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Association between antithyroid peroxidase antibody and recurrent miscarriage

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Abstract. – OBJECTIVE: Thyroid disease is the second most commonly affected disease in childbearing women, after diabetes, and thyroid autoimmunity in pregnancy has been connected with adverse pregnancy outcomes such as miscarriage, recurrent miscarriage, preterm birth, and low IQ. The study seeks to determine the correlation between anti-thyroid peroxidase antibodies and unexplained recurrent miscarriage.

PATIENTS AND METHODS: 124 women were included in this case-control study, divided into 62 women who have experienced unexplained recurrent miscarriages and 62 healthy women without a history of miscarriage. Screening for TSH and anti-TPO antibody were done for both groups.

RESULTS: The prevalence rate of positive anti-TPO antibody in women with recurrent miscarriage was 19.4%, while in women without miscarriage was 6.5% (which is considerably higher in cases than in women without recurrent miscarriage with a p-value of 0.03 and an odd ratio of 3.48 (95% CI; 1.06-11.48).

CONCLUSIONS: A statistically significant relationship between anti-TPO antibodies and recurrent miscarriage has been detected. We recommend screening for TSH and thyroid antibodies for women with recurrent miscarriages and further studies on the effect of levothyroxine therapy for euthyroid women with antibody positive.

Key Words:
Recurrent miscarriage, Auto-immune thyroid disease (AITD), Anti-thyroid peroxidase antibody (anti-TPO antibody).

Introduction

Miscarriage is the loss of a pregnancy that can occur from the onset of conception until 24 weeks of gestation. When a miscarriage happens consecutively three or more times, it is called a recurrent miscarriage. It is worth mentioning that 1% of couples suffer from recurrent miscarriages[1]. Studies have shown that various factors contribute to recurrent miscarriage, such as age, smoking, and obesity that are categorized as epidemiological reasons, antiphospholipid syndrome, genetic factors, anatomical aspects like a congenital uterine anomaly and cervical weakness, inherited thrombophilic factor, endocrine diseases like diabetes mellitus and thyroid disease[1]. However, the majority of miscarriage causes are not diagnosed. 50% or more of recurrent miscarriages occur for no apparent reason. To diagnose the cause of recurrent miscarriage in those couples, tests for parental karyotypes, hysterosalpingography or hysteroscopy, and antiphospholipid antibody are done; nevertheless, even when all of the tests are negative, the miscarriage still happens[2].

Scholars[3] concluded that thyroid peroxidase antibody is positive in women who have normal thyroid hormone levels and unexplained recurrent miscarriages. After diabetes, thyroid disorder is the second most commonly diagnosed endocrine condition in childbearing women[3]. The most frequent cause of thyroid disease is thyroid autoimmunity, which is identified as the existence of anti-thyroid antibodies, precisely thyroid peroxidase antibodies and/or thyroglobulin antibodies[4]. The prevalence of thyroid autoimmunity causes negative pregnancy outcomes such as miscarriage, recurrent miscarriages, preterm birth, and low IQ[5]. Thyroid antibodies can be found in the serum of euthyroid women in about 5-20%, and in women with a history of recurrent miscarriage (17-33%)[6].

Thyroid peroxidase is an enzyme that is crucial for iodinating tyrosine residues to form thyroid hormones. Thyroglobulin is a glycoprotein that contains these tyrosine residues. It is generated by thyroid cells and stored in the thyroid colloid[7].

The mechanism underlying the relationship between recurrent miscarriage and anti-thyroid peroxidase antibody is still not precisely detected. However, there are two hypotheses that explain this

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association. The first hypothesis states that thyroid peroxidase antibody simply reflects a different level of autoimmune process that causes subfertility or pregnancy loss, while the second hypothesis states that the association with miscarriage is attributable to a subtle thyroid hormone deficiency[8].

Euthyroid individuals with a serum TSH level higher than 3 ml U/L have higher TPO-Ab titers and also a higher rate of changing to overt hypothyroidism in the future, in spite of the fact that they have normal TSH[9]. Thyroxine therapy might be beneficial in preventing miscarriage and increasing the term delivery rate for women with a history of unexplained recurrent miscarriage and anti-thyroid peroxidase antibody positive before and during conception[10]. In contrast to this, other studies[11-13] have found that thyroxine treatment is ineffective in women with a history of unexplained recurrent miscarriage and normal thyroid function test with thyroid antibody positive.

The current study was therefore carried out in order to determine the correlation between anti-thyroid peroxidase antibodies and unexplained recurrent miscarriages.

Patients and Methods

Study Sample

This case-control study included 120 participants, calculated depending on the prevalence of recurrent miscarriage among reproductive-age women who relied on previous studies[14]. Participants were divided into two groups: the first group of 60 cases included women who had had three or more consecutive miscarriages before 22 weeks of gestation with or without live birth; the second group (as control) of 60 healthy fertile multigravida women who had had three or more term live birth without complications (preterm labor, premature rupture membrane, stillbirth, preeclampsia, gestational diabetes, any chronic disease) and a history of miscarriage.

Ethical Consideration

Data collection started after approval of the research protocol by the Research Protocol Ethics Committee, Kurdistan Board of Medical Specialties, Ministry of Higher Education and Scientific Research, Kurdistan Region Government, Iraq, with approval no. 1182 (11.9.2021 until 10.6.2022) in the Outpatient and Emergency Department of Sulaimani Maternity Teaching Hospital and private clinics in Sulaimani City, Kurdistan Region, Iraq.

120 women participated in the research after an investigation; nevertheless, 7 participants in the case group and 6 participants in the control group were excluded from the study since they had TSH > 4.27 and were diagnosed with overt hypothyroidism.

Women with a history of a uterine anomaly, fibroid, cervical weakness, autoimmune diseases like antiphospholipid syndrome, overt thyroid disease (defined as TSH <0.27 OR >4.27), any chronic disease like hypertension, diabetes, or any other endocrine disease like PCOS and hyperprolactinemia either physiological or medical hyperprolactinemia and chromosomal abnormality of both parents were also excluded.

Data Collection Procedure

Samples were collected from 120 women who volunteered to participate in the study. Their consent was obtained verbally and they were also informed about the right to refuse participation.

After demographic data and BMI full history were taken from participants, gravity, parity, and the number of miscarriages in the first or second trimester, gynecological history, past surgical history, drug history, and their health record laboratory investigation of cases with recurrent miscarriage were analyzed. Clinical examination was performed to exclude anyone with a history of medical illness, an abnormal BMI, or any other exclusion criteria. All data were entered into the questionnaire. After agreement from participants, 1 cc peripheral blood samples were collected from all women, and the serum was separated by centrifuge and kept in a low-temperature freezer at -36°C for sample protection until the day which is taken for a lab to investigate for titer of anti-thyroid peroxidase antibody and thyroid-stimulating hormone in serum of cases and control women, which are done in Smart Medical Building/Laboratory Department. TSH and Anti-thyroid peroxidase antibody titers were measured by Elecsys TSH kits and Elecsys Anti-TPO from Roche diagnostic kits (Munich, Germany), using electrochemiluminescence (ECL) (Cobas 6000.e601 module) analyzer series, 2151000062; HITACH (Tokyo, Japan). Both of them had run through and were guaranteed by laboratory quality control.

An anti-thyroid peroxidase antibody titer greater than 35 was considered positive because laboratory reference values and the normal value of the thyroid-stimulating hormone test (0.27-4.27 mIU/ml) were analyzed.

Statistical Analysis

The statistical analysis was performed by the SPSS program, version 21 (IBM Corp., Armonk, NY, USA). Compliance of quantitative random variables with the Gaussian curve (normal distribution) was analyzed using Kolmogo-Smirnov and Shapiro – Wilk tests. The data presented in tabular forms shows the frequency and relative frequency distribution of different variables among both groups. Chi-square tests were used to compare the categorical data between these two groups of study participants (cases and controls) for different variables.

The statistical significance of the difference in mean between the two groups (cases and controls) was assessed using an independent sample *t*-test. Non-normally distributed quantitative variables, such as TSH and Anti-TPO antibody titer was described by the median in addition to the mean. In such conditions, the median and interquartile ranges (IQR) were used for compared groups. The difference in the median (IQR) of the two groups was assessed by a non-parametric test (Mann-Whitney) *p*-value of 0.05 were used as a cut-off-point for the significance of statistical tests.

Results

A total number of 120 participants were assessed for TSH anti-TPO titer, 2 of them represented the case group having recurrent miscarriages, 40 of them had just first-trimester miscarriage, and 22 of them had first and second-trimester miscarriages, while 62 of them represented the control group without miscarriage; all of them were euthyroid (TSH between 0.2-4.27 mlU/L). The sociodemographic status for both groups and a comparison of characteristics are shown in (Table I).

The mean age of both groups was 32.59 ± 5.81 with an age range of 18-47 years and no significant difference between the age of both groups (33.11±6.37 *vs.* 32.06±5.19) *p*-value=0.32. Among all participants 76.6% of them lived inside the city center, others were from the urban area, 61.3% were housewives, 97 of them were Kurdish and the remaining 23 were Arabic, no one was a smoker, and no one was alcoholic.

Of a total of 120 participants, 16 of them had positive anti-TPO antibody titer (12.9%), 12 were in the case group and 4 were in the control group. Additionally, there were 12 cases of positive anti-TPO in the recurrent miscarriage group, 9 of

Table I. Sociodemographic status for both groups and comparison of characteristics.

		Cases	Controls	Total	*p*-value
Age	Mean ± SD	33.11 ± 6.37	32.06 ± 5.19	32.59 ± 5.81	0.32
	18 - 27 Years	14 (22.6%)	16 (25.8%)	30 (24.2%)	0.42
	28 - 37 Years	32 (51.6%)	36 (58.1%)	68 (54.8%)	
	38 - 47 Years	16 (25.8%)	10 (16.1%)	26 (21.0%)	
Occupation	Housewife	45 (72.6%)	31 (50.0%)	76 (61.3%)	0.01
	Employed	17 (27.4%)	31 (50.0%)	48 (38.7%)	
Residency	Rural	10 (16.1%)	19 (30.6%)	29 (23.4%)	0.06
	Urban	52 (83.9%)	43 (69.4%)	95 (76.6%)	
Gravida	0 - 2	2 (3.2%)	1 (1.6%)	3 (2.4%)	< 0.001
	3 - 4	22 (35.5%)	61 (98.4%)	83 (66.9%)	
	5 or more	38 (61.3%)	0 (0%)	38 (30.6%)	
Para	nulliparous	19 (30.6%)	0 (0%)	19 (15.3%)	< 0.001
	1 - 3	40 (64.5%)	60 (96.8%)	100 (80.6%)	
	4 or more	3 (4.8%)	2 (3.2%)	5 (4.0%)	
Previous NVD	None	33 (53.2%)	17 (27.4%)	50 (40.3%)	< 0.001
	One - two	21 (33.9%)	12 (19.4%)	33 (26.6%)	
	Three - four	8 (12.9%)	33 (53.2%)	41 (33.1%)	
Previous C/S	None	43 (69.4%)	34 (54.8%)	77 (62.1%)	< 0.001
	One - two	18 (29.0%)	13 (21.0%)	31 (25.0%)	
	Three	1 (1.6%)	15 (24.2%)	16 (12.9%)	
Total	62 (100%)	62 (100%)	124 (100%)		

Table II. Comparison of anti- TPO result in study participants (Cases and controls).

		Cases	Controls	Total	*p*-value
Anti TOP titer	Positive	12 (19.4%)	4 (6.5%)	16 (12.9%)	0.03
	Negative	50 (80.6%)	58 (93.5%)	108 (87.1%)	
Odds ratio (95% CI)	3.48 (1.06-11.48)				0.03
Total	62 (100%)	62 (100%)	124 (100%)		

Table III. Association between age group and thyroid peroxidase antibody titer in both group.

		Anti- TPO Antibody in case group	
		Positive	Negative
TSH	Median (IQR)	3.15 (2.1)	1.75 (1.83)
	p-value		0.02

them were those with first-trimester miscarriages and 3 of them were those who had first and second-trimester miscarriages.

The prevalence of positive anti-TPO antibodies in women with recurrent miscarriage was 19.4%, while in women without miscarriage was 6.5% (which is considerably higher than the control group with p=0.03 and the odd ratio of 3.48 (95% CI; 1.06-11.48) (Table II).

There was no significant association between age group and thyroid peroxidase antibody in both groups as shown in Table III.

The median of TSH in women with anti-TPO positive was higher than in women with anti -TPO negative in the recurrent miscarriage group (3.15 *vs.* 1.75) p=0.02 (Table IV).

The median of TSH in cases including anti-TPO positive and negative was 2.1, while in the control group was 1.8, with p=0.15 hence there is no statistically significant difference in median (or average) TSH between cases and controls (irrespective of anti-TPO).

Discussion

Many families and communities bear a heavy physical, emotional, and financial burden due to recurrent miscarriages. Proper management and treatment are needed; however, according to the 2011 RCOG guideline[1], 75% of women with unexplained recurrent miscarriages have an excellent prognosis for a successful future pregnancy with only support and no medication[1].

Association between anti-TPO antibodies and recurrent miscarriage has been reported by many systematic reviews and meta-analyses[14,15]. In a 2011 meta-analysis by Thangaratinam et al[14], it was demonstrated that in women with normal thyroid function and thyroid, autoantibodies miscarriage risk is expected to become three times higher and the risk of preterm birth is increased twice.

In the present study prevalence of thyroid antibody positive in recurrent miscarriage in women was 19.4%.

Table IV. Comparison between median of TSH in ant-TPO positive and negative cases of recurrent miscarriage.

Age		Anti-TPO Antibody				Total
		Cases		Controls		
		Positive	Negative	Positive	Negative	
1	18 - 27 Years	1	13	1	15	30
2	28 - 37 Years	9	23	2	34	68
3	38 - 47 Years	2	14	1	9	26
	p-value	0.18		0.88		

This prevalence was also in the same range found in other studies6, which vary from 17-33%.

In a case-control study conducted by Ticconi et al[15], the prevalence of thyroid antibody in recurrent miscarriage was 28.7% and the prevalence in women without abortion was 13%, study prevalence of anti-TPO antibody was 6% in the control group without miscarriage, which is in the same range as the total population range (6-20%)[6].

Thyroid peroxidase antibody is considered the most sensitive marker for the detection of thyroid autoimmunity, with TPO antibodies being the most prevalent and commonly tested for 6.

In 2006 Ghafoor et al[16] did a cross-sectional analytical study on 1,500 pregnant women for the role of thyroid antibodies and the outcome of pregnancy concluded that thyroid autoimmune illness among pregnant women may lead to low birth weight of neonates and increased miscarriage frequency in pregnant women.

In contrast to our study, Esplin et al[17] conducted a study and found that circulating thyroid autoantibodies are not significantly different in women with recurrent miscarriage and fertile women. They also state that testing for anti-thyroid antibodies is not necessary for diagnosing the reason behind recurrent miscarriage.

As women get older, the quality and the number of oocytes decrease. Moreover, maternal age and the number of previous miscarriages are two distinct risk factors for a subsequent miscarriage.

The study found that women over the age of 35 are more likely to have miscarriages. However, this difference was not statistically significant. Additionally, the study found that there is no association between anti-TPO antibodies and age groups. This is similar to a meta-analysis of 10 studies that found that there was no significant difference between age groups and anti-thyroid peroxidase antibodies[14].

Another finding in the current study was that the median of TSH in women with an anti-TPO positive was significantly higher than in women with an anti-TPO negative in the recurrent miscarriage group (3.15 *vs.* 1.75); this is also similar to the finding of the meta-analysis which is performed by Thangaratinam in 2011[14] The findings support the hypothesis that anti-TPO antibodies are linked to pregnancy loss.

The American Thyroid Association Guidelines 2011 and the Endocrine Society Guidelines 2012 dose not recommended universally TPO-Ab testing in the evaluation and treatment of women with recurrent miscarriage[18].

Limitations

We excluded other causes of recurrent miscarriage for example antiphospholipid syndrome and it was very difficult to identify cases of unexplained recurrent miscarriage.

In couples who have experienced successive pregnancy loss, chromosomal abnormalities of the embryo account for 30-57% of further miscarriages[1], chromosomal analysis of the embryo which was miscarried was not done by all 62 recurrent miscarriage participants, as it is very costly and not applicable for every miscarriage. This was one of the limitations of our study, along with a small sample size and all women were from a developing country.

Conclusions

There is a statistically significant association between anti-TPO antibodies and recurrent miscarriage. We recommend TSH and thyroid antibody investigation for women with recurrent miscarriages. Also, the European Society for Human Reproduction and Embryology Recurrent pregnancy loss recommends thyroid screening with TSH and anti-TPO antibodies. Further studies on the effect of levothyroxine therapy for euthyroid women with anti-thyroid peroxidase antibody positive are needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

Requisite IRB approval was obtained from the research protocol ethics committee, Kurdistan Board of Medical Specialties, Ministry of Higher Education and Scientific Research, Kurdistan Region Government - Iraq, with approval No. 1182.

Informed Consent

With prior consent, the patients were included in the study.

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