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RESEARCH ARTICLE

Evaluation of Anti Thyroid Antibodies Among Iraqi Women Suffering From Recurrent Abortion

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Abstract

Autoimmunity has been directly associated in the etiology of several reproductive conditions including unexplained infertility and recurrent abortion. This study focused on anti-thyroid autoantibodies and thyroid hormones as immunological and hormonal causative factors for recurrent abortion. The study was case control study included one hundred women 20-30 years old who history of recurrent abortion during the first trimester as cases , and 100 healthy multigravida without any pervious abortion as a control group. They were selected in the third trimester of pregnancy in the Al-Yarmouk teaching Hospital, Baghdad, from September 2013 to March 2014.For all women in the two groups serum concentration for anti thyroglobulin antibodies(TG-abs),anti thyroid peroxidase antibodies(TPO-abs),thyroid function tests in addition to antibodies to Toxoplasma ,Cytomegalovirus,Cardolipin and Phospholipid antibodies were evaluated using ELISA kits. Results revealed that there was a statistically significant relation between recurrent abortion and anti TPO autoantibodies (X2= 10.039, *P*= 0.002), where the majority of positive results were in cases as follow: 26(13%)of cases were positive for TPO antibodies compared to 6(3.0%) of control group. Also there was a statistically significant relation between recurrent abortion and anti TG autoantibodies (X2= 11.317, *P*= 0.002), where the majority of positive results were in cases as follow: 20(10%) of cases were positive for TG antibodies compared to 4(2%) of control group. The results showed statistically significant relationship between anti-TPO Abs and anti-TG Abs with TSH level (X2=9.968, P=0.002, and X2=5.977, P= 0.032, respectively). Free T4 levels were normal in the two groups. On other hand, there was no statistically significant relationship between thyroid antibodies and Toxoplasma, CMV, Cardiolipin and phospholipid antibodies among women in Baghdad city . We concluded that recurrent abortion has a statistically significant relation with antithyroid autoantibodies (TG-abs and TPO-abs), where 32% of cases were positive for one or both (anti TPO antibodies and/or anti TG antibodies), while the positivity in control where 9.0%.

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Introduction

Recurrent abortion is defined as the occurrence of three or more consecutive spontaneous abortions prior to 20th week of gestation, it occurs in 0.5 to 1 % (**1**).The cause of recurrent abortion is unexplained in up to 60% of studied couples (**2**).Because the acceptance of the fetal allograft within the uterus remains one of the major physiologic

response to pregnancy, the immune system has long been thought to be responsible for many miscarriages (**3**). In particular, the Lupus anticoagulant has been associated with an increased abortion rate, even in women who did not present with systemic lupus erythematosus (**4**).Furthermore, recent studies have shown the presence of numerous autoantibodies in women who are habitual aborters (**5,6**). The relationship of autoimmune thyroid disease to pregnancy has been the object of considerable interest with the recognition of the postpartum thyroid disease syndrome (**7,8**). Thyroid autoantibodies have been shown to be a useful markers in predicting women at risk for clinical miscarriage (**9,10**).

Thyroid peroxidase (TPO) is a glycoprotein with molecular weight of 100-107KD present on the thyroid cell surface and is important antigenic target in autoimmune thyroid disease. It is responsible for the Iodination of tyrosine residues on thyroglobulin (Tg) and the intramolecular coupling reaction of Iodinated tyrosine leading to the formation of thyroxine (T4) and triiodothyronine (T3).Thyroglobulin (Tg) is a large glycoprotein dimer (each subunit is 330KD) which is synthesized and secreted by the cell membrane. It is Iodinated on tyrosine residues by thyroid peroxidase. Basal production of thyroid hormones results from pinocytosis of Iodinated Tg and hydrolysis by lysosomal enzymes. Post-translation modifications, together with the degree of TG Iodination, are important determinate of the immunogenicity of the TG molecule (**10**).The aim of this work is to test the value of measuring thyroid antibodies,Tg-Ab, and TPO-A, as predictor of abortion in those women with history of recurrent unexplained recurrent abortion.

Materials and methods

In this case control study, 100 women aged 20-30 years, who referred to al-Yarmouk teaching hospital or the clinic affiliated to al-Mustansiryia University of Medical college were selected. Out of 200 women, 100 who had history of unexplained recurrent (>=3 recurrent abortion) abortion during the first trimester selected as the cases women, and 100 pregnant women with gestational age less than 20 weeks were include as the controls. And according to age, body mass index, gestational age and the number of pregnancy, were matched with case group. Patients with non-spontaneous abortion and patients treated with thyroid medication (levothyroxine, Methimazole and propylthiouracil), according to information had obtained from the questionnaires and tests, were excluded from study. After completion of the questionnaires and consent by the patient, on the day of abortion, from each sample, 5 ml blood was taken for measurement of thyroid function test (T3,T4,TSH,FT3 and FT4 concentrations according to experimental kit .In order to assess anti-thyroid antibodies level, blood samples were taken from each participant Anti-thyroid peroxidase antibody (Anti-TPO-Ab) >100 IU/ml and anti-thyroglobulin antibody (Anti-TG-Abs) >150 IU/ml were defined as Positive. Data expressed as mean ±SD were analyzed using Chi square test, Student t-test and Fisher's exact test when appropriate. Sensitivity, specificity, positive predictive value and negative predictive value were used for evaluation of thyroid autoantibodies positivity as predictors of abortion .

Results and Discussion

Serum anti-TPO Abs and anti -TG Abs levels

The analysis of the results indicated that 32 (16.0%) of the study population were positive for anti TPO antibodies while 168 (84.0%) were negative for anti TPO antibodies. Women with positive anti TPO antibodies were distributed as follows: 26(13 %) were cases and 6 (3%) were controls. In contrast, women with negative anti TPO antibodies were 74 (37 %) cases and 94 (47 %) controls (Table 1).On the other hand, Table 2 showed that 24 (12.0%) of the study population were positive for anti TG antibodies while 176 (88.0%) were negative for anti TG antibodies. Women with positive anti TG antibodies were distributed as follows: 20 (10 %) were in cases and 4 (2%) were in control group. In contrast, women with negative anti TG antibodies were 80 (40%) cases and 96 (48%) controls.

Table (1): Distribution of anti TPO antibody among recurrent abortion cases and control .

Anti TPO Abs	Positive *	Negative
Abortion cases	26(13 %)	74 (37%)
Control	6 (3%)	94 (47%)
Total	32 (16%)	168 (84%)

*Positive: TPO level >100 lU /ml .Negative: TPO level ≤ 100 lU /ml

Table (2): Distribution of anti TG antibody among recurrent abortion cases and control.

Anti TG Ab	Positive*	Negative
Abortion case	20 (10%)	80 (40%)
Control	4(2 %)	96 (48%)
Total	24(12 %)	176(88 %)

*Positive : TG Abs level > 150 lU /ml. Negative : TG Abs level ≤ 150 lU/ml.

Relation between anti-TPO Abs and anti-TG Abs

Results revealed statistically significant relation between anti-TPO Abs and anti-TG Abs for both cases and control group, where X2=17.497, P= 0.002 in cases and X2=22.72 ,P= 0.002 in control group. The percentage of cases with positive anti-TPO Abs and anti-TG Abs was 14.0%, while the percentage of those of negative anti-TPO Abs and anti-TG Abs was 68.0% (Table 3). The percentage of cases with positive anti-TPO Abs and negative anti-TG Abs was 12.0%, while the percentage of those with negative anti-TPO Abs and positive anti-TG Abs was 6.0%. This means that 32.0% of cases were positive with one or both of anti-TPO Abs and/or anti-TG Abs, while 68.0% were negative for these Abs. On the other hand the percentage of controls with positive anti-TPO Abs and anti-TG Abs was 2.0%, while the percentage of those of negative anti-TPO Abs and anti-TG Abs was 92.0%. The percentage of controls with positive anti-TPO Abs and negative anti-TG Abs was 4.0%, while the percentage of those with negative anti-TPO Abs and positive anti-TG Abs was 2.0%. This means that 8.0% of controls were positive with one or both of anti- TPO Abs and/or anti-TG Abs.

Table (3) :Relation between anti TPO Abs and anti TG Abs

Recurrent abortion cases	No. of negative TG Abs (%)	No. of positive TG Abs (%)	Total (%)	P value
Positive TPO	12	14	26	0.002
Negative TPO	68	6	74	
Total	80	20	100	
Control				
Positive TPO	4	2	6	0.002
Negative TPO	92	2	94	
Total	96	4	100	

Relationship between anti-TG Abs and Abs to phospholipid among cases

Results also showed a significant relation between anti-TG Abs and Abs to phospholipid (X2= 13.61 *P*< 0.002) as shown in (Table 4), where 16 (16.0%) of cases were positive for both anti-TG Abs and Abs to Cardiolipin, and 4 (4.0%) of them were positive for anti-TG Abs and negative for Abs to phospholipid.

Table 4 :Relationship between anti-TG Abs and Abs to phospholipid among cases

Variable	No .of Positive TG Abs (%)	No . of Negative TG Abs (%)	**P value**
Positive phospholipids. Ab	16 (16)	6(6)	P<0.002
Negative phospholipids Ab	4(4)	74(74)	
Total	20(20)	80(80)	

Relationship between anti-TG Abs and Abs to Cardiolipin among cases

Results also showed no significant relation between anti-TG Abs and Abs to phospholipids (X2= 1.622 *P*= 0.212) as shown in (Table 5), where 5 (5.0%) of cases were positive for both anti-TG Abs and Abs to Cardiolipin, and 15 (15.0%) of them were positive for anti-TG Abs and negative for Abs to Cardiolipin.

Table 5 :Relationship between anti-TG Abs and Abs to Cardiolipin among cases

Variable	No .of Positive TG Abs (%)	No . of Negative TG Abs (%)	P value
Positive Cardiolipin Ab	5 (5)	10(10)	NS P>0.24
Negative Cardiolipin Ab	15(15)	70(70)	
Total	20(20)	80(80)	

Relationship between anti-TPO Abs and Abs to Cardiolipin IgM among cases

Results revealed that there was no statistically significant relation between anti-TPO Abs and Abs to Cardiolipin (X2= 1.064, *P*= 0.314) as shown in (Table 6), where 5 (5 %) were positive for both anti-TPO Abs and Abs to Cardiolipin, and 21 (21%) were positive for anti-TPO Abs and negative for Abs to Cardiolipin.

Table 6:Relationship between anti-TPO Abs and Abs to Cardiolipin IgM among cases

Variable	No .of Positive TPO ABS (%)	No .of Negative TPO ABS (%)	P value
Positive Cardiolipin Ab	5 (5)	10(10)	0.312
Negative Cardiolipin Ab	21(21)	64(64)	
Total	26(26)	74(74)	

Relationship between anti-TPO Abs and Abs to phospholipid among cases)

Results revealed that there was a statistically significant relation between anti-TPO Abs and Abs to phospholipids (X2= 16.32, *P*< 0.002) as shown in (Table 7), where 18(18%) were positive for both anti-TPO Abs and Abs to phospholipid, and 8 (8%) were positive for anti-TPO Abs and negative for Abs to phospholipid

Table 7: Relationship between anti-TG Abs and Abs to phospholipid among cases

Variable	No .of Positive TPO Abs (%)	No . of Negative TPO Abs (%)	P value
Positive phospholipids. Ab	18 (18)	4(4)	0.002
Negative phospholipids Ab	8(8)	76(76)	
Total	26(26)	80(80)	

Comparison between Anti TPO Abs levels and Anti TG Abs levels Comparison between Anti TPO Abs levels among cases and controls

Table 8 shows the levels of serum Anti TPO Abs in both cases and controls. Anti TPO Abs levels of cases were higher than that of controls (128.5±86.8 vs. 64.1 ± 37.1, % difference= 133.9, **t** =3.46, **P**=0.001). These changes were statistically significant

Comparison between Anti TG Abs levels among cases and controls

As depicted from (Table 9), Anti TG Abs levels of cases were higher than that of controls (113.9±74.1vs.44.8± 31.3, % difference=136.7, **t**=3.5, **P**=0.001).

Table 8: The levels of serum Anti TPO Abs among cases (n=100) and controls (n=100)

Anti- TPO levels (IU/ml)	Mean ±SD	Range		% difference	T-test	P -value
		Min.	**Max.**			
Abortion cases	**128.5±86.8**	**6**	**644**	**134.6**	**3.5**	**0.001**
Controls	**86.8± 28.5**	**4**	**442**			

Table 9: The levels of serum Anti TG Abs among cases (n=100) and controls (n=100)

Anti- TG levels (IU/ml)	Mean ±SD	Range		% difference	T-test	P -value
		Min	**Max**			
Abortion cases	**113.9± 74.1 •**	**6**	**532**	**138.4**	**3.48**	**0.001**
Controls	**56.8± 38.5**	**5**	**236**			

Iraqi women with a history of RA were compared to multigravida women without any abortion (control) in the Baghdad city. The study showed that the majority of positive results for both anti TPO antibodies and anti TG antibodies were in RA cases. We found that 32.0% of cases (n=100) were positive for one or both anti TPO antibodies and/or anti TG antibodies. On the other hand, 8.0% of control group (n=100) were positive for one or both anti TPO antibodies and/or anti TG antibodies. There was significant relationship between the presence of anti thyroid antibodies anti TPO antibodies and anti TG antibodies and RA, where (X2=10.039, *P*= 0.002) for anti TPO autoantibodies. There was a statistically significant relation between recurrent abortion and anti TPO autoantibodies, as well as there was a statistically significant relation between recurrent abortion and anti TG autoantibodies (X2= 11.317, *P*= 0.001).

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 **(11).** 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 **(12).** In the most studies for determining the relationship between autoantibodies and miscarriage, both of anti-TPO and anti-TG antibodies have been measured, in addition to TSH level and FT4 level. Our results were in agreement with other researchers who found a relationship between thyroid autoantibodies and abortion**(3,9).**so an association between thyroid autoantibodies and increased risk of spontaneous abortion can be suggested. The higher the levels of antithyroid autoantibodies the higher the abortion rate. (table 1) This association have been found to be highly significant in present study p<0.001.These findings are in agreement with previous reports who suggested such assumption (**13-18**). Although Esplin et al (**19**) showed that no difference in positivity for antithyroid antibodies in non pregnant women with and without history of recurrent abortion. Wilson et al (**20**) showed that thyroid peroxidase titers and avidity was significantly higher in women with recurrent abortion who later miscarried compared to women with recurrent abortion whose pregnancies continued and showed that the titer and avidity declined in those who continue to term. Also Roberts et al (**21**)showed that women with recurrent miscarriage had a higher incidence of thyroid antibodies suggesting an association between autoimmunity and recurrent miscarriage. Several mechanisms have been suggested to explain the role of thyroid autoantibodies in recurrent abortion.

Anti-Cardiolipin (ACA) is probably the most classicall anti phospholipids (APL), as it is used for the definition of APS. It is highly associated with recurrent abortion (**22**). Previous studies have subsequently confirmed the adverse effects of ACA on pregnancy with the experimental mouse model. The experimental induction of APA causes the

increased resumption rate and at the same time decreases placental and embryo weight in pregnant mice (**23**).). In our study we found that 15% of the cases were positive for antibodies to Cardiolipin. Our results showed that there is no relationship between anti TPO autoantibodies and abs to Cardiolipin (P=0.302). Also, we had not found relationship between anti TG autoantibodies and abs to Cardiolipin (P=0.203). It was reported that APA was found in 10-15 % of women with fetal death (**4**).

Although APA may be a risk factor for pregnancy losses (**24**). An association of APA with recurrent idiopathic pregnancy losses was reported and fetal death is associated with APA(**25**). our results showed that there was no significant difference in the distribution of (TG-abs and TPO-abs) in women who had suffered from recurrent pregnancy loss and positivity of abs. to Cardiolipin. In other prospective studies, investigators did not find any relation between APA and fetal growth restriction (26).Kdous and his colleagues reported that the exact mechanism of APA remains controversial (27).from above results the results revealed that there is an association between abortion and presence of antithyroid antibodies in mother's serum.

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