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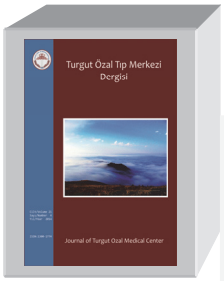

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The Effect of the Presence of Mother's Thyroid Autoantibodies on Intrauterine Fetal Death

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Absract
Aim: The aim of this study is to evaluate on the effects of mother's thyroid autoantibodies on intrauterin fetal death.
Meterial and Methods: A total of 200 pregnant women aged between 18 and 40 in their 6th-12th weeks of pregnancy were included in this study. The first group (study group) included 100 pregnant women who had previously experienced pregnancy loss in the second trimester in their previous pregnancies (termination of the pregnancy in the 13th-28th gestational weeks due to intrauterine fetal death). The second group (control group) also had 100 patients.
Results: There is no statistically significant difference between the two groups in terms of age, number of pregnancies, and miscarriages (p>0.05). The avarage serum level of anti-thyroid peroxidase in the study group was higher than the control group (p=0.009). But when anti-thyroglobulin and anticardiolipin IgG levels were evaluated, we have seen that there was no difference between the two groups (p>0.05). Moreover, the ratio of pregnant women who had positive anti-thyroid peroxidase was %3 in the control group and %11 in the study group. This was considered statistically high (p=0.027). The correlation between the presence of thyroid autoantibodies and antiphospholipid antibody positivity was statistically low.
Conclusion: Thyroid autoantibodies can be one of the autoimmune factors that are responsible for pregnancy loss. They show their effects by making a direct impact on the fetoplasental unit, causing thyroid malfunction, or creating an imbalance in the immune system of the mother. However, the role of thyroid autoantibodies on pregnancy losses are not clear and controlled studies are needed.
Key Words: Intrauterin Fetal Death; Thyroid Autoantibodies; Antiphospholipid Antibodies.

Annenin Tiroid Otoantikor Pozitifliğinin İntrauterin Fetal Ölüm Üzerine Etkisi

Özet
Amaç: Annenin tiroid otoantikor pozitifliğinin intrauterin fetal ölüm üzerine etkisinin araştırılması
Gereç ve Yöntemler: Çalışmaya 18-40 yaş arası 6-12. gebelik haftalarında 200 gebe dâhil edildi. En az bir kez 13 hafta ve üzeri intrauterin ölü fetus tanısıyla gebelik sonlandırması uygulanan hastalarla (çalışma grubu, n=100), en az 2 canlı doğumu olan ve ikinci trimester gebelik kaybı olmayan hastaların (kontrol grubu n=100) tiroid otoantikor titreleri ve antifosfolipid antikor titreleri karşılaştırıldı.
Bulgular: İki grup arasında yaş, gebelik sayısı, düşük sayısı açısından istatistiksel anlamlı fark saptanmadı (p > 0.05). Anti-tiroid peroksidaz serum düzeyleri ortalaması çalışma grubunda kontrol grubuna göre anlamlı yüksek bulunmuştur (p = 0.009). Anti-tiroglobulin ve antikardiyolipin IgG düzey ortalamaları karşılaştırıldığında iki grup arasında fark bulunmamıştır (p > 0.05). Kontrol grubunda anti - tiroid peroksidaz pozitif gebe sayısı %3 iken, çalışma grubunda %11 olup, bu farklılık istatistiksel olarak yüksek bulunmuştur (p = 0.027). İki grup arasında anti-tiroglobulin, antikardiyolipin Ig-G ve lupus antikoagulan pozitifliği açısından istatistiksel anlamlı fark bulunmamıştır (p > 0.05). Tiroid otoantikor pozitifliği ile antifosfolipid antikor pozitifliği arasında zayıf korelasyon saptanmıştır.
Sonuç: Tiroid otoantikorları gebelik kayıplarından sorumlu otoimmün faktörlerden biri olabilirler. Bu etkilerini direkt fetoplasental üniteyi etkileyerek veya tiroid fonksiyonlarını hafif derecede bozarak ve ya annenin immün sisteminde dengesizlik yaratarak yapabilirler. Ancak tiroid otoantikorlarının gebelik kayıplarındaki rolü ve patogenezi çok açık olmayıp geniş çapta çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: İntrauterin Fetal Ölüm; Tiroid Otoantikorları; Antifosfolipid Antikorlar.

INTRODUCTION

Genetical, anatomical, hormonal, infectious, environmental, and autoimmune factors have been implicated in the etiology of pregnancy loss. However, the causes of recurrent pregnancy loss (RPL) in 40-50% of couples have not been found (1, 2). When it is unable to determine the etiology of pregnancy loss, autoimmune factors are thought to be responsible for the loss. Pregnancy loss, in absence of symptomatic autoimmune diseases, has often been associated with possible presence of subclinical diseases and autoantibody positivity. Within this context, the most studied non-organ-specific autoantibodies are antiphospholipid antibodies (anti-TPO).

Significant changes occur in thyroid function during pregnancy. Thyroid dysfunction during pregnancy may affect the course of pregnancy, feto-maternal mortality and morbidity, and quality of life of the newborn. Lately, several studies have been published concerning the association between thyroid autoantibodies (TA), one of the organ-specific autoantibodies, and conditions such as recurrent miscarriage, fetal death, infertility, implantation failure, and preterm birth (3-8). However,

the affect of TA on intrauterine fetal death has not been a focus of study very often and therefore the number of publications on this issue is limited. As is known, chromosomal abnormalities are very rare in the second trimester pregnancy loss compared to first-trimester abortions. In addition, prognosis of pregnancy is even worse in women with previous pregnancy loss in the second trimester of their pregnancies (preterm delivery, stillbirth, neonatal death at high risk). The purpose of this study is to compare TA antiphospholipid antibody titers of intrauterine fetal death cases with the autoantibody titers of pregnancies without pregnancy loss and to determine the role of titers in pregnancy loss.

MATERIALS AND METHODS

Our study covers the period between May 2008 and May 2009. The study was conducted at Etlik Zubeyde Hanim Maternity Ward and Gynecology Training and Research Hospital after getting the ethics committee approval of the hospital. We have included 200 patients who were admitted to the clinic in their early 6th-12th gestational weeks. We have obtained consent from all our patients to conduct the study. We have created two groups of patients. The first group (study group) included 100 pregnant women who had previously experienced pregnancy loss in the second trimester in their previous pregnancies (termination of the pregnancy in the 13th-28th gestational weeks due to intrauterine fetal death). The second group (control group) also had 100 patients. We have included pregnancies that did not have prior pregnancy loss in the second trimester but a parity history of two live births. We have excluded those with hypertension, diabetes, systemic lupus erythematosus, antiphospholipid antibody syndrome, known systemic diseases like kidney or liver diseases, history of thrombophilia, drug use history for treatment hypothyroidism in previous pregnancies, overt hypothyroidism or hyperthyroidism diseases, and those with history of pregnancy loss accompanied by fetal anomalies. We have confirmed the intrauterine fetal deaths over 13 weeks by checking the information in the anamnesis records and ultrasound results. We examined the blood samples for routine biochemistry, thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3) at the biochemistry and nuclear medicine laboratories of our hospital. The thyroid functions were measured by using Roche Diagnostics Gmbh Germany kits and chemiluminescence method. TSH: 0.2-4.2 µIU/ml, fT4: 12-22 pM, and fT3: 1.0-6.8 pM were considered as normal values for thyroid functions. Anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg), and ACA IgG evaluations were done on an autoantibody analyser (ORGENTEC brand, Alegria model) using ELISA method by diluting the samples at a proportion rate of 1/100. The normal values for autoantibodies were anti-TPO: 0-75 IU/ml, anti-Tg: 0-150 IU/ml, and ACA Ig G: 0-10 IU/mL. LA was studied at the nuclear medicine laboratories of our hospital by using coagulometer with Amax Accuclot drWT screen kits. The normal values for LA is 31-44 seconds in our laboratory so over 44 seconds was considered positive.

Statistical analysis: The data obtained in this study were evaluated with SPSS 17 package software. The compliance of the distribution of data obtained to the normal distribution was examined by using the Kruskal Wallis test. The descriptive statistics were expressed in mean±standard deviation (SD) for continuous measurement variables, in median values (minimum-maximum) for discrete variables, and in the number of observations (%) for categorical variables. To see whether there were any statistically significant differences between the control and study groups in terms of features identified by measurements, we have used the Student's t test or Mann-Whitney U test. For the categorical comparisons we have made use of the Chi-square test. To test the presence of correlation, we have applied Spearman correlation analysis. $P<0.05$ value was considered statistically significant for all the results.

RESULTS

A total of 200 pregnant women were included in the study. The mean age of patients in the control group was 31.5±4.5 years and 30.6±5.2 years in the study group. We did not detect any statistically significant differences between the groups in terms of age, number of pregnancies (gravida), and abortion. The number of births (parity) in the control and the study groups were 2.3±0.6 and 1.4±1.0, respectively. Parity was considerably higher in the control group ($p<0.001$). The mean value of late pregnancy loss in the study group was 1.1±0.3. The demographic characteristics of the pregnant women included in the study are presented in Table 1.

The thyroid function test results of the pregnant women are shown in Table 2. Having compared the average TSH and fT3 levels, we have seen no statistically significant difference between the groups ($p>0.05$). However, the average fT4 levels were notably higher in the study group than the control group ($p=0.031$); the mean fT4 value was 15.4 pM in the control group while this value was 16.4 pM in the study group. The ratio of pregnant women with subclinical hypothyroidism was 11% in the control group, and 16% in the study group; the difference between the groups was not statistically significant ($p=0.301$).

Table 3 shows the statistical analysis of serum levels of TA and antiphospholipid antibodies. According to this table, the difference between the mean values of anti-TPO serum levels in the control group (13.5 IU/ml) and the study group (22.6 IU/ml) was statistically notable ($p=0.009$). However, the difference between the mean anti-Tg serum levels in the control group (37.4 IU/ml) and the study group (33 IU/ml) was not statistically significant ($p=0.589$). Again, there was no statistically significant difference ($p=0.165$) between the groups in terms of mean ACA IgG serum levels (3.6 IU/ml in the control group and 3.1IU/ml in the study group).

Table 1. Demographic characteristics of the patients

	Control Group (n=100)	Study Group (n=100)	p
Age (years)	31.5 ± 4.5	30.6 ± 5.2	0.285
Gravida	4.1 ± 1.2	3.0 ± 1.2	0.363
Parity	2.3 ± 0.6	1.4 ± 1.0	**<0.001***
Abortions	0.3 ± 0.6	0.5 ± 0.7	0.089
PL in later periods (13-28 weeks)	-	1.1 ± 0.3	

* P<0.05: Statistically significant difference

Table 2. Thyroid function test results

	Control Group (n=100)	Study Group (n=100)	p	Odds ratio (CI)
Free T3 (pM)	4.7 (1.0-7.7)	4.2 (1.2-7.8)	0.285	
Free T4 (pM)	15.4 (9.3-22.8)	16.4 (10.6-20.6)	0.031*	
TSH (µIU/ml)	2.6 (0.13-7.1)	2.1 (0.9-9.6)	0.30	
Subclinical hypothyroidism	11 (%11)	16 (%16)	0.301	1.54(0.68-3.51)

* P<0.05: Statistically significant difference

Table 3. Analysis of serum levels of TA and antiphospholipid antibodies

	Control Group (n=100)	Study Group (n=100)	p
Anti-TPO (IU/ml)	13.5(2.5-943)	22.6(1.6-1674)	0.009*
Anti –Tg (IU/ml)	37.4(11-278)	33(9.1.1239)	0.589
ACA IgG (IU/ml)	3.6(0.7-68.6)	3.1(1.0-113.8)	0.165

Abbreviations: Anti-TPO: antiphospholipid antibodies; **Anti Tg:** anti-thyroglobulin antibodies; **ACA:** anticardiolipin antibodies.
* P<0.05: Statistically significant difference

In one of the control group patients with subclinical hypothyroidism, we observed positive anti-TPO and anti-Tg together; meanwhile anti-TPO was positive in one of the cases while two patients had positive anti-Tg. Six of the study group patients with subclinical hypothyroidism had positive results both for anti-Tg and anti-TPO; three of these patients had only positive anti-TPO. The percentage of pregnant women with anti-TPO positive in the control group was 3%; this value was 11% in the study group, which is higher and, thus, statistically significant (p=0.027). The number of anti-Tg-positive pregnant women were 37% and 32% in the control and study groups respectively and there is no significant difference between the two groups. In terms of the number of pregnant women with positive ACA, there is no difference between the two groups either (p=0.059). The LA presence was indicated as positive-negative. LA was positive in three women in the control group; the same parameter was positive in seven pregnant women in the study group and this difference is not statistically significant (p=0.166) (Table 4).

Table 4. Positivity for thyroid antibodies and antiphospholipid antibodies

	Control Group (n=100)	Study Group (n=100)	p	Odds ratio (CI)
Anti-TPO	3%3	11%11	0.027*	4.0 (1.1-14.8)
Anti Tg	37%37	32%32	0.457	0.8 (0.5-1.4)
ACA Ig G	1%1	6%6	0.059	6.4 (0.8-53.5)
LA	3%3	7%7	0.166	2.4 (0.6-9.7)

Abbreviations: Anti-TPO: antiphospholipid antibodies; **Anti Tg:** anti-thyroglobulin antibodies; **ACA:** anticardiolipin antibodies; **LA**: lupus anticoagulants.
* P<0.05: Statistically significant difference

There was poor correlation between LA or ACA IgG positivity and anti-TPO positivity with a correlation coefficient of 0.261. Similarly, there was weak correlation between LA or ACA IgG positivity and anti-Tg positivity with a correlation coefficient of 0.171.

DISCUSSION

Autoimmune factors are thought to be responsible for the unidentified aetiologies in pregnancy loss. The bottom line of our study was the statistically significant relationship between anti-TPO antibody titers and the pregnancy losses due to intrauterine fetal death. However, we did not find a significant difference between the two groups in other autoantibody titers.

It was Stegnaro-Green et al.'s (on 552 pregnant women) and Lejeune et al.'s (on 363 pregnant women) studies in the 1990s that initiated the course of pregnancies in terms of TA in the first trimester; at the time, they concluded that pregnancy loss rate was higher in pregnant women with positive TA (5, 6). In 1991, Glino et al. showed that pregnancy loss was 4 times more in women with positive TA than those with negative TA (by

13.3% to 3.3%) (9). In 1993, Singh et al. evaluated 487 women who had received reproductive assistance and reported that pregnancy loss rate was 32% in the autoantibody-positive group while this rate was 16% in the antibody-negative group, which, they thought, was statistically revealing. As a result, they associated thyroid autoimmunity with pregnancy loss in the early stages of pregnancy (10). In an article published by Stagnaro-Green and Glinoer, it was suggested that euthyroid patients with positive autoantibodies who experienced pregnancy loss could be explained by the mild hypothyroidism spontaneously caused by antibodies during the loss as well as by the imbalance in the immune system (11). Mecacci et. al.'s 1999 article puts forward a comparative study of 69 women with a history of pregnancy loss and another 69 multigravida woman who never had negative experiences during labour. Comparing the patients in terms of antiphospholipid antibodies, the study showed that, similar to our own results, the TA positivity was three times higher in the RPL group and four times higher in the group with fetal death history. They also linked the obstetric complications with the presence of anti-TPO. The antiphospholipid antibody incidence was normal in TA positive women (12). Pratt, Busen, and Stecker's reports were consistent with these results as well (13, 14). In our study, the rate of patients with subclinical hypothyroidism was 11% in the control group and 16% in the study group; this difference was not considered statistically significant. Mecacci et al. also state that the TSH levels were higher in all three sub-groups of the study group with positive TA compared to the women with negative TA. However, they could not find any differences between the groups in terms of thyroid hormone levels (ft3 and fT4) regardless of TA. In the study at hand, we could not detect any significant differences with regards to fT3 and TSH levels although fT4 hormone levels were considerably higher in the study group.

Mecacci et al. have stated that TA brings about pregnancy loss either by interacting with placental hormones (human chorionic gonadotropin, hCG and human chorionic thyrotropin, hCT) or by changing maternal immunology through lymphocytes hyperactivity (12). Kutteh et al. have similarly reported that less than 20% of antithyroid antibody-positive pregnant women had hypothyroidism related to RPL and antibody positivity (7).

Several studies have been planned to determine polyclonal B cell activation and abnormal T cell function that cause pregnancy loss and infertility in TA positive women. In these studies, as it was the case in our study, there was but a poor correlation in the union of organ-specific antibodies (antiTPO and anti-Tg) and non-organ-specific antibodies. These studies have associated ACAs with pregnancy loss in later periods while antithyroid antibodies have been associated with pregnancy loss in the first trimester (13, 14). Negro et al.'s 2005 study puts forward the idea that anti-TPO positivity in euthyroid women increases the risk of miscarriage and preterm labour by reducing thyroid functions and that levothyroxine therapy can actually reduce such risks (15). Indeed, studies conducted in recent years have shown that TA may affect the formation and course of pregnancies particularly in infertile patients and patients who had spontaneous abortion (16, 17, 18).

As opposed to the studies that claim the strong link between RPL and TA, there are also studies that argue that such a de facto relationship does not exist (19, 20).

As a result, it can be stated that TA may be one of the responsible autoimmune factors in pregnancy loss. The consequences of TA may manifest themselves by directly influencing fetoplacental unit, by mildly disrupting thyroid functions, or by creating imbalance in mother's immune system. However, it should also be claimed that the real effect of TA on pregnancy loss or the pathogenesis of these effects are not clear for the time being. Therefore there is need for more and larger controlled studies to clarify this issue.

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