD-PTU	GD-MTZ	HC	p
Age (Mean±SD)	41.1±12.7	38±13.3	34.93±13.68	0.083
Gender				0.641
Male (n, %)	9 (30 %)	11 (36.7 %)	12 (%40)	
Female (n, %)	21(70 %)	19 (63.7%)	18 (%60)	
fT3 (pg/mL)	7.88±4.22	10.21±7.42	3.07±0.50	<0.001
fT4 (ng/dL)	3.60±7.10	4.53±7.63	1.19±0.21	<0.001
TSH (mIU/L)	0.01±0.01	0.01±0.02	1.96±0.99	<0.001
TAS (μmol H ₂ O ₂)	2.26±0.70	3.11±0.09	1.36±0.17	<0.001
TOS (mmol Equiv./L)	8.85±4.50	8.89±4.19	7.80±2.55	0.804
OSI (arbitrary unit)	0.42±0.19	0.28±0.13	0.27±0.08	<0.001
OPN (ng/mL)	49.87±31.29	56.10±47.51	31.74±17.31	0.010

Data were expressed as mean ±SS. P<0.005 was regarded as statistically significant. GD, Graves' disease; HC, healthy control; GD-PTU, GD group taking propylthiouracil; GD-MTZ, GD group taking methimazole; fT3, free triiodothyronine; fT4, free thyroxine, TSH, thyroid stimulating hormone; TAS, total antioxidant status; TOS, total oxidant status; OPN, osteopontin.

increased ($p=0.001$), while OSI and TOS levels decreased ($p<0.001$ and $p=0.008$, respectively; Table 2). In GD group taking MTZ treatment, TAS levels were significantly decreased ($p=0.029$) but, OSI and TOS levels did not change by Wilcoxon test analysis (Table 3).

Serum OPN levels were high in patients with GD. OPN levels did not differ between the GD groups before treatment ($p=0.551$). OPN levels decreased on the third month of the treatment in the GD group taking PTU ($p=0.001$); however OPN levels in the group taking MTZ did not change significantly when compared to the pretreatment value by Wilcoxon test analysis ($p=0.199$).

There was no correlation between serum OPN levels and TAS, TOS, OSI levels by Spearman correlation analysis ($r=0.073$, $p=0.496$; $r=-0.135$, $p=0.304$; and $r=0.05$, $p=0.972$, respectively).

DISCUSSION

This is the first reported study that compares the effects of PTU and MTZ treatments in GD patients on oxidative stress and serum OPN levels.

OPN is a potent proinflammatory protein that is closely related with various autoimmune diseases. In systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis, elevated OPN levels have been shown (17-20). Impaired balance between the proinflammatory and antiinflammatory cytokines in GD plays an important role in the pathogenesis of this

disease (21). Xu *et al.* have demonstrated that serum OPN levels and the number of OPN receptors in the peripheral blood mononuclear cells increase in GD, and also that OPN levels correlate with the clinical parameters of GD (5). OPN increases the production of proinflammatory cytokines and chemokines (CCL20 and CCL21) via the NF- κ B signal pathway (5, 22, 23). Additionally, it stimulates the production of immunoglobulins by increasing CD40 ligand expression (24). OPN affects the various stages that are responsible for the GD pathogenesis, and the control of this protein may therefore be important in the remission of the disease. Being similar with the results of other studies, OPN level in the present study was determined to be high in GD ($p=0.035$). Serum OPN levels decreased in both PTU- and MTZ-treated groups after ensuring the euthyroid state; however this decrease was more marked in the group treated with PTU ($p=0.001$).

Oxidative stress has been shown to increase in GD in many of the previous studies (8, 9, 25). In this study TOS and OSI were evaluated as oxidant markers, and TAS as antioxidant marker. OSI levels determined in our study were increased in GD group ($p<0.001$). In order to ensure remission in GD, it is important to decrease the oxidative stress or to compensate it by antioxidant defense (8). In our study, TAS value increased, and OSI and TOS levels decreased in the PTU-treated group (Table 2). In the MTZ-treated group, TAS level decreased significantly ($p<0.029$); OSI and TOS levels also decreased, but these decreases were not statistically significant. OPN and oxidative stress,

Table 2. Laboratory findings in pretreatment and end of the third month of treatment in the PTU-treated group

Parameters	Pretreatment	Third month of the treatment	p
fT3 (pg/mL)	7.88 $\pm$ 4.22	3.21 $\pm$ 0.59	<0.001
fT4 (ng/dL)	3.60 $\pm$ 7.10	1.19 $\pm$ 0.22	<0.001
TSH (mIU/L)	0.01 $\pm$ 0.01	1.48 $\pm$ 1.071	<0.001
TAS (μ mol H ₂ O ₂)	2.26 $\pm$ 0.69	3.05 $\pm$ 0.35	0.001
TOS (mmol Equiv./L)	8.85 $\pm$ 4.50	6.09 $\pm$ 2.85	0.008
OSI (arbitrary unit)	0.42 $\pm$ 0.19	0.26 $\pm$ 0.14	0.001
OPN (ng/mL)	49.80 $\pm$ 31.20	39.60 $\pm$ 19.70	0.001

Data were expressed as mean $\pm$ SS. $P<0.005$ was regarded as statistically significant. fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid stimulating hormone; TAS, total antioxidant status; TOS, total oxidant status; OPN, osteopontin.

Table 3. Laboratory findings in pretreatment and end of the third month of treatment in the MTZ-treated group

Parameters	Pretreatment	Third month of the treatment	p
fT3 (pg/mL)	10.21 $\pm$ 7.42	3.25 $\pm$ 0.55	<0.001
fT4 (ng/dL)	4.52 $\pm$ 7.62	1.12 $\pm$ 0.21	<0.001
TSH (mIU/L)	0.01 $\pm$ 0.02	1.40 $\pm$ 1.057	<0.001
TAS (μ mol H ₂ O ₂)	3.11 $\pm$ 0.09	2.81 $\pm$ 0.730	0.029
TOS (mmol Equiv./L)	8.99 $\pm$ 4.19	7.70 $\pm$ 4.309	0.238
OSI (arbitrary unit)	0.28 $\pm$ 0.13	0.25 $\pm$ 0.14	0.471
OPN (ng/mL)	56.10 $\pm$ 47.50	45.30 $\pm$ 27.90	0.199

fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid stimulating hormone; TAS, total antioxidant status; TOS, total oxidant status; OPN, osteopontin.

which are known to play a role in GD development, decreased more markedly in the PTU-treated group, and this result led to the conclusion that PTU treatment may be more effective in the treatment of GD. In a study evaluating the 10-year remission rates of MTZ and PTU treatments in the GD patients, remission ratios determined in the MTZ (n=20) and PTU (n=40) groups were 35% and 50%, respectively (26). We could not show a correlation between serum OPN levels and oxidative stress; this result made us consider that these two parameters affect GD via different mechanisms. The limitation of this study is that the follow-up period of patients with GD is relatively short.

The more prominent decreases of serum OPN and OSI levels in the PTU-treated GD patients lead to the suggestion that PTU treatment may be more effective in ensuring remission of this disease. More detailed and long-term studies are needed on this subject.

Conflict of interest

The authors have not declared any conflict of interest .

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