Original Article

Anti-Thyroid Peroxidase and Risk of Recurrent Spontaneous Abortion

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Abstract

Background: Approximately 2-4% of all women have recurrent spontaneous abortion (RSA); however, the cause is determined in only 50% of cases. Recent studies have shown an association between thyroid autoantibodies as a sign of thyroid autoimmunity and abortion. The aim of the present study was to determine whether circulating anti-thyroid peroxidase (anti-TPO) was associated with RSA.

Materials and Methods: In this observational analytic study, Sera from 58 non-pregnant women with a history of RSA and also 58 healthy, fertile subjects with at least one live birth as control (Aging from 18 to 45 years) were tested for thyroid peroxidase antibodies by means of a standard Anti-TPO ELISA kit. We used data collection forms and SPSS software for data analysis.

Results: Of 116 women, 8 (13.8%) of the control subjects and 12 (20.7%) of the women with a history of RSA had positive results for anti-TPO. There was not any significant association between presence of anti-TPO and RSA.

Conclusions: We did not find any correlation between the presence of TPO antibodies and abortion in women with a history of RSA. On the basis of this study, testing for anti-TPO doesn't seem to be useful in the evaluation of patients with a history of RSA.

Keywords: Anti-Thyroid Peroxidase, Recurrent Spontaneous Abortion.

Introduction

Miscarriage is a common occurrence and as many as 31% of pregnancies end in a miscarriage when sensitive human chorionic gonadotropin (HCG) assays are used, of which about one-third will be noticed by the mother (1). The diagnostic criterion of recurrent spontaneous abortion (RSA) is at least two consecutive pregnancies ending in spontaneous abortion which occurs in 2-4% of pregnant women (2). About 80% of spontaneous abortions occur in first trimester of pregnancy. Certain factors, such as uterine abnormalities, hormonal disturbances, chromosomal abnormalities, and infectious agents are considered to be the factors causing RSA (3). Nonetheless, 30-50% of the RSA occur in women with unrecognizable risk factors (2). The most frequent side effects of abortion are infectious (48%), bleeding (21%), embolism (11%) and psychic problems (3).

Much attention has focused on the immune system in RSA (4). It is determined that 15% of more than 1000 women with RSA showed recognized autoimmune factors (3). Also a significant association between thyroid antibodies and risk of abortion has been shown by various but not all studies (1, 2, 5). Autoimmune thyroid disorders are characterized by the presence of antithyroid antibodies, specifically antithyroid peroxidase (anti-TPO) and antithyroglobulin (anti-TG) (5).

Saguaro Green et al. found that the presence of TPO or TG antibodies in the first trimester of pregnancy is a risk factor for subsequent spontaneous pregnancy loss (6). Esplin et al., studied the association between thyroid autoantibodies and recurrent pregnancy loss, and found that the presence of these antibodies was not associated with pregnancy loss (7).

Potential theories that have been proposed for the spontaneous abortion in women who carry thyroid autoantibodies include:

1. There may exist a subtle degree of hypothyroidism.
2. Thyroid autoantibodies directly cause RSA.
3. Women with thyroid autoimmunity and subfertility become pregnant at older ages (often after 30 years), which by itself, may constitute

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an additional factor to explain the greater rate of spontaneous miscarriage.
4. The presence of thyroid antibodies represent an epiphenomenon that reflects an underlying overall autoimmune imbalance, resulting in more frequent rejection of the fetus (6). It is unclear, however, whether any relationship exists between thyroid autoantibodies and RSA. The object of our study was to determine whether anti-TPO is associated with RSA.

Materials and Methods

In this observational analytic study, case patients with RSA and fertile control subjects (Age ranged from 18 to 45 years) were enrolled at the Royan research center and Akbarabady hospital, Iran university of medical sciences in Tehran, Iran. The study group included 58 non-pregnant women in reproductive age with unexplained first trimester RSA (≥2 consecutive pregnancy losses & ≤1 previous live birth) who were referred to the Royan research center for evaluation. The study was approved by Royan ethics committee, and all subjects gave informed consent at initial presentation. All women had an evaluation including history and physical examination, hysterosalpingogram or hysteroscopy, paternal karyotype analysis, luteal phase progesterone determination and other hormonal evaluation (Prolactin, Estradiol, LH, FSH, Testosterone and DHEAs). Clinical criterion for exclusion was known thyroid disease in the subjects.

The control population also consisted of 58 non-pregnant, reproductive aged women with one previous live birth without a history of reproductive problems.
Patients answered a questionnaire regarding previous thyroid illness, family history and related symptoms. Physical examination was performed to look for the presence of goiter and /or thyroid nodules.

Laboratory evaluation
Blood samples were drawn from all women at the initial visit and allowed to clot. Sera were separated by centrifugation and stored at a temperature of -20°C degrees until assayed for determining the presence of Anti-TPO. The samples underwent only one freeze thaw cycle.
All serum samples were evaluated for the presence of anti-TPO using AccuBind™ ELISA Microwells kit (MONOBIND, INC. Costa Mesa, CA 92627, and USA). Samples were diluted according to the manufacturer's instruction and assayed in duplicates. Diluted serum samples and standards were added 25µl per well and 100µl of TPO-Biotinylated conjugate solution, consequently and then plates were incubated at room temperature for 60 minutes. Then plates washed 3 times by Wash Buffer and 100µl of Enzyme-Anti-h-IgG conjugate solution were added to each well and plate incubated again at room temperature for 30 minutes. After 3 times washing, 100µl of working substrate TMB solution were added to every wells and plates and incubated for 15 minutes at room temperature in darkness. At the final stage, the colorimetric reaction stopped by addition of 50 µl of Stop Solution (1N HCl) per well and the optical density of each well was measured at 450/630 nm, using a micro plate reader. A positive assay test was defined as ≥40 U/ml for anti-TPO.

Statistical analysis
Statistical analysis was performed by means of SPSS software program. Individual characteristics of both groups were tested using χ^2, Mann Whitney and student t-tests accordingly. Laboratory results were compared between 2 groups by means of χ^2 analysis. P values of <0/05 were considered statistically significant.

Results

Patients' age ranged from 18 to 45 years (mean ± S.D; 32.65±6.4) with 33.6% (39 of 116) being older than 35 years (Table 1).

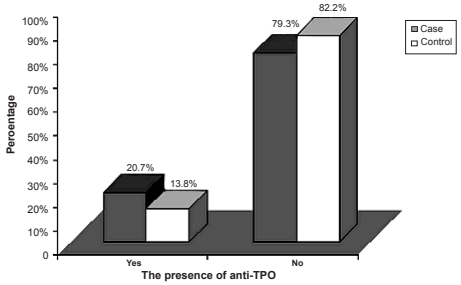

Fig 1: The percentage of anti-TPO in RSA patients and normal controls

The mean age in the recurrent abortion group was less than control population [32.05±6 years versus 33.24±6.8], However this difference was not statistically significant. As expected, the history of one live birth was greater in the control group [15/5% and 100% respectively].
At the present study we did not find significant association between anti-TPO level and RSA. Of 116 women, 20 (17/2%) were TPO ab positive. Anti-TPO were found in 12 (20.7%) of women with RSA and 8 (13.8%) of control group (Table and Figure 1). There was no significant association between age with RSA and presence of anti-TPO.

Table 1: Risk of miscarriage according to age and anti-TPO

	Women who miscarried	Control group	
characteristic	N (%)	N (%)	P. value
Age (year)			
<20	1 (1/7)	1 (1/7)	
20-35	41 (70/7)	34 (58/6)	NS
>35	16 (27/6)	23 (39/7)	
Anti-TPO			
Yes	12 (20/7)	8 (13/8)	NS
No	46 (79/3)	50 (86/2)	

NS=not significant

Discussion

By studying patients admitted in Royan research center, we determined the levels of TPO antibodies before pregnancy in women with a history of RSA. In accordance to some other studies, our study showed that there was no significant relationship between the presence of anti-TPO and RSA (20.7% in women with RSA and 13.8% in the control population).

RSA, defined as≥2 consecutive spontaneous abortions, affects approximately 2-4% of women (2). The etiology of RSA is determined in 50% of the cases that may include genetic anomalies, hormonal abnormalities and uterine factors (3). Anticardiolipin antibodies in women with or without systemic lupus erythematous have also been associated with RSA. Although the pathogenesis of this association remains unclear, treatment during pregnancy with heparin, low dose aspirin, corticosteroids, or a combination has been documented to decrease the abortion rate (6).

The result of our study was concordant with some other studies in this field (8, 10). In the study by Esplin et al, no association was found between the presence of thyroid antibodies and miscarriage (7). Muller et al. studied 173 non-pregnant women and confirmed these findings (8). Reznikoff-Etievant et al. in Paris evaluated 678 women with RSA for the presence of antiphospholipid and antithyroid antibodies, but only 2.9% of the women were thyroid autoantibody positive (9). Rushworth and Backos conclude that the future risk of pregnancy loss in women with unexplained RSA is not affected by their thyroid antibody status (10).

There are two working hypothesis concerning the pathophysiological roles of TG and TPO antibodies in pregnancy loss: 1) Biochemical interaction between hormones and elevated thyroid antibodies may directly result in pregnancy loss, because thyroid function is normal in many patients when thyroid antibodies are detected. 2) Other investigators consider TG and TPO antibodies to be secondary markers of autoimmune disease rather than the actual cause of pregnancy loss (11). Alternatively, the presence of thyroid antibodies may be a consequence of the failed pregnancy or may reflect a separate primary problem with the pregnancy (7). Antithyroid antibodies are known to occur in normal, healthy population, and these autoantibodies are five times more common in women than in men. Because of prominent prevalence of antithyroid antibodies in normal women, interpreting the significance of these antibodies in women with reproductive problems remains difficult (5). It is also suggested that the presence of thyroid autoantibodies reflects a generalized activation of the immune system particularly of T cells, which are ultimately responsible for the loss of the pregnancy (7) However, the matter remains undecided, as other studies with statistically significant results showed no influence of autoimmunity (11). According to the findings of Vaquero et al. patients with thyroid antibodies treated with thyroid replacement therapy, had a lower rate of RSA, compared with those treated with IVIG (2). These findings suggest that miscarriage in such patients is related to a thyroid dysfunction, rather than a generalized over reaction of the immune system.

Our study did not reflect the results of other researchers, as though most of them in their studies found a relationship between autoantibodies and abortion. Dendrinos et al. showed the incidence of thyroid antibodies significantly more in women with RSA than age matched controls (12). Maria et al. in their studies found that anti-TPO antibody is the most common antibody in patients with the history of habitual abortion (p=0.01) (13). Carolis et al. realized that patients with anti-thyroid antibodies (regardless of antibody type), in addition to reduced fecundity had lower percentage of successful pregnancies compared to the patients with antiphospholipid antibodies alone (p=0.003) (14).

In the most studies for determining the relationship between autoantibodies and miscarriage, both of anti-TPO and anti-TG antibodies have been measured (2, 5, 6, 14). As in the study of Carolis et al, 5.9% of patients had only anti-TPO, 3.9% only anti-TG and 16.7% had both anti-TPO and anti-TG (14). In another study on 700 non-pregnant women with RSA, 6.6% had positive anti-TPO, and anti-TG were

detected in 7.7% of them, meanwhile 8.3% had positive results for both the anti-TPO and anti-TG antibodies (5). Since the aim of our study was to determine the association between anti-TPO and RSA, the difference in aims might be affecting our results.

Muller et al. by setting the TPO cut-off value at 80 U/m, did not find significant relationship between the presence of TPO antibodies and subsequent miscarriage. They recalculated this correlation using a cut-off value at 100 U/ml; again no correlation was found (8). Siero Netto et al used cut-of value>40 U/ml to report positive anti-TPO in their research and found a correlation between thyroid autoantibodies and miscarriage (11). We also used cut-off value>40 U/ml in our study to detect the high level of anti-TPO. This means that the negative findings of our study do not relate to our cut-off value selection.

William et al. indicated an increased prevalence of antithyroid antibodies in women with RSA compared with controls [22.5% and 14.5% respectively](5). In our study, the percentage of positive anti-TPO in RSA group was 20.7 and 13.8 in control population. As it is realized, the percentage of antibodies in our study is similar to William's research. But our results are in contrast to theirs. The cause might be related to small sample size of our study.

The possibility of geographic differences also can be an explanation for our negative results as compared with some other researches.

In some studies, an increasing risk of RSA, with increasing maternal age has been observed (1, 4). However in our study, 33.6% of patients were older than 35, but there was no association between age and RSA.

In the present study, we also did not find any correlation between age and presence of anti-TPO. Some, but not all studies have revealed an increase in the prevalence of autoantibodies with age. In the study of William, most of the patients with RSA demonstrated an elevated autoantibody titer as age increased up to the age range of 31-35(5). Kontiainen et al. found an increase in the amount of anti-TPO with age. However, this correlation was not statistically significant (15). It is postulated that autoimmunity could be related to older aged pregnancies because of its association with infertility.

Conclusion

No association was found between the presence of anti-TPO and miscarriage in women with a history of RSA. On the basis of this study, testing for anti-TPO doesn't seem to be useful in the evaluation of patients with a history of RSA.

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