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Investigation of antiphospholipid antibody syndrome markers in the etiology of recurrent miscarriage

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Abstract

This study aimed to determine the markers of antiphospholipid antibody syndrome (APS), which has an important place in the etiology of recurrent miscarriage, and to provide guidance for the treatment of the related disorders. This prospective observational study was performed on 120 female patients admitted to the Obstetrics and Gynecology outpatient clinics due to recurrent pregnancy loss. Blood samples were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique to find out the levels of APS-associated anticardiolipin (ACA), anti-beta2-glycoprotein I (aβ2GPI), anti-annexin V (aANXV), anti-prothrombin (aPT), and antiphosphatidylserine (aPS) antibodies. A statistically significant difference was found between increased age groups and the number of miscarriages ($p=0.03$, $p<0.05$). There were no significant differences between the age groups by the time of miscarriage ($p=0.35$, $p<0.05$). ACA positivity (75%, n = 90) and anti-annexin V positivity (50.8%, n = 61) were higher than the positive results found in other tests($p=0.01$, $p<0.05$). Antiphospholipid antibodies (APAs) showed significantly different positivity levels by the age groups ($p=0.01$, $p<0.05$). The positivity levels of APAs were significantly different by the number of miscarriages ($p=0.01$, $p<0.05$). Other antibody levels were not statistically significantly different. The frequency of positive APA levels increases with advancing age and a high number of miscarriages in association with the etiology of recurrent miscarriage. Among the subgroups of these antibodies, ACA and anti-annexin V can serve as highly appropriate diagnostic markers in recurrent miscarriage.

Keywords: Antiphospholipid, recurrent miscarriages, etiology, anticardiolipin antibody

Introduction

Antiphospholipid antibody syndrome (APS) is an autoimmune disease that is characterized by the presence of antiphospholipid antibodies (APAs) and recurrent miscarriage, frequently occurring in the general population. Pregnancy complications in obstetric APS include unexplained recurrent pregnancy loss and fetal death due to severe preeclampsia, eclampsia, intrauterine growth restriction, or other consequences of placental failure [1]. In the literature, no relation was found between antiphospholipid antibodies (anticardiolipin, anti-beta2-glycoprotein I) and late fetal losses [2]. Failure to fully demonstrate the risk factors in the etiology of pregnancy loss hinders the application of appropriate interventions precisely for miscarriage prevention. Therefore, a clinical diagnosis of miscarriage is made at rates ranging from 8% to 15% in the presence of pregnancy loss [3]. Elucidation of the immunological causes in the etiology will contribute to the management of RPL(Recurrent Pregnancy Loss) patients. Studies have shown increased rates of APAs in RPL patients. These antibodies induce trophoblastic apoptosis and target the vascular endothelium, inducing the generation of anomalous spiral arteries [4]. The relationship between fertility disorders and autoimmune diseases is well described in the literature. However, it is very difficult to talk about humoral autoimmunity in the absence of any autoimmune disease criteria; This may involve the positivity of APA as there is little evidence in the literature [5]. Various types of evidence have shown that the risk of miscarriage increases in the presence of autoimmune diseases and associated autoantibody positivity. The most well-known autoimmune disease shown to be associated with this condition is antiphospholipid antibody syndrome [6]. APS is characterized by recurrent thrombosis or complications of pregnancy (miscarriage and fetal death, preeclampsia, placental insufficiency, and fetal growth restriction) in addition to the presence of antiphospholipid antibodies [7]. The antigenic targets of APAs are negatively charged phospholipids and phospholipid-binding proteins in the serum [7]. The antigens

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to which APAs bind are partially understood. Autoimmune factors inducing the synthesis of antibodies targeting phospholipids (cardiolipin, phosphatidylserine, etc.) or plasma proteins (beta2-glycoprotein I, prothrombin, and annexin V, etc.) that bind to phospholipids are risk factors for recurrent miscarriage. However, the mechanisms of antiphospholipid antibody-mediated pregnancy failure remain to be a subject of research [8]. Recurrent miscarriage induces the synthesis of IgG, IgM, and IgA type antibodies against phospholipids. These antibodies disrupt cell functions, resulting in inflammation and coagulation. However, the relationship between antiphospholipid antibodies and spontaneous abortions of unknown etiology is unclear [6]. This study aims to identify APS markers that play an important role in the etiology of recurrent abortions and to guide clinical diagnosis and treatment.

Materials and Methods

Study Population

In the study, 310 pregnant women who applied to the gynecology and obstetrics outpatient clinic of Afyon Kocatepe University Faculty of Medicine were evaluated. Pregnant women with an etiological cause and a history of infection, systemic autoimmune rheumatic diseases (systemic lupus erythematosus, rheumatoid arthritis, etc.), and those who had a miscarriage 1 were excluded from the study. 190 pregnant women were excluded for these reasons. 120 patients who had 2 or more miscarriages were included in the study. The study included 120 patients 26,55±6,52 old, who were admitted to the obstetrics and gynecology outpatient clinic with recurrent miscarriage (miscarriage number ≥ 2) were included in the study. Patients were assigned into 4 age groups as follows: 20-25 years (n = 51), 26-30 years (n = 42), 31-35 years (n = 16), and 36 years and older (n = 11). This study was conducted prospectively in compliance with the Declaration of Helsinki after obtaining the approval of the institutional Ethics Committee. This study was done in Afyon Kocatepe University Medical Faculty's medical microbiology laboratory.

Ethical approval

This observational study was conducted by the ethical principles stated in the "Declaration of Helsinki" and permission was obtained from the Ethics Committee of Afyon Kocatepe University Faculty of Medicine for the use of patient data for publication purposes (Date-Decision no: 14.09.2007-115).

Power analysis

The prospective observational study universe comprised patients presenting to the obstetrics and gynecology outpatient clinic due to recurrent pregnancy loss. During the study period, 310 female patients were admitted to the obstetrics and gynecology outpatient clinic due to recurrent miscarriage. It was concluded that 118 patients to be included in the study using a simple random sampling method would be explanatory for the study universe in a 95% confidence interval and with a 3% error margin. In light of these data, the study was carried out on the samples collected from 120 patients. The patients included in the study provided a 0.95 sampling power at an effect size of 0.50 (studies having a 0.70 sampling power are considered to have a small, medium, or large effect size at an effect size of less than 0.10, 0.25, and 0.40). In summary, it is observed that the study is appropriately powered with a considerable appropriate effect size.

The statistical power of the study and the effect size was calculated with G*Power Version 3.1.7.

Laboratory analysis

The blood sample sera were separated by centrifugation at 5000 rpm for 5 minutes. Blood samples were screened for antibodies associated with APS using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The Orgentec kit (ORGENTEC Diagnostika GmbH, Germany) was used for measuring the antibody levels. The tests were automatically conducted using a fully automated Alegria device (ORGENTEC Diagnostica GmbH, Germany) based on the ELISA method. The test strips were designed for a single assay. The strips consisted of 8 wells with barcodes. The wells were coated with individual antigens (cardiolipin, beta2-glycoprotein, phosphatidylserine, prothrombin, and annexin V), depending on the test type. The assays were performed as indicated in the kit procedure. Quantitative results for each type of antiphospholipid antibody were interpreted based on the upper and lower limit values specified in the kit procedure. The positive cut-off levels (U/ml) specified for APAs were as follows; ACA IgM: ≥ 7 Positive, ACA IgG and IgA: ≥ 10, aβ2GPI IgM, IgG, and IgA: ≥ 5, Antiprothrombin IgM, IgG, and IgA: ≥ 10, Antiphosphatidylserine IgM and IgG: ≥ 10, Anti-annexin V IgM and IgG: ≥ 5.

Statistical Analysis

The numeric data were coded and entered into the statistical software. SPSS (Statistical Package for Social Science, Chicago, II, USA) 22.0 Windows package program was used for carrying out the statistical analyses. The critical decision-making value was set at 0.05. Descriptive statistics are presented with mean, deviation, frequency, and percentage. Also, a chi-square analysis was performed to examine the number and time of miscarriage by the patient's age. Kolmogorov-Smirnov test was performed to test the assumption of normality of distribution of mean age and a low number of patients. According to the results, it was determined that the distributions of age and number of lows showed normal distribution. Also, due to the sufficient number of samples and the appropriate average standard deviation rates, the tests performed were selected from the tests that show a parametric test approach. Correlation analysis was applied to examine the relationship between the patients' ages and their abortion numbers.

Results

The study was conducted on 120 female patients, who were examined for recurrent pregnancy loss. The mean age of the patients was found to be 26,55±6,52, and their number of miscarriages found to be 3,42±1,38 (Table 1). The distribution of the study patients by the age groups was as follows: 43% (n = 51) were 20-25 years old, 35% (n = 42) were 26-30 years old, 13% (n = 16) were 31-35 years old, and 9% (n = 11) were 36 years or older.

Table 1. Patient characteristics

Age	Mean ± SD
	26.5±6.52
Number of Miscarriage	Mean ± SD
	3.42±1.38
Type of Miscarriage (n:120)	
Early miscarriage	108 %90
Late miscarriage	2 %2
Early and late miscarriage	10 %8

The distribution of the study patients by the number of miscarriages was as follows: 1% (n = 1) of the patients had 7 miscarriages, 1% (n = 1) had 6, 2% (n = 2) had 5, and 6% (n = 7) had 4 miscarriages. Those patients having a history of 4, 5, 6, or 7 miscarriages were brought together to form a single group. Of the patients who had recurrent pregnancy loss, 53% (n = 63) had 3 and 38% (n = 46) had 2 miscarriages. Early miscarriage was observed in 90% (n = 108) of the patients and late miscarriage was observed in 2% (n = 2) of the patients. A history of both early and late miscarriage was present in 8% (n = 10) of the patients.

The number and time of spontaneous abortions were analyzed by the age groups. The number of miscarriages by the age groups was statistically significant. The patients having 2 (n = 21) and 3 (n = 29) miscarriages comprised 46% of the age group of 20-25 years and the proportion of patients having 4 or more miscarriages (n=5) was 45% in the age group of 36 years and older (p=0.03, p<0.05) (Table 2).

Table 2. Relationship between the number of miscarriages and age

Number of Miscarriages		20-25 years	26-30 years	31–35 years	≥36 years	p
2 miscarriages	n	21	17	6	2	0.03*
	%	46%	37%	13%	4%	
3 miscarriages	n	29	23	7	4	
	%	46%	37%	11%	6%	
≥ 4 miscarriages	n	1	2	3	5	
	%	9%	18%	27%	45%	

Those patients having a history of 4, 5, 6, or 7 miscarriages were brought together to form a single group.

It was observed that the mean age of the patients was not at different levels according to their early or late miscarriage (p = 0.16) (Table 3).

Table 3. Relationship between the time of miscarriage and age

Time of miscarriage	n	X±s.d.	p
Early miscarriage	108	26.08±7.54	0,16
Early and Late Miscarriage	10	27.05±9.22	

*Late Miscarriage n=2 excluded from the analysis

There were no significant differences between the age groups and the time of miscarriage (p=0.35, p>0.05) (Table 4).

There was a significant difference between the sensitivity of the tests. ACA (anticardiolipin antibody) total (IgM%/IgG%) seropositivity was 75% (n = 90) and anti-annexin V total (IgM%/IgG%) seropositivity was 50.8% (n = 61). The seropositivity of ACA and that of anti-annexin V were higher than those of other tests. Moreover, the positivity of ACA IgM isotype (42.5%, n = 51) and the seropositivity of anti-annexin V IgM isotype (40%, n = 48) were higher than the other isotypes (p=0.01, p <0.05) (Table 5) (Figure 1).

The distribution of antiphospholipid antibody positivity was different in 4 different age groups. ACA was positive at the lowest percentage (56%) in the 31-35 age group compared to the other age groups. aβ2GPI (anti-beta2-glycoprotein I) showed positivity in more patients in the age groups of 20-25 years (12%) and 31-35 years (13%). Compared to the other age groups, antiphosphatidylserine positivity was the highest (9%) in the patient group at the age of 36 years and older. Antiprothrombin positivity rates were higher in the age group of 31-35 years (38%) and the patient group at 36 years of age or older (36%) compared to the other age groups. Anti-annexin V positive patients were found at the highest ratio (64%) in the patient group at 36 years of age and over compared to the other age groups (p=0.01, p<0.05) (Table 6) (Figure 2).

Table 4. Relationship between the time of miscarriage and age

Time of miscarriage		20-25 years	26-30 years	31–35 years	≥36 years	p
Early miscarriage	n	48	37	13	10	0.35
	%	44%	34%	12%	9%	
Early and Late Miscarriage	n	3	4	3	0	
	%	30%	40%	30%	0%	
Late Miscarriage	n	0	1	0	1	
	%	0%	50%	0%	50%	

The distribution of antiphospholipid antibody positivity was different from the number of miscarriages. Both ACA and aβ2GPI positivity rates were the highest (71% and 13% respectively) in the patients having 3 miscarriages compared to the other groups. Antiphosphatidylserine positivity was also the highest (5%) in the patient group with 3 spontaneous abortions. Antiprothrombin positivity was found at the lowest percentage (22%) in the patient group with 2 spontaneous abortions. Anti-annexin V was positive at the highest percentage (60%) in patients with 3 spontaneous abortions (p=0.01, p<0.05) (Table 7) (Figure 3).

Table 5. IgM-IgG-IgA analysis of antiphospholipid antibodies in the patients

APAs		IgM / IgG / IgA	IgM	IgG	IgA	IgG+IgM+IgA	p
Early miscarriage	n	90	51	28	0	11	
	%	75%	42.5%	23.3%	0%	9.2%	
Early and Late Miscarriage	n	12	2	3	6	1	
	%	10%	1.7%	2.5%	5.0%	0.8%	
Late Miscarriage	n	4	4	0	0	0	0.01*
	%	3.3%	3.3%	0%	0%	0%	
Anti-prothrombin	n	30	21	4	4	1	
	%	25%	17.5%	3.3%	3.3%	0.8%	
Anti-annexin V	n	61	48	8	0	5	
	%	50.8%	40.0%	6.7%	0%	4.1%	

Table 6. The frequencies of antiphospholipid antibodies by the age groups

			Ages				
APAs		**Positive**	**20-25 years**	**26-30 years**	**31–35 years**	**≥36 years**	**p**
ACA	n	90	37	36	9	8	
	%	75%	73%	86%	56%	73%	
aβ2GPI	n	12	6	3	2	1	
	%	10%	12%	7%	13%	9%	
antiphosphatidylserine	n	4	2	0	1	1	0.01*
		3.3%	4%	0%	6%	9%	
Anti-prothrombin	n	30	9	11	6	4	
	%	25%	18%	26%	38%	36%	
Anti-annexin V	n	61	25	20	8	7	
	%	51%	49%	48%	50%	64%	

*Chi-square analysis

Table 7. Antiphospholipid antibody positivity by the miscarriage number

APAs		Positive	2 miscarriage	3 miscarriage	≥4 miscarriage	p
ACA	n	90	29	45	7	
	%	75%	63%	71%	64%	
aβ2GPI	n	12	3	8	1	
	%	10%	7%	13%	9%	
Antiphosphatidylserine	n	4	1	3	0	0.01*
	%	3.30%	2%	5%	0%	
Anti-prothrombin	n	30	10	17	3	
	%	25%	22%	27%	27%	
Anti-annexin V	n	61	19	38	4	
	%	51%	41%	60%	36%	

*Chi-square analysis

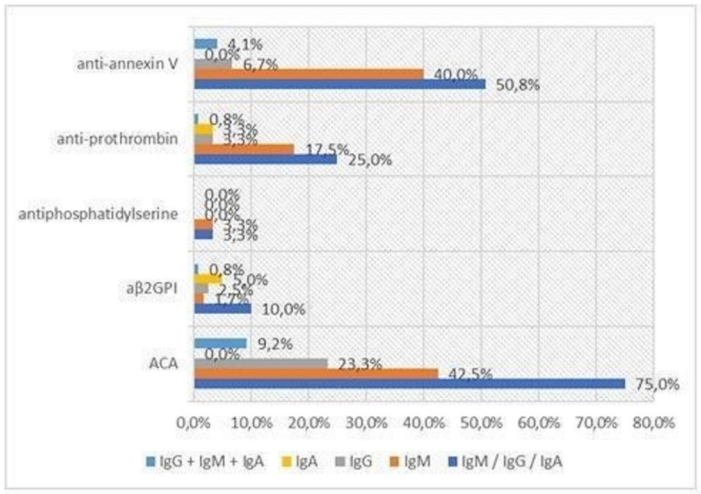

Figure 1. Test analysis results by the age groups

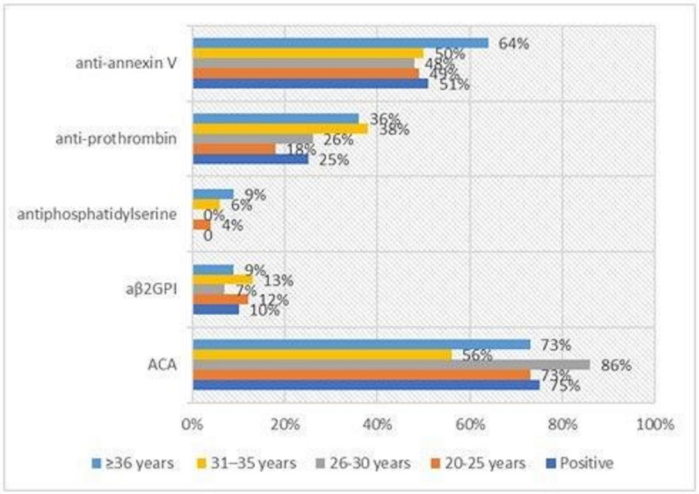

Figure 2. The frequencies of antiphospholipid antibodies by the age groups

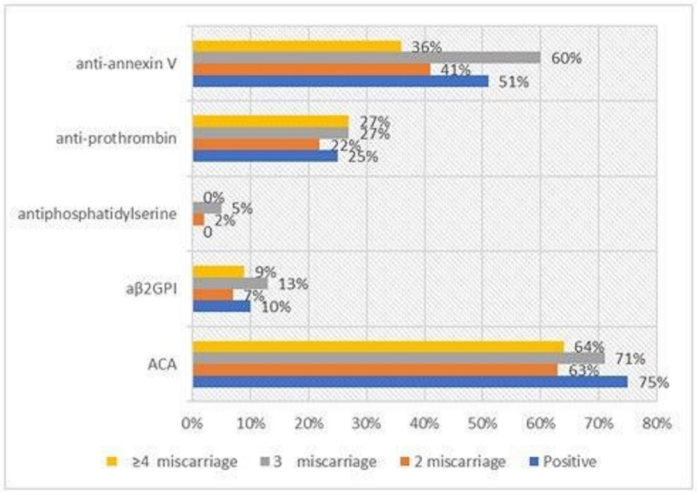

Figure 3. Antiphospholipid antibody positivity by the miscarriage number

Discussion

The coexistence of recurrent miscarriage and positivity of antiphospholipid antibodies is continuously stressed as characteristics of APS. If we think that 80% of the miscarriages are in the first trimester; APS may be considered as the most important treatable cause of recurrent first-trimester miscarriage. Also, It is a major cause of early-onset preeclampsia and intrauterine growth restriction due to abnormal placental function. It is observed that APAs may not directly affect the early stages of embryonic implantation in this patient group but it may affect subsequent trophoblast migration and implantation based on the time of miscarriage [9]. Phospholipids are found in the cell membrane and they help the transformation process into syncytiotrophoblasts. However, disorders in implantation and trophoblast defects develop in association with the antiphospholipid antibodies, resulting in miscarriage. The relationship between placental dysfunction and antiphospholipid antibody positivity has been the focus of many studies and is still a matter of debate [6].

This study investigated the role of APAs in the etiology of recurrent miscarriage. The positivity of antiphospholipid antibodies was analyzed in four age groups by the number and time of spontaneous abortions. In our study, statistically significant differences were found in the number of abortions by the age groups of the patients. In this study, it was observed that pregnant women who had 2 and 3 miscarriages were mostly in the age range of 20 to 30 years. Women with 4 or more miscarriages were more likely to be 36 years or older. This finding might result from the age of pregnancy mostly occurring from 20 and 30 years of age and also due to the high ratios of antiphospholipid antibody positivity in women having 2 and 3 miscarriages. This finding was similarly positive in the pregnant women group having 4 or more miscarriages, showing a high patient ratio with antiphospholipid antibody positivity. This study also examined the relationship between the time of miscarriage and the age of the patients. It was observed that 90% of the patients had an early miscarriage. In the literature, the rate of early miscarriage was 80% [3]. This study could not find out a relationship between the age groups and early or late spontaneous abortions. This finding may be due to the factors associated with the time of emergence of the antiphospholipid antibodies, suggesting that the time of APAs synthesis is an independent parameter of the age of the patient. Franklin et al. conducted a study on 79 recurrent miscarriage patients in 2002 and they examined the role of APAs in women with 2 or more miscarriages by the pathophysiology and obstetric history. Their study could find no significant associations among the patient's age, previous pregnancies, and previous spontaneous abortions based on the comparison of the demographic characteristics of the patients [10]. Another study on APS patients was conducted by Van den Boogaard et al., finding out no significant differences by the clinical obstetric parameters. That study could not find out a significant variable in association with the gestational age, the number of spontaneous abortions, and the type of positive antiphospholipid antibodies. Furthermore, that study demonstrated that women with two consecutive miscarriages are at the same risk as women with three or more spontaneous abortions [11]. Currently, it is not known whether diagnostic test results for APS can be supported by clinical determinants derived from obstetric history [11].

Antiphospholipid antibody positivity is associated with recurrent miscarriage and is used as diagnostic criteria. Also, determining APA positivity is important in the evaluation of autoimmune pregnancy losses. However, the results are highly variable among patients [12]. In our study, APA positivity was different among the age groups. While the APAs were positive in patients having 2

spontaneous abortions, the ratio of patients with APA positivity increased in patients with 3 miscarriages. This finding shows that the ratio of patients with APA positivity increases with the increasing number of spontaneous abortions, explaining the direct relationship between recurrent miscarriage and APA positivity. When the percentage of patients with positive APA levels were examined by the number of spontaneous abortions, significantly high ratios of patients with especially the positivity of ACA and the positivity of anti-annexin V antibodies were observed in the group of pregnant women with 2, 3, 4, and more spontaneous abortions. This finding indicates that; of the APAs, mostly ACA and annexin V antibodies were associated with recurrent miscarriage. However, in patients with recurrent miscarriage, ACA and annexin V antibodies should be tested and these antibodies should be included in diagnostic tests.

Several studies have found out statistically significant relationships between ACA and RPL. It has been reported that the risks of fetal growth delay and preeclampsia increase in pregnant women with ACA positivity. These antibodies cause thrombosis of placental vessels. Nielsen et al. performed a study on 147 patients in 2005, reporting a 41% ACA positivity rate based on ELISA test results. Of these positive ACA antibodies, 32% were ACA IgM and 13% were ACA IgG. It has been found out that ACA IgM is more sensitive than ACA IgG and, consequently, ACA IgM is more strongly correlated with recurrent miscarriage compared to ACA IgG [13]. In our study, ACA was found to be 75% positive. Of these ACA positive antibodies, 42.5% were ACA IgM and 23.3% were ACA IgG. ACA IgM and ACA IgG positivity were found in combination with a ratio of 9.2%. Based on our study results, we can argue that ACA IgM antibodies are found with higher ratios and should be considered as the main parameter for making the diagnosis.

IgM antibodies in these patients are found in high concentrations in the primary immune response but IgM positivity in APS should not be considered transient as it is in infectious diseases. IgM positivity is long-term in APS. The IgM isotype of APAs is the most important biological marker for recurrent miscarriage and is associated with pathogenicity. A literature review shows that the IgM isotype of antiphospholipid antibodies is considered a potential clinical risk for APS and it shows a more significant correlation with thrombosis. Despite the already defined diagnostic criteria for APS, difficulties remain in identifying patients at risk for thrombosis. A study reviewed 1288 articles in the literature in Pubmed and reported the results by the antibody types (ACA, aβ2GPI), the isotypes (IgM, IgG), and the type of thrombosis. It has been reported that strong evidence is obtained about the relationship between thrombosis and the ACA IgM isotype and the aβ2GPIantibodies [14]. aβ2GPI inhibits the proliferation of trophoblasts and maternal spiral artery invasion. Consequently; large necrosis, infarcts, and thrombosis occur in the placenta [14]. We believe that this study of ours will be guiding for the researches in developing a better classification system in the future.

Most antiphospholipid antibody studies have focused on the detection of IgM and IgG isotypes and a few studies are reporting the pathogenic importance of the IgA isotype [15]. The association between APS and IgA is observed, albeit slightly. This study also shows that IgA isotypes are less commonly found positive compared to the other antibody isotypes [15].

Several pathogenic mechanisms have been identified in APS, reporting the important role of annexin V. Annexin V is a phospholipid-binding protein that is highly expressed by the vascular endothelium and placental syncytiotrophoblasts, showing strong anticoagulant characteristics in antithrombotic activities. Annexin V, also known as the placental anticoagulant protein, is a strong vascular anticoagulant protein. APAs reduce annexin V quantities on cell surfaces and consequently accelerate coagulation in trophoblasts and endothelial cells. This may cause thrombotic events and pregnancy loss in APS [16]. Studies have reported that the anti-annexin V positivity results in placentation disorders in recurrent miscarriages [17]. It has been reported that anti-annexin V plays a role in recurrent miscarriage [18]. Bizzaro et al. conducted a study in 2005, examining samples from 1038 patients by the ELISA method. They reported that 25% of the patients had anti-annexin V IgG and 27.5% had anti-annexin V IgM [19]. In our study, anti-annexin V was found to be 50.8% positive in total. While 40% of these were anti-annexin V IgM, 6.7% were anti-annexin V IgG. This high ratio of anti-annexin V positivity in our study and the findings from other studies in the literature suggest that investigation of the role of anti-annexin V in miscarriage will unfold a better understanding of the placental physiology.

Phosphatidylserine is a phospholipid on the inner surface of the cell membrane and anti-phosphatidylserine autoantibodies have been detected in patients with APS. Studies in animal models show that antiphosphatidylserine antibodies cause pathogenic events in trophoblasts. One study reported the positivity rates for antiphosphatidylserine and aβ2GPI antibodies as 4% and 12%, respectively; suggesting that antiphosphatidylserine and aβ2GPI antibodies should be considered as risk factors in recurrent miscarriage [20]. Velayuthaprabhu et al. in 2005 found the ACA IgA and antiphosphatidylserine IgG levels were 40% and 19%, respectively, in women with recurrent miscarriage [21]. A study has reported that anti-prothrombin IgG and IgM isotypes may be associated with pregnancy loss in recurrent miscarriage. These antibodies are suggested to play an important role in the etiology and pathogenesis of miscarriage [22]. Anti-prothrombin and aβ2GPI antibodies have prothrombotic properties and their potential relationships with recurrent miscarriage have been reported [23]. Prothrombin (coagulation factor II) is a plasma protein having a phospholipid structure and it triggers the conversion of fibrinogen to fibrin. Anti-prothrombin antibodies are associated with thrombosis in patients with APS. Prothrombin antibodies cause prothrombin aggregation on the surface of the activated cell. Singh et al. study on 50 patients with a history of recurrent miscarriage found the positivity rates for aβ2GPI and anti-prothrombin as 12% and 6% respectively; highlighting the relationship between APS and thrombotic events in pregnant women. That finding shows the varying rates of APA positivity in patients with recurrent miscarriage [24]. Our study found out that the frequencies of anti-prothrombin, aβ2GP, and antiphosphatidylserine positivity were 25%, 10%, and 3.3% respectively. In light of our study findings and the results reported by previous studies in the literature, we think that these antibodies (anti-prothrombin, aβ2GPI, and antiphosphatidylserine) will be guiding for the management of spontaneous abortions and enable to make the diagnosis easily.

The data suggest that ACA and anti-annexin V may be more strongly associated with APS and may be more common in this disorder. The positivity for aβ2GPI, anti-prothrombin, and antiphosphatidylserine are observed at low rates. In our study, we observed that ACA and anti-annexin V antibodies correlated better with the clinical symptoms of the patients in our geographical region. Our results showed the major role played by APAs in recurrent miscarriage.

Conclusion

This study may guide for obtaining a better understanding of the etiology of APS in recurrent miscarriage in women having no history of chromosomal or anatomic malformations in the fetus. It can be argued that ACA and anti-annexin V antibodies play an important role in the etiology of recurrent miscarriage. Therefore, they are highly suitable diagnostic markers for recurrent miscarriage. Anti-prothrombin, aβ2GPI, and antiphosphatidylserine antibodies may be seen at low rates in the etiology of miscarriage. We think that the investigation of these antibodies may help in diagnosis and treatment. IgM and IgG isotypes of antiphospholipid antibodies were observed more frequently than the IgA isotype. In particular, the IgM isotype is an important marker and should be used as a diagnostic tool; however, the role of the IgA isotype in APS is very low.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
This observational study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and permission was obtained from Ethics Committee of Afyon Kocatepe University Faculty of Medicine for the use of patient data for publication purposes (Date-Decision no: 14.09.2007-115).

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