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Original Research

Screening for Adrenocortical and Thyroid Peroxidase Antibodies to Look for Underlying Autoimmune Etiologies in Women under 35 with Idiopathic Dimished Ovarian Reserve

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Abstract

Background: Autoimmune disorders are more common in premature ovarian insufficiency (POI) than in the general population. The most important association is with autoimmune Addison's disease. Measurement of adrenocortical antibodies (ACA) and/or 21-hydroxylase antibodies (21-OH) is recommended in every POI patients as they appear to be the marker with the highest diagnostic sensitivity for autoimmune POI. Also thyroid peroxidase autoantibodies (TPO-Ab) should be assayed due to the common association between thyroid disease and POI. The underlying etiologies of diminished ovarian reserve (DOR) in young women can be expected to be similar to the etiology of POI since they represent a continuum in the phenotypic expression of premature ovarian aging. **Methods**: This pilot case-control study was conducted between January 2019 and April 2020. The study group consisted of patients under the age of 35, who was infertile and diagnosed with idiopathic DOR by ovarian reserve tests during infertility work up. Controls were patients of the same age range who diagnosed with isolated tubal factor or male infertility and had functional ovarian reserve test results during infertility work up. Patients with a history of ovarian surgery, cancer, genetic or autoimmune disease were excluded. Abnormal ovarian reserve tests are defined as antral follicle count <5 and anti-mullerian hormone (AMH) <1.2 ng/dL corresponding to group 3 according to POSEIDON criteria. In total, 35 DOR patients and 35 controls were included in the study. ACA and TPO-Ab screening were performed in serum samples using indirect immunofluorescence method. Demographics and family history of autoimmune diseases were also evaluated. **Results**: A higher rate of ACA positivity was detected in the DOR group (34.3%) compare to controls (17.1%), although it was not found to be statistically significant ($p = 0.101$, $p < 0.05$). The incidence of family history of autoimmune diseases in first degree relatives was positively correlated with ACA positivity ($p = 0.006$, $p < 0.05$). In DOR group, autoimmune disease history in the family was significantly higher in ACA (+) patients compared to ACA (–) individuals ($p = 0.03$, $p < 0.05$). TPO-Ab positivity rates were similar between 2 groups (17.1% *vs* 20%, $p = 0.759$, $p < 0.05$). **Conclusions**: Even if there is no specific treatment option yet for autoimmune ovarian damage, screening for ACA or 21-OH antibodies may be considered in young women with idiopathic DOR, especially those with a family history of autoimmune disease, based on knowledge that identification of women with autoimmune POI is clinically important for the identification of subclinical autoimmune Addison's cases.

Keywords: diminished ovarian reserve; autoantibodies; premature ovarian insuffiency

1. Introduction

Premature ovarian insufficiency (POI) is a clinical syndrome defined as amenorrhea due to loss of ovarian function before the age of 40. Physical and psychological problems caused by high gonadotropin and low estradiol levels affect the quality of life [1]. Its incidence increases with age, and it is seen at a rate of 1% before the age of 40 and 0.1% before the age of 30 [2,3]. The diagnosis is made by detecting two follicle-stimulating hormone (FSH) values higher than 25 IU/L at intervals of at least four weeks in women under 40 years of age with oligo/amenorrhea for more than four months [4]. Although most cases are described as idiopathic, iatrogenic causes such as numerical and structural chromosome anomalies, fragile X premutation, autoimmune diseases, infections, radiotherapy, chemotherapy and surgery are shown among the etiologies that can be associated with POI [5]

Occult POI is characterized by infertility, regular menstrual cycles and increased serum FSH levels and considered as the early stage of POI. Occult POI can be observed in the diminished ovarian reserve (DOR) patient population presenting with infertility. Not all DOR women will develop POI, although POI may be considered as the continuum of the pathology, therefore it should be taken into account especially in DOR patients presenting with infertility. The majority of these women cannot be diagnosed until they apply with the complaint of infertility and this may cause a delayed diagnosis. Considering the literature, it is of great importance to evaluate the ovarian reserve in this particular patient group [5].

DOR is defined as reduced fertility capacity compared to women of the same age group. Poor ovarian response (POR) will occur as a result of DOR. Poor ovarian responders (PORs) embody 9–24% of patients undergoing ovarian stimulation for *in vitro* fertilization (IVF). Poor responders have a reduced potencial to produce an adequate number of oocytes. Therefore there are many other techniques for reproduction in poor responders including cryoconservation of oocytes and embryos, embryo transfers, oocyte vitrification. Ovarian stimulation combined with vitrification of oocytes and embrios could increase the chances for these patients. Also age negatively impacts the rates of oocyte survival, therefore especially DOR/poor responder patients may choose to undertake oocyte vitrification for preserving fertility, future opportunities and IVF success [6,7].

Melatonin reduces the oxidative stress on follicles causing a good oocyte quality. Also myo-inositol acts as a second messenger of FSH and improves oocyte quality and fertilization rate [7]. Therefore melatonin and myo-inositol supplements have an affective role to manage women with poor ovarian response and a positive impact on clinical pregnancy rate in infertile women [8,9].

Gullo *et al.* [10] and Burgio *et al.* [11] conducted a study that showed couples undergo medically assisted reproduction had higher anxiety levels, higher depression levels and high infertility-related stress [10,11]. Identification of women with DOR before medically assisted reproduction enables more personalization of treatments and protocols to be arranged by predicting POR.

1.1 Autoimmunity

It is estimated that approximately 10–30% of women with POI are accompanied by an autoimmune disease [12, 13]. Addison's disease is the autoimmune disease with the strongest autoimmune relationship to POI. Since untreated Addison's disease is associated with life-threatening complications, it is highly recommended to determine the associated adrenal autoimmunity/subclinical Addison's disease in individuals diagnosed with POI.

Addison's disease (autoimmune adrenalitis) is the most common cause of primary adrenal insufficiency. It is characterized by the presence of serum antibodies against adrenal cortex, steroidogenic cells and enzymes. The identified antibodies are called "adrenocortical antibodies (ACA)". The presence of ACAs in peripheral blood samples is important in terms of both determining the etiology of primary adrenal insufficiency and identifying high-risk groups for future autoimmune Addison's disease [14]. 21-hydroxylase ntibodies have been identified as the most common among ACAs and can be detected in approximately 0.5% of the healthy population [15]. The incidence of ACA, which can be detected in individuals with normal adrenal functions was low and around 2%. ACAs were found to be 60–80% in peripheral blood samples of patients with primary adrenal insufficiency caused by autoimmune Addison's disease. In order to detect subclinical autoimmune adrenal insufficiency in these patients, the measurement of ACAs by indirect immunofluorescence technique or the measurement of 21-hydroxylase antibodies (21-OH) autoantibodies by immunoprecipitation test constitute the recommended screening tests [16].

In the POI group unrelated to adrenal autoimmunity, the most common association is thyroid autoimmunity with a rate of 25–40%. Hashimoto thyroiditis is the most common thyroid disorder and Hashimoto thyroiditis is present in 14% to 27% of women at the time of initial diagnosis of POI. Therefore, it is recommended to measure thyroid hormone levels and investigate the presence of thyroid peroxidase autoantibodies (TPO-Ab) after the diagnosis of POI. All women with TPO-Abs should be referred to the endocrinologist for further evaluation and follow-up [17].

1.2 Aim

The genetic and autoimmune etiologies of POI have been well documented in the literature, but there are no studies yet suggesting that the same etiologies apply to DOR. However, it can be expected to be similar to the etiology of POI since they represent a continuum in the phenotypic expression of premature ovarian aging. There is no standard yet to identify the underlying genetic and autoimmune causes of DOR. This pilot-case study aims to investigate the presence of ACA and TPO-Ab in patients with DOR, to demonstrate the presence of autoimmune etiology, and to investigate its correlation with the presence of autoimmune disease and genetic history in the family. In individuals with ACA positivity, it will be possible to develop screening, follow-up and treatment plans for autoimmune diseases that may develop in the future, especially Addison's disease.

2. Materials and Methods

This pilot case-control study was conducted between January 2019 and April 2020. The study group consisted of 35 women under the age of 35, who was diagnosed with idiopathic DOR by ovarian reserve tests during infertility workup. Controls were 35 women of the same age range who diagnosed with isolated tubal factor or male infertility and had functional ovarian reserve (FOR) test results during infertility workup.

All study participants gave informed and written consent.The study was conducted in agreement with the local and international guidelines and regulations, including the Declaration of Helsinki and the principles of good clinical practice. Approval of the Ethics Committee was acquired (2020/1055, Istanbul Faculty of Medicine, Ethics Committee). Women over 35 years of age with a known history of ovarian surgery, radiotherapy or chemotherapy, and a known history of autoimmune disease were excluded from the study.

Table 1. Demographics of the study and control groups.

n = 70	FOR group		DOR group	
	Average ± SD	Min–Max	Average ± SD	Min–Max
Age	30.8 ± 2.52	24–35 (30)	32.31 ± 2.53	26–35 (33)
BMI	24.69 ± 3.68	17–34.5 (24.4)	24.8 ± 3.78	17.8–35 (24.2)
Menarche age	13.17 ± 1.22	12–16 (13)	12.77 ± 1.35	11–17 (13)
Antral follicle	11.23 ± 6.17	2–20 (10)	3.83 ± 2.9	0–12 (3)
AMH	1.95 ± 2.28	1.1–12.2 (1.05)	0.49 ± 0.33	0.01–1 (0.4)
FSH	8.87 ± 4.15	1.76–12.6 (7.93)	10.06 ± 5.04	1.46–22.7 (8.2)
LH	7.13 ± 4.43	0.1–25 (6.1)	6.83 ± 3.14	1.3–14.1 (5.7)
TSH	1.9 ± 0.92	0.55–4.27 (1.6)	2.79 ± 1.65	0.7–10 (2.4)
Estradiol	55.47 ± 48.51	8.6–270 (45.6)	71.11 ± 85.74	5–440 (48)
Prolactin	19.29 ± 10.17	3.98–42.9 (16)	18.2 ± 12.49	3–60 (12.2)

BMI, Body Mass Index.

The age, height, weight and menstrual cycle order of the groups were questioned by the first independent researcher. Routine gynecological examination and transvaginal-ultrasonography (USG) were performed to determine the number of antral follicles in the ovaries. Each woman's serum anti-mullerian hormone (AMH) level in the last 6 months, and serum FSH, luteinizing hormone (LH), estradiol (E2), prolactine (PRL) and thyroid-stimulating hormone (TSH) levels on the 3rd day of the cycle were examined. Peripheral blood samples taken from all women participating in the study were centrifuged and frozen in serum at –20 degrees. Subsequently, the presence of ACA in these serum samples was investigated by indirect immunofluorescence method. Autoantibodies against thyroid peroxidase (TPO-Ab) were analyzed by chemiluminescence immunoassay.

Statistical analysis made by NCSS (Number Cruncher Statistical System) 2007 (NCSS 2007, LLC, Kaysville, UT, USA). When evaluating the study data, descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum) were used and the distribution of the data were evaluated with the Shapiro-Wilk Test. Mann-Whitney U test was used to compare two groups of quantitative data that did not show normal distribution. Chi-square test was used to determine the relationship between qualitative data.Statistical significance was evaluated at the level of $p < 0.05$.

3. Results

The demographics of the patient group are demonstrated in Table 1. While the mean age of the patients with DOR (n = 35) participating in the study was 32.31 ± 2.53, the mean age of the control FOR group (n = 35) was 30.8 ± 2.52.

20% (n = 7) of the control group were TPO-Ab positive. While 17.1% (n = 6) of the study group were TPO-Ab positive , 82.9% (n = 29) of them were negative. There was no statistically significant relationship with the presence of TPO-Ab between the study and control groups ($p > 0.05$) (Table 2).

Table 2. Summarizes the comparison of TPO-Ab prevalance between study and control groups.

		Group		*p*
		DOR (n = 35)	FOR (n = 35)	
TPO-Ab	Positive	6 (17.1%)	7 (20%)	0.759
	Negative	29 (82.9%)	28 (80%)	

34.2% (n = 12) of the study group were ACA positive , 65.8% (n = 23) were ACA negative. While 17.1% (n = 6) of the control group were ACA positive. A higher rate of ACA positivity was detected in the DOR group (34.3%) compare to controls (17.1%), although it was not found to be statistically significant ($p = 0.101$, $p < 0.05$) (Table 3).

Table 3. Summarizes the comparison of ACA prevalance between study and control groups.

		Group		*p*
		DOR (n = 35)	FOR (n = 35)	
ACA	Positive	12 (34.2%)	6 (17.1%)	0.101
	Negative	23 (65.7%)	29 (82.9%)	

Comparison of Biochemical and USG Parameters Between ACA (+) DOR and NOR Groups is shown in Table 4.

It was statistically significant that the AMH level and the antral follicle count of the patients in the study group with ACA (+) were lower than the control group ($p = 0.001$; $p < 0.01$, $p = 0.001$; $p < 0.01$ respectively). There was no statistically significant difference between the FSH levels of the groups ($p > 0.05$) (Tables 2,4).

The incidence of unknown family history of infertility was positively correlated with ACA positivity in the control group ($p = 0.044$, $p < 0.05$). Family history of early menopause and autoimmune disease rates were similar between 2 groups ($p = 0.658$ and $p = 0.135$ respectively, $p < 0.05$) (Table 5).

Table 4. Comparison of biochemical and USG parameters between ACA (+) DOR and NOR groups.

	n		Average ± SD	Min–Max (Median)	*p*
AMH	FOR	6	4.38 ± 4.03	1.1–12.2 (3.15)	0.001
	DOR	12	0.38 ± 0.23	0.1–0.8 (0.4)	
Antral follicle	FOR	6	17 ± 5.62	6–20 (20)	0.001
	DOR	12	3.67 ± 2.23	0–7 (3)	
FSH	FOR	6	7.26 ± 2.94	4.33–12.4 (6.43)	0.111
	DOR	12	10.35 ± 5.33	1.46–20.7 (8.45)	

Table 5. Comparison of family history between ACA (+) and ACA (–) patients in control group.

		FOR		*p*
		ACA (–) (n = 29)	ACA (+) (n = 6)	
Family history of early menopause	(–)	26 (74.2%)	5 (14.3%)	0.658
	(+)	3 (8.6%)	1 (2.9%)	
Unknown family history of ınfertility	(–)	25 (71.4%)	3 (8.6%)	0.044
	(+)	4 (11.4%)	3 (8.6%)	
History of autoimmune disease in first degree relative	(–)	23 (65.7%)	3 (8.6%)	0.135
	(+)	6 (17.1%)	3 (8.6%)	

Table 6. Comparison of family history between ACA (+) and ACA (–) patients in study group.

		DOR		*p*
		ACA (–) (n = 23)	ACA (+) (n = 12)	
Family history of early menopause	(–)	19 (54.3%)	10 (28.6%)	0.957
	(+)	4 (11.4%)	2 (5.7%)	
Unknown family history of ınfertility	(–)	17 (48.6%)	8 (22.9%)	0.652
	(+)	6 (17.1%)	4 (11.4%)	
History of autoimmune disease in first degree relative	(–)	18 (51.4%)	5 (14.3%)	0.030
	(+)	5 (14.3%)	7 (20%)	

In DOR group, autoimmune disease history in the family was significantly higher in ACA (+) patients compared to ACA (–) individuals ($p = 0.03$, $p < 0.05$). Family history of early menopause and unknown infertility rates were similar between the two groups ($p = 0.957$ and $p = 0.652$ respectively, $p < 0.05$) (Table 6).

The incidence of family history of autoimmune diseases in first degree relatives was positively correlated with ACA positivity ($p = 0.006$, $p < 0.05$) (Table 7).

4. Discussion

Infertility is a multidisciplinary condition that touches both men and women's lives. It may result in a decrease in quality of life and create issues of anxiety, stress, depressive disorders, self blame and isolation. Oocyte abnormality or maturity might not always been the cause of fertilization failure in IVF procedures. Sperm defects, spermatozoon-oocyte interactions also have been shown as possible causes of IVF failure. Goudakou *et al*. [18] conducted a study that showed even in normal spermatozoas there might be cryptic sperm defects that cause fertilization failure which needs identifying. Gullo *et al*. [19] conducted a study showing both men and women affected by infertility equally and gender impact assessment is affective for future health practices and gender-oriented strategies in assisted reproductive technology (ART) [11,19].

Autoimmune pathogenesis is shown as the underlying etiology between 10 and 30% in cases of POI. Early identification of ovarian autoimmunity may enable the creation of treatment options without irreversible loss of ovarian functions [20]. The incidence of concurrent autoimmune diseases varies between 10–55% in women diagnosed with POI. The study focused on two factors to indicate that the underlying etiologies in women with diminished ovarian reserve were similar to POI and that autoimmunity and genetic predisposition should be investigated in the group of patients with DOR.

In the study, the anamnesis of the study and control groups were taken in terms of the history of autoimmune disease in the family, early menopause in the family and the presence of unknown infertility. In line with the findings of studies on similar subjects, thyroid disorders in family histories have been found to be common.In addition to thyroid disorders, family history of type 1 diabetes mellitus has also been found.

Table 7. Comparison of family history between ACA (+) and ACA (–) patients.

		ACA		*p*
		Positive (n = 18)	Negative (n = 52)	
Family history of early menopause	(–)	15 (14.3%)	45 (64.4%)	0.503
	(+)	3 (4.3%)	7 (10%)	
Unknown family history of ınfertility	(–)	11 (15.7%)	42 (60%)	0.090
	(+)	7 (10%)	10 (14.3%)	
History of autoimmune disease in first degree relative	(–)	10 (14.3%)	41 (58.6%)	0.006
	(+)	8 (11.4%)	11 (15.7%)	

Patients with DOR or occult POI are evaluated in the POI spectrum, even as precursor conditions of POI, and we encounter more complaints of infertility than POI symptoms. There is no accepted universal diagnostic criterion for these patient groups. Abnormal ovarian reserve tests are defined as antral follicle count <5 and AMH <1.2 ng/dL corresponding to group 3 according to POSEIDON criteria [21].

Premature ovarian insufficiency may develop as a result of increased ovarian reserve depletion rate as a result of delayed diagnosis in DOR and occult POI patient groups. For this reason, it is increasingly important to determine the underlying etiology in these patients.

In the study, the presence of ACA was investigated between 35 study group patient under 35 years of age with low ovarian reserve and 35 control group patient with normal ovarian reserve. While ACA positivity was detected in 34.3% (n = 12) of the study group with DOR, this rate was found to be 17.1% (n = 6) in the FOR group.In the study, ACA positivity was found to be higher in the group with DOR in accordance with the literature. In a study of Gao *et al*. [22], a total of 250 women with idiopathic POI was compared to a total of 256 healthy women and significantly higher ACA positivity was found in women with POI. It is thought that the small sample group in our study caused the study to be insufficient to obtain meaningful results.

Early menarche is among the risk factors that can be shown for DOR and POI.The mean menarche age was 12.77 $\pm$ 1.35 in the DOR group and 13.17 $\pm$ 1.22 in the control group. Weghofer *et al*.'s [23] review also proved that age at menarche has a major significance on infertile women to have DOR risk later in life.

When study and control groups were compared, no statistically significant relationship was found between TPO-Ab presence and groups ($p > 0.05$). In addition, there was no statistically significant relationship between ACA positive and negative individuals with TPO-Ab presence ($p > 0.05$). The most common autoimmune association in individuals diagnosed with POI is autoimmune thyroid diseases. However, the main pathophysiology that associates the decrease in ovarian reserve with TPO-Ab and thyroid hormones has not yet been elucidated. It has been observed that the results vary with many studies on DOR and thyroid autoimmunity. In a study conducted on 5000 Belgian women, no significant relationship was found between DOR and thyroid autoimmunity. In a study by Bahri *et al*. [24] involving 775 healthy women in reproductive age, it was found that TPO-Ab levels were higher in the group with low ovarian reserve after 12 years of follow-up of the population compared to other groups. There are many studies in the literature on the relationship between low ovarian reserve and hypothyroidism and thyroid autoimmunity.

It is known that the prevalence of accompanying autoimmune disease is high in DOR and POI cases with autoimmunity in the etiology. In the study, a statistically significant relationship was found with the family history of autoimmune disease in the group with ACA positivity. In a study by Košir *et al*. [25] involving 37 women, a family history of autoimmune disease was detected in up to 55% of the 20 POI diagnosed study groups. When the study group with DOR was considered as a separate subgroup, ACA positivity was detected in 34.3% (n = 12).

Adrenocortical antibody (ACA) is one of the sensitive markers showing the presence of subclinical Addison's disease or showing the risk of developing Addison's disease later in life [26]. ACA positive patient groups with spontaneous POI are at risk for Addison's disease. In a study of Gao *et al*. [22], indirect immunofluorescence and the presence of ACA were compared among a total of 406 women who were diagnosed with 250 idiopathic POI and 256 healthy control groups. While ACA positivity was 19.2% in the idiopathic POI group, this rate was 5.9% in the control group ($p < 0.01$). What is noteworthy is that adrenal insufficiency symptoms developed after 3 years in one of the 15 POI and ACA positive patients whose adrenal functions were followed up [21].

In the literature, it is recommended to further evaluate the cases with positive ACA in order to investigate subclinical Addison's disease. In the study, a statistically significant relationship was found between the ACA-positive group and the family history of autoimmune disease ($p = 0.006$, $p < 0.05$). It is known that genetic predisposition is one of the primary causes of autoimmunity as well as its involvement in the etiology of DOR and POI. There are also data in the literature that support the close relationship between autoantibody positivity and autoimmune disease.

5. Conclusions

There is no study in the literature evaluating the correlation between ACA screening and antibody positivity, family history and genetic predisposition in patients with DOR. The dilution of the samples and the small number of samples were the disadvantage of this pilot-case study and caused the study to be insufficient to obtain meaningful results.

However, in line with the data obtained, it would not be wrong to take a detailed anamnesis in individuals with DOR and to detail the family history, and then should be individualized to include ACA and TPO-Ab research in the etiological and diagnostic evaluation in patients with DOR risk factors and family history.

6. Future Directions

Based on knowledge that identification of women with autoimmune POI is clinically important. By screening these individuals, it will be possible for clinicians to identify subclinical Addisons's disease which has a life threatening risk for adrenal insufficiency, also it would allow clinicians to follow-up patients positive for antibodies annually. In order to obtain more significant data correlated with the literature, it would be appropriate to conduct further studies on a larger population.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

OD and CA designed the research study. IE performed the research. CY, FGU and CE provided help and advice on the aim of the study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All study participants gave informed and written consent. The study was conducted in agreement with the local and international guidelines and regulations, including the Declaration of Helsinki and the principles of good clinical practice. Approval of the Ethics Committee was acquired (2020/1055, Istanbul Faculty of Medicine, Ethics Committee).

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Conflict of Interest

The authors declare no conflict of interest.

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