Evaluation of Serum Homocysteine Levels in Pregnant Patients with Graves' Disease

Graves Hastalığı Olan Gebelerde Serum Homosistein Düzeylerinin Değerlendirilmesi

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ABSTRACT Objective: In several studies, serum homocysteine levels were found normal or low in hyperthyroidism and low in pregnancy. Hyperhomocysteinemia and thyrotoxicosis may individually cause various complications in pregnancy. In this study we aimed to evaluate serum total homocysteine (tHcy) levels in pregnant with Graves' disease. **Material and Methods:** Twenty-two newly diagnosed thyrotoxic pregnant women (gravida 1, pregnancy weeks 12^{th}-16^{th}, average age 24.0 ± 2.8) and 20 healthy pregnant women (gravida 1, pregnancy weeks 12^{th}-16^{th}, average age 23.1 ± 3.0) were included in the study. Fasting blood tests were performed for thyrotropin (TSH), free thyroxine (FT4), free triiodothyronine (FT3), TSH-receptor antibody (TRAb), homocysteine (tHcy), vitamin B_{12}, folate, and albumin. **Results:** There were no significant differences between the 2 groups with regard to age (24.0 ± 2.8 and 23.1 ± 3.0 respectively) (p= 0.265). Vitamin B_{12}, folate, and albumin levels were not significantly different between the thyrotoxic and control group. The overall mean homocysteine concentration of the thyrotoxic pregnant subjects (9.6 ± 2.9 μmol/L) was significantly higher than control group (5.0 ± 1.9 μmol/L) (p< 0.001). Homocysteine levels were positively correlated with FT3 levels (r= 0.390, p= 0.01) and negatively correlated with TSH levels in both thyrotoxic and non-thyrotoxic groups (r= -0.490, p= 0.01). However, no correlation was found between FT4 and tHcy level. Average TRAb level of the patients was 16.6 ± 3.2 IU/L (minimum 12.8 IU/L and maximum 23.4 IU/L). No correlation was found between TSH receptor antibody and tHcy level. **Conclusion:** In this study, we observed that serum homocysteine levels in thyrotoxic pregnant women with Graves' disease were higher than control group. There is a need for large scale studies comparing pre- and post-treatment homocysteine levels in hyper-hypothyroid cases of pregnant and non-pregnant individuals.

Key Words: Graves disease; homocysteine; thyrotoxicosis; pregnancy

ÖZET Amaç: Birçok çalışmada serum homosistein seviyeleri hipertiroidizmde normal ya da düşük, gebelikte ise düşük bulunmuştur. Hiperhomosisteinemi ve tirotoksikoz birbirinden bağımsız olarak gebelikte pek çok komplikasyondan sorumlu olabilmektedir. Graves hastalığı olan gebelerde serum total homosistein (tHcy) düzeylerini değerlendirmek amacıyla bu çalışmayı planladık. **Gereç ve Yöntemler:** Yeni tanı almış 22 tirotoksik gebe hasta (gravida 1, 12-16 gebelik haftası) ile 20 sağlıklı gebe (gravida 1, 12-16 gebelik haftası) çalışmaya dahil edildi. Açlık serum tirotropin (TSH), serbest tiroksin (sT4), serbest triiyodotironin (sT3), TSH reseptör antikoru (TRAb), vitamin B_{12}, folat ve tHcy düzeyleri çalışıldı. **Bulgular:** Tirotoksik gebeler ile kontrol grubunun yaş ortalamaları benzerdi (sırasıyla 24.0 ± 2.8 ve 23.1 ± 3.0, p= 0.265). Tirotoksik hastalar ile kontrol grubu arasında serum folat, vitamin B_{12} ve albumin düzeylerinde anlamlı farklılık yoktu. tHcy düzeyleri tirotoksik gebelerde (9.6 ± 2.9) kontrol grubuna (5.0 ± 1.9) oranla anlamlı düzeyde daha yüksekti (p< 0.001). Tirotoksikozlu ve kontrol gebe grupların her ikisinde sT3 düzeyi ile tHcy düzeyi arasında istatistiksel olarak anlamlı pozitif korelasyon (r= 0.390, p= 0.01) ve TSH düzeyi ile tHcy düzeyleri arasında istatistiksel olarak anlamlı negatif yönde korelasyon izlendi (r= -0.490, p= 0.01). Bununla birlikte sT4 düzeyi ile tHcy düzeyi arasında ilişki saptanmadı. Hastaların ortalama TRAb düzeyi 16.6 ± 3.2 IU/L (minimum 12.8 IU/L ve maksimum 23.4 IU/L) olarak saptandı. TSH reseptör antikoru ile tHcy düzeyi arasında ilişki bulunmadı. **Sonuç:** Çalışmamızda Graves hastalığı saptanan tirotoksik gebelerde normal gebelere göre serum tHcy düzeyleri daha yüksek bulundu. Bu konuda gebe ve gebe olmayan bireylerde hiper-hipotiroidi durumlarında tedavi öncesi ve sonrası homosistein düzeylerinin karşılaştırıldığı geniş çaplı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Graves hastalığı; homosistein; tirotoksikoz; gebelik

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Thyroid hormones increase oxidative system as they induce basal metabolic rate and specific mitochondria enzymes, consequently result in free radical formation.[1] Overt hypothyroidism and thyrotoxicosis have well-documented adverse impacts on pregnancy outcomes.[2-4] Graves' disease (GD) is an autoimmune disorder causing hyperthyroidism, in which the thyrotrophin receptor antibody (TRAb) acts as a stimulator of the thyroid gland. Similar to other autoimmune diseases, the prevalence of GD is much more common in females than males, particularly in women of childbearing age. Because untreated GD during pregnancy results in an increased frequency of complications such as miscarriage, stillbirth, preterm delivery and intrauterine growth retardation.[5] Generally, in human studies, serum homocysteine levels were found normal or low in hyperthyroidism.[6-10]

Homocysteine is an amino acid whose effect is similar to that of free radicals, which is recently acknowledged to be included in oxidative system and which is not classified under proteins.[11-13] Hyperhomocysteinemia has also been associated with complications in pregnancy, such as neural tube defects, repeated miscarriages, abruptio placentae, fetal death, preeclampsia and intrauterine growth retardation.[14,15] Various studies show that homocysteine concentrations are physiologically lower in normal pregnancies.[16,17] As we have not encountered studies on homocysteine levels in pregnant women with hyperthyroidism, we studied if there is a difference between the serum homocysteine levels of women with thyrotoxic pregnancy and normal pregnancy.

MATERIAL AND METHODS

STUDY GROUP AND DESIGN

Twenty-two newly diagnosed thyrotoxic pregnant women (gravida 1, pregnancy weeks 12^{th}-16^{th}, average age 24.0 ± 2.8) and 20 healthy pregnant women (gravida 1, pregnancy weeks 12^{th}-16^{th}, average age 23.1 ± 3.0) were included in the study. Pregnant women with diabetes mellitus, impaired glucose tolerance, connective tissue disease, hypertension, coagulation disorder, atherosclerotic disease, smoking history, active infection and inflammation findings and nodules in thyroid ultrasonography larger than 1 cm were excluded. The diagnosis of GD was based on the clinical signs of hyperthyroidism combined with suppressed serum thyrotropin and positive thyrotropin receptor antibodies. Informed consent was obtained from all participants.

LABORATORY ANALYSIS

Venous blood samples were obtained after fasting overnight for 8-10 hours and a resting period of 20 minutes. After 30 minutes they were centrifuged for 10 minutes at 3000 g and were assayed after being separated. Homocysteine specimens were centrifuged at 4°C for 10 minutes at 3000 g and sera were stored at -20°C until assayed. We measured serum thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxin (FT4), serum vitamin B_{12} and serum folate levels by an immunoluminometric assay on a random-access analyzer (Architect i2000; Abbott Diagnostics Division). Biological principle of the procedure was chemiluminescent microparticle immunoassay (CMIA). Patients who had TSH levels lower than 0.35 μIU/mL and FT3 and FT4 levels above reference ranges (> 3.71 pg/mL and > 1.48 ng/dL; respectively) were accepted as having hyperthyroidism. TSH TRAb was studied with RADIM brand kit and radioimmunassay (RIA) method (normal < 10 IU/L). Serum albumin was analyzed on the Olympus-AU2700 with photometric method using original kits in biochemistry autoanalyser. Homocysteine assays were studied using Recipe Chemicals kit following HPLC method and utilizing Shimadzu brand HPLC device (reference ranges for homocysteine 5.5-17 μmol/L).

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS version 13.0 for Windows) was used for statistical analyses. Descriptive statistics are expressed as mean +/- standard deviation. Continuous variables were compared with Mann-Whitney U test. The chi-square test was used for categorical variables. p values of less than 0.05 were considered, statistically significant. Correlations between vari-

ables were tested using the Spearman rank correlation test.

RESULTS

There were no significant differences between the 2 groups with regards to age (24.0 ± 2.8 and 23.1 ± 3.0 in group 1 and 2 respectively) (p= 0.265). Serum albumin, folate and vitamin B_{12} levels were similar between pregnant women with thyrotoxicosis and the control group (Table 1). However, tHcy levels were significantly higher in the pregnant women with thyrotoxicosis (9.6 ± 2.9) than the control group (5.0 ± 1.9) (p< 0.001).

Statistically significant negative correlation was followed between TSH level and tHcy levels (r= -0.490, p= 0.01). Statistically significant correlation was found between FT3 level and tHcy level (r= 0.390, p= 0.01). However, no correlation was found between FT4 and tHcy level. Average TRAb level of the patients was 16.6 ± 3.2 IU/L (minimum 12.8 IU/L and maximum 23.4 IU/L). No correlation was found between TSH receptor antibody and tHcy level (Table 2).

DISCUSSION

Previous studies showed that homocysteine (tHcy) level decreased in pregnancy.[16,17] The mechanism responsible for this decrease in tHcy concentration is not known but increased use of metionin by fetus, increase in estrogen and cortisol hormones in pregnancy, decrease in albumin level was related to decreased B_{12} vitamin and folate level and physiological pregnancy hemodilution.[18,19] There are contradictory conclusions about the homocysteine levels of the hyper-hypothyroidy patients. Generally serum homocysteine levels were found normal or high in hypothyroidism and normal or low in hyperthyroidism.[6-10] In animal studies, however, serum homocysteine levels were high in hyperthyroidism.[20,21] The mechanism of the homocysteine increase in hypothyroid patients is not entirely clear, but studies suggest it may be related to decreased renal excretion of homocysteine due to decreased glomerular filtration rate or decreased activity enzymes involved in homocystein metabolism.[18,22] The mechanism of the increase in homocysteine levels in hyperthyroidism seems to be more complicated. Colleran et al. found in their study that there was an increase in homocysteine levels in thyrotoxic individuals with newly diagnosed Graves' disease.[23] It is known that Graves' disease may be accompanied by pernicious anemia as well as other autoimmune diseases. In this case, there may be absolute vitamin B_{12} deficiency and due homocysteine increase. However, in this study, none of the patients had low vitamin B_{12} level. Although there was no vitamin B_{12} deficiency in the

TABLE 1: Comparison of groups in terms of age and laboratory results.

	Thyrotoxic group (n= 22)	Control group (n= 20)	p
Age (years)	24.0 ± 2.8	23.1 ± 3.0	0.265[a]
FT3 (pg/mL)	3.92 ± 1.14	2.61 ± 0.38	< 0.001[b]
FT4 (ng/mL)	1.40 ± 0.56	1.25 ± 0.65	0.06[b]
TSH (µU/mL)	0.03 ± 0.02	1.89 ± 1.09	< 0.001[b]
Vitamin B_{12} (pg/mL)	209.5 ± 36.3	235.0 ± 86.9	0.595[b]
Folate (ng/mL)	8.8 ± 3.0	8.9 ± 3.8	0.792[b]
tHcy (µmol/mL)	9.6 ± 2.9	5.0 ± 1.9	< 0.001[b]
Albumin (g/L)	35.0 ± 4.6	36.2 ± 3.9	0.683[b]

TSH: thyroid-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; TR-Ab: anti-TSH receptor antibody; tHcy: total homocysteine

[a] Student's t-test, [b] Mann-Whitney U test.

TABLE 2: Correlation coefficients and significance levels between the age, thyroid functions and autoantibodies, vitamin B_{12}, folate and tHcy levels

	Total homocysteine	
Variables	rho	p
Age (years)	0.296	0.057
FT3 (pg/mL)	0.489	0.001
FT4 (ng/dL)	0.183	0.247
TSH (µIU/mL)	-0.605	< 0.001
TR-Ab (IU/L)	0.178	0.480
TPO-Ab (IU/L)	0.179	0.438
Tg-Ab (IU/L)	0.115	0.619
Folate (ng/mL)	0.054	0.732
Vit B_{12} (pg/mL)	0.043	0.785
Albumin (g/L)	0.151	0.296

TSH: thyroid stimulating hormone; FT3: free triiodothyronine; ft4: free thyroxin; TR-Ab: anti-TSH receptor antibody; TPO-Ab: anti-thyroperoxidase antibody ; Tg-Ab: anti-thyroglobulin antibody.

mentioned study, positive correlation was found with homocysteine levels in methylmalonic acid levels which is the marker of vitamin B_{12} deficiency.[23] Also in this study, which revealed similar results, although there was no absolute vitamin B_{12} deficiency, it was considered that potential functional vitamin B_{12} deficiency may be the cause for the increase in homocysisteine levels. It has also been stated that there may be a homocysteine increase in autoimmune diseases in line with inflammatory activation, as well as it can contrarily act as immune-stimulating molecule in homocysteine.[24] It was considered that another reason for high homocysteine in our patients with hyperthyroidism depending on Graves' disease can be the bi-directional relationship between autoimmune diseases and homocysteine.

There are numerous reports on experimental studies showing that FT4 and hyperthyroidism affect folate metabolism and the enzymes involved. The observations that methylenetetrahydrofolate reductase is increased in hyperthyroidism and decreased in hypothyroidism may be relevant for the relation between the tHcy level and thyroid status.[25] This enzyme is responsible for the formation of 5-methyltetrahydrofolate, which functions as methyl donor during remethylation of homocysteine to methionine.[26] During endemic goiter in humans, plasma tHcy increases, whereas most other amino acids except for methionine decrease.[22] Turkey is in endemic goiter region. Yet it is considered that the reason why pregnant women with Graves' disease have high homocystein levels than those without, lies not in endemic goiter but thyrotoxicosis, autoimmune or functional vit B_{12} deficiency.

In this study, serum tHcy levels were higher in thyrotoxic pregnant women than normal pregnant women. However sufficient information has not been achieved concerning the homocysteine in pregnant women with GD. As it is known that hyperhomocysteine causes neural tube defects, repeated miscarriages, abruptio placentae, fetal death, preeclampsia and intrauterine growth retardation in pregnant women. It should be borne in mind that homocysteine levels can be high in risky pregnancies accompanied by Graves' disease and can cause complications, as there is no clear data available so far on changes in homocysteine levels in pregnant women with Graves' disease.

In conclusion, there is a need for large scale studies comparing pre- and post-treatment homocysteine levels in hyperthyroid cases of pregnant and non-pregnant individuals.

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