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CLINICAL ARTICLE

Gynecology

Vascular dysfunction in women with recurrent pregnancy loss: Possible association with antiphospholipid antibodies

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Abstract

Objective: Antiphospholipid antibodies (aPL) are recognized to have a pivotal role in recurrent pregnancy loss (RPL) and cardiovascular disease. Therefore, we assessed the vascular function of women with RPL and examined the association with each type of aPL.

Methods: In this retrospective study, 569 women with RPL and 55 healthy women who had never experienced pregnancy loss were recruited. We performed blood tests for aPL and acceleration plethysmography (APG) to evaluate peripheral vascular function.

Results: The differential pulse wave index (DPI), indicating vascular elasticity, was significantly lower in women with RPL (115.6 ± 4.1) compared to the control group (117.0 ± 2.3). DPI in RPL women with anti-β_2 glycoprotein I (aβ_2GPI) IgG was significantly lower than those without. Remained blood volume (RBV), indicating post-vasoconstriction blood content, was significantly higher in RPL women with aβ_2GI IgG than in those without. Regression analysis showed aβ_2GPI IgG and body mass index (BMI) linked negatively with DPI.

Conclusion: Women with RPL have subclinical vascular dysfunction even at reproductive age. It is possible that aβ_2GPI IgG is associated with vascular dysfunction in RPL women.

KEYWORDS
accelerated photoplethysmography, anti-β_2 glycoprotein I antibody, antiphospholipid antibodies, recurrent pregnancy loss, vascular dysfunction

1 | INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies.[1] The causes of RPL are categorized as genetic, anatomic, endocrinologic, immunologic, microbiologic, environmental, and so on.[2,3] Antiphospholipid antibodies (aPL) are known to play a central role in pregnancy loss.[4] These antibodies may interfere with the implantation and development of the embryo, leading to miscarriage.[5] aPL may lead to the formation of blood clots, which cause placental dysfunction, fetal growth restriction (FGR) and fetal death.[6]

It has been known that antiphospholipid antibodies (aPL) can interfere with the normal function of endothelial cells and affect

microcirculation.[7,8] In our previous study, the elevation of pulsatility index (PI), which is a measurement used in the field of vascular ultrasound, indicating impaired uterine perfusion was observed in non-pregnant women with RPL.[9] We also reported that increased PI of the uterine arteries was prominent in RPL women with aPL before pregnancy[10] and during pregnancy.[11]

We reported that arterial stiffness of the large vascular vessels assessed by pulse wave velocity (PWV) was increased in women with RPL.[12] We also reported elevated plasma levels of adrenomedullin, a biomarker of systematic vascular disorders, in women with RRL, especially in those with aPL.[10]

Antiphospholipid antibodies are also known to be associated with systemic vascular disorders. Some studies have shown that women who experience pregnancy loss including stillbirth may be at an increased risk of developing cardiovascular diseases in the future.[13–15] Women with RPL, especially those with aPL, are likely to develop cardiovascular events such as stroke later in their lives.[16] Therefore, it may be important to evaluate systemic vascular dysfunction in women with RPL, although they are relatively young.

aPL are a heterogeneous group of autoantibodies that are directed against proteins that bind phospholipids, such as lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), anti-β_2 glycoprotein I antibodies (aβ_2GPI), and antiphosphatidylserine antibodies (aPS) and antiphosphatidylethanolamine antibodies (aPE),[7,17–19] The pathogenesis of aPL may vary depending on the types and target phospholipids and/or glycoproteins. Although the clinical significance of the various types of aPL in cardiovascular diseases is of increasing interest,[20] the pathogenic effects of them have not been fully elucidated.

There are various techniques to assess vascular function, including flow-mediated dilation (FMD), plethysmography, and acceleration plethysmography (APG). The main purpose of APG is to observe the mechanical movement of the heart and the kinetics of blood flow.[21] APG can yield important information about vascular function, which is obtained from the second-order derivatives of the photoplethysmographic (PPG) signal.[22,23] Previous studies have suggested that various indices of APG can be used to estimate the risk of chronic heart disease in the general population.[24] The parameters of the APG waveform can be used to provide clinical insight into arterial stiffness, arterial compliance, vascular aging, and the mental stress associated with the autonomic nervous system.[25–27]

Among indices of APG, the differential pulse wave index (DPI), is used to assess the state of the microcirculation and it provides information about the health of small blood vessels. It is commonly used clinically to assess peripheral vascular function and to detect blood flow abnormalities such as arterial disease, diabetes-related vascular complications, or other circulatory disorders.[28,29] DPI is closely related to blood vessel aging and its values decline with age, and a higher DPI reflects improved vascular health.[23] Stress power (SP) reflects blood vessel extensibility. The higher the absolute value of SP, the better the vascular condition. Relative blood volume (RBV) denotes the volume of blood remaining after contraction of blood vessels; therefore, a low absolute value means that blood flows well into the vessels. Blood vessel tension (BVT) is a measure of the elasticity in contracting and releasing the blood vessels. In general, the greater the value of BVT, the better the condition of the vessels.[23,30]

The aim of this study was to evaluate vascular function in women with RPL, analyze its relationship with aPL and specify the type of aPL significantly associated with vascular dysfunction in women with RPL.

2 | MATERIALS AND METHODS

2.1 | Study participants

In this retrospective study, data were collected from 569 RPL women who visited the outpatient clinic of Okayama University Hospital between 2012 and 2021. The control group consisted of 55 healthy women who had their own children and had not experienced pregnancy loss. The women who smoked were excluded from the subjects. This study was carried out with the approval of the ethics committee of the Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University (1511–009). All procedures were performed after the informed consent of each subject.

2.2 | Blood tests

Blood tests for glucose tolerance, lipid metabolism, and aPL were carried out at the initial infertility consultation. Blood glucose was measured by glucose oxidase assay (Glucoroder-Nx, Sinotest, Mitsubishi Kagaku Iatron, Tokyo, Japan) and serum insulin levels were measured by EIA (Sysmex Corporation, Kobe, Japan). Total cholesterol, triglycerides, free cholesterol, high-density lipoprotein-cholesterol (HDL-C) (Kyowa Medix, Tokyo, Japan), low-density lipoprotein-cholesterol (LDL-C) (Daiichi Chemicals, Tokyo, Japan), cholesterol ester, phospholipids, lipoprotein b (Wako Pure Chemicals, Osaka, Japan) and free fatty acid (Mitsubishi Kagaku Iatron, Tokyo, Japan) were determined in serum using reagents available on the market and the Hitachi 7170 analyzer (Hitachi, Tokyo, Japan). Anticardiolipin antibodies (aCL) IgG and IgM were detected on ELISA (MBL, Nagoya, Japan) and anti-β_2 glycoprotein I antibodies (aβ_2GPI) IgG were measured by ELISA (Yamamsa, Choshi, Japan), the antiphosphatidylethanolamine antibody detected on ELISA (SRL, Tokyo, Japan) and antiphosphatidylserine antibodies detected on ELISA (Finggal-Link, Tokyo, Japan). LAC was analyzed by dilution of Russell's viper venom time (Medical & Biological Laboratories, Nagoya, Japan).

2.3 | Acceleration plethysmography (APG)

APG was performed using a pulse analyzer (TAS9, YKC Inc., Tokyo, Japan).[21] Subjects rested for 10 min in a quiet room prior to recording a surface electrocardiogram (ECG) in the sitting position for 5 min. The transmission method involves the amount of infrared light absorbed in the fingertip region (fingertip volume pulse wave) to measure the degree of blood in the blood vessels due to cardiac output.

TABLE 1 Clinical features of women with RPL.

	Recurrent pregnancy loss						Control[g] ($n=55$)	*p* value				
	Total[a] ($n=569$)	Pregnancy loss			Stillbirth							
		2[b] 279 (49.0%)	3[c] 184 (32.4%)	≥4[d] 106 (18.6%)	With[e] 146 (25.7%)	Without[f] 423 (74.3%)		a versus g	b versus c	b versus d	c versus d	e versus f
Age (years)	35.0 ± 4.7 [22–45]	34.5 ± 4.8 [22–44]	35.5 ± 4.9 [23–45]	35.5 ± 4.2 [26–44]	34.9 ± 4.7 [23–44]	35.0 ± 4.8 [22–45]	34.0 ± 6.2 [24–46]	0.236	0.052	0.123	0.914	0.823
BMI	21.3 ± 3.3	21.4 ± 3.3	21.5 ± 3.4	21.1 ± 3.5	21.4 ± 3.4	21.3 ± 3.3	21.2 ± 3.0	0.954	0.657	0.174	0.124	0.893
Insulin (μU/mL)	7.1 ± 7.0	7.7 ± 8.7	6.3 ± 4.2	6.8 ± 5.7	10.8 ± 13.5	6.4 ± 4.7			0.581	0.488	0.849	0.916
HOMA-R	1.9 ± 2.6	2.1 ± 3.4	1.7 ± 1.5	1.7 ± 1.7	3.5 ± 5.3	1.6 ± 1.6			0.947	0.568	0.918	0.802
Total cholesterol (mg/dL)	189.1 ± 30.4	186.9 ± 27.4	190.9 ± 31.2	191.9 ± 36.4	187.9 ± 24.1	189.5 ± 32.3			0.479	0.563	>0.99	0.836
HDL-C (mg/dL)	68.2 ± 16.5	69.7 ± 17.3	66.7 ± 17.4	67.4 ± 12.2	63.6 ± 11.7	69.5 ± 17.4			0.155	0.739	0.426	**0.008**
LDL-C (mg/dL)	111.6 ± 28.2	114.5 ± 31.6	110.6 ± 24.9	106.5 ± 24.0	113.0 ± 34.8	111.3 ± 26.3			0.758	0.388	0.683	0.829
Triglyceride (mg/dL)	73.5 ± 34.3	74.8 ± 39.4	73.3 ± 31.1	70.3 ± 24.3	74.9 ± 33.8	73.0 ± 34.6			0.763	0.835	>0.99	0.598

Note: BMI, calculated as weight in kilograms divided by the square of height in meters. Mean ± S.D.[range]. Superscript a-g indicates the respective groups. Bold values indicates statistically significant of $p < 0.05$.

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; HOMA-R, homeostasis model assessment-ratio; LDL-C, low-density lipoprotein-cholesterol; RPL, recurrent pregnancy loss.

TABLE 2 APG indices of women with RPL.

	Recurrent pregnancy loss						Control[g] (n = 55)	p value							
	Total[a] (n = 569)	Pregnancy loss			Stillbirth			a versus g	b versus c	b versus d	b versus g	c versus d	c versus g	d versus g	e versus f	e versus g	f versus g
		2[b] 279 (49.0%)	3[c] 184 (32.3%)	≥4[d] 106 (18.6%)	With[e] 146 (25.7%)	Without[f] 423 (74.3%)											
DPI	115.6 ± 4.1	115.4 ± 4.4	115.6 ± 4.0	116.1 ± 3.3	115.2 ± 4.6	115.7 ± 3.8	117.0 ± 2.3	**0.009**	0.941	0.220	**0.008**	0.275	**0.009**	0.132	0.262	**0.005**	**0.017**
SP	−71.4 ± 17.9	−71.7 ± 18.3	−70.9 ± 18.3	−70.4 ± 16.1	−72.1 ± 20.1	−71.2 ± 17.1	−70.5 ± 13.9	0.663	0.358	0.868	0.719	0.534	0.818	0.788	0.735	0.787	0.911
RBV	−25.4 ± 17.7	−24.2 ± 19.2	−-5.2 ± 16.8	−29.0 ± 14.6	−24.2 ± 19.3	−25.8 ± 17.1	−28.1 ± 13.6	0.421	0.51	0.069	0.193	0.227	0.345	0.935	0.419	0.223	0.402
BVT	−40.5 ± 16.4	−40.9 ± 17.5	−40.1 ± 15.6	−40.1 ± 14.9	−39.9 ± 16.9	−40.7 ± 16.2	−41.0 ± 14.1	0.819	0.665	0.875	0.827	0.787	0.615	0.824	0.679	0.622	0.794

Note: Mean ± S.D.[range]. Superscript a-g indicates the respective groups. Bold values indicates statistically significant of $p < 0.05$.

Abbreviations: APG, acceleration photoplethysmogram; BVT, blood vessel tension; DPI, differential pulse wave index; RBV, relative blood volume; RPL, recurrent pregnancy loss, SP, stress power.

Statistically significant differences are shown in bold.

2.4 | Statistical analysis

Statistical analysis was performed with SPSS version 27.0 (IBM Corp. Armonk. NY, USA). The data were examined using the *F*-test and the Kolmogorov–Smirnov test and found to be normally distributed. All subsequent statistical analyses were performed using student's *t*-test or paired t-test. Linear regression analysis was used to assess the effect of various factors on each APG index, and *p* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Clinical features of women with RPL

There were no significant differences in age and body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) between the control and RPL groups (Table 1). In addition, there were no significant differences between the control and the groups of RPL in insulin, homeostasis model assessment-ratio (HOMA-R), total cholesterol, low-density lipoprotein-cholesterol (LDL-C) and triglyceride. Among women with RPL, high-density lipoprotein-cholesterol (HDL-C) in women with stillbirth were significantly lower than in women without stillbirth ($p = 0.008$).

3.2 | APG indices of women with RPL

The DPI was significantly higher in women with RPL ($p = 0.009$), including those with two, three pregnancy losses, and those with or without stillbirths, compared to the control group ($p = 0.008$, $p = 0.009$, $p = 0.005$ and $p = 0.017$, respectively) (Table 2).

3.3 | Age and APG indices in women with RPL

Absolute values of SP were significantly higher in women aged 20–29 and 30–39 than in those aged ≥40 group ($p < 0.001$ and $p < 0.001$, respectively) (Table 3). In addition, absolute values of RBV were significantly lower in the 20–29 age group than in the 30–39 age groups and ≥40 age group ($p = 0.047$ and $p = 0.003$, respectively), and BVT was significantly higher in the 20–29 and 30–39 age groups than in the ≥40 age group ($p = 0.029$, $p < 0.001$ and $p = 0.004$, respectively).

3.4 | Various risk factors and APG indices in women with RPL

Women in the RPL groups were categorized according to BMI into underweight (<18.5), normal range (18.5–25), and overweight groups (≥25) (Table 3). Among RPL women, the absolute values of SP were significantly higher in the underweight group compared to the overweight group and normal range group ($p = 0.028$ and

TABLE 3 Various risk factors for vascular function and APG indices in women with RPL.

	DPI	*p* value	SP	*p* value	RBV	*p* value	BVT	*p* value
Age								
20–29[a] (*n* = 86, 15.1%)	115.5 ± 3.7	a versus b:0.577	−74.6 ± 17.0	a versus b:0.170	−21.5 ± 18.6	**a** versus **b:0.047**	−35.7 ± 15.1	**a** versus **b:0.029**
30–39[b] (*n* = 374, 65.7%)	115.7 ± 4.0	a versus c:0.902	−72.2 ± 18.0	**a** versus **c:<0.001**	−25.2 ± 17.9	**a** versus **c:0.003**	−40.3 ± 16.7	**a** versus **c:<0.001**
≥40[c] (*n* = 109, 19.2%)	115.3 ± 4.6	b versus c:0.496	−66.0 ± 17.2	**b** versus **c:<0.001**	−29.0 ± 15.4	b versus c:0.051	−44.9 ± 15.3	**b** versus **c:0.004**
BMI								
Underweight[a] (*n* = 100, 17.6%)	115.9 ± 3.3	a versus b:0.893	−74.9 ± 20.6	**a** versus **b:0.028**	−22.8 ± 18.9	a versus b:0.191	−38.2 ± 18.3	a versus b:0.138
Normal[b] (*n* = 398, 69.9%)	115.7 ± 3.9	a versus c:0.252	−71.0 ± 16.8	**a** versus **c:0.023**	−25.3 ± 16.7	**a** versus **c:0.005**	−39.9 ± 15.8	**a** versus **c:<0.001**
Overweight[c] (*n* = 71, 12.5%)	114.8 ± 5.7	b versus c:0.211	−68.8 ± 19.2	b versus c:0.390	−29.4 ± 20.8	**b** versus **c:0.013**	−47.0 ± 15.8	**b** versus **c:<0.001**
sBP								
>120 (*n* = 69, 12.1%)	114.9 ± 4.8	0.136	−67.7 ± 20.4	**0.032**	−22.7 ± 22.1	0.651	−46.2 ± 16.4	**0.002**
≤120 (*n* = 500, 87.9%)	115.7 ± 3.9		−71.9 ± 17.4		−25.8 ± 17.0		−39.7 ± 16.3	
dBP								
>80 (*n* = 23, 4.0%)	114.9 ± 4.8	0.710	−64.8 ± 18.4	0.079	−21.3 ± 24.9	0.618	−48.5 ± 17.1	**0.016**
≤80 (*n* = 546, 96.0%)	115.6 ± 4.0		−71.7 ± 17.8		−25.6 ± 17.3		−40.1 ± 16.3	
HOMA-R								
≥2.5 (*n* = 12, 12.9%)	116.8 ± 4.1	0.952	−77.9 ± 21.8	0.657	−27.4 ± 23.9	0.581	−36.8 ± 16.5	0.681
<2.5 (*n* = 81, 87.1%)	116.2 ± 3.2		−71.0 ± 17.2		−26.8 ± 15.0		−40.5 ± 16.5	
HDL-C								
≤40 (*n* = 2, 0.9%)	113.5 ± 2.1	n.a.	−57.5 ± 10.6	n.a.	−28.0 ± 4.2	n.a.	−43.5 ± 16.3	n.a.
>40 (*n* = 234, 99.1%)	115.6 ± 4.1		−72.5 ± 18.7		−23.7 ± 17.7		−39.5 ± 15.8	
LDL-C								
≥140 (*n* = 29, 12.4%)	115.3 ± 3.4	0.312	−70.4 ± 15.8	0.296	−31.0 ± 18.0	**0.008**	−42.1 ± 16.2	0.310
<140 (*n* = 204, 87.6%)	115.8 ± 3.8		−72.7 ± 19.1		−22.6 ± 17.4		−39.1 ± 15.7	

Note: BMI, calculated as weight in kilograms divided by the square of height in meters. Mean ± S.D. [range]. Superscript a-c indicates the respective groups. Bold values indicates statistically significant of $p < 0.05$.

Abbreviations: APG, acceleration photoplethysmogram; BMI, body mass index; BVT, blood vessel tension; dBP, diastolic blood pressure; DPI, differential pulse wave index; HDL-C, high-density lipoprotein-cholesterol; HOMA-R, homeostasis model assessment-ratio; LDL-C, low-density lipoprotein-cholesterol; n.a., not applicable; RPL, recurrent pregnancy loss; RBV, relative blood volume; sBP, systolic blood pressure; SP, stress power.

$p = 0.023$, respectively). The absolute values of RBV were significantly higher in the overweight group compared to the underweight and normal range groups ($p = 0.005$ and $p = 0.013$, respectively). The BVT was significantly lower in the overweight group compared to the underweight and normal range groups ($p < 0.001$ and $p < 0.001$, respectively).

Among women with RPL, the analysis of the APG index concerning risk factors affecting vascular function indicated that the absolute values of SP were significantly lower in women with systolic blood pressure (sBP) >120 mmHg than those with ≤120 mmHg ($p = 0.032$). Conversely, BVT was significantly lower in women with sBP >120 mmHg than in women with sBP ≤120 ($p = 0.002$), and BVT was significantly lower in women with diastolic blood pressure (dBP) >80 mmHg than in women with dBP ≤80 mmHg ($p = 0.016$).

The absolute values of RBV were significantly higher in women with abnormal LDL-C (≥140 mg/dL) than those with normal LDL-C (<140 mg/dL) ($p = 0.008$).

3.5 | Various types of antiphospholipid antibodies and APG indices in women with RPL

Among women with RPL, the group with aβ_2GPI IgG exhibited significantly lower DPI ($p = 0.002$) and significantly lower absolute value of RBV compared to the group without it ($p = 0.006$) (Table 4). Additionally, DPI was significantly higher in the group with kininogen-dependent antiphosphatidylethanolamine antibody IgG [K (+) aPE IgG] than in the group without It ($p = 0.017$), while it was significantly lower in the group with kininogen-independent antiphosphatidylethanolamine antibody IgM (K [−] aPE IgM) than in the group without it ($p = 0.010$). RPL women with at least one aPL had significantly lower DPI than those without aPL or control women ($p = 0.013$ and $p < 0.001$, respectively).

3.6 | Analysis of factors associated with APG indices

This study employed linear regression analysis to evaluate the impact of various factors on APG indices. For DPI, independent variables included age, BMI, presence of aβ_2GPI IgG and presence of K (−) aPE IgM antibodies. Among these, the predictor variable BMI had a significant negative correlation with DPI, with a regression coefficient of −0.26 (95% CI: −0.44 to −0.09). Similarly, aβ_2GPI IgG was negatively associated with DPI, with a regression coefficient of −2.57 (95% CI: −3.94 to −1.21). Through multiple regression analysis, it is possible to obtain: DPI = 122.38−0.26× (BMI) −2.57× (aβ_2GPI IgG [0: negative,1: positive]).

Regarding SP, the independent variables included age, BMI, and sBP. Age was positively correlated with SP, with a regression coefficient of 0.52 (95% CI: 0.21–0.83). The multiple regression analysis gives: SP = −111.37 + 0.52 (age).

In the case of RBV, the independent variables included age, BMI and LDL-C. There was a negative correlation between RBV and LDL-C, with a regression coefficient of −7.25 (95% CI: −14.31 to −0.20). The regression analysis gives: RBV = −11.52–7.25 (LDL-C [0: normal, 1: abnormal]).

Finally, the independent variables for BVT included age, BMI and sBP. The regression coefficient for age was −0.57 (95% CI: −0.85 to −0.29), for BMI was −0.48 (95% CI: −0.90 to −0.05), and for sBP was −0.20 (95% CI: −0.33 to −0.07). Age, BMI, and sBP were significantly negatively correlated with BVT. The multiple regression analysis yields: BVT = 11.66−0.57(age) −0.48(BMI) −0.20(sBP).

4 | DISCUSSION

In the present study, RPL women had lower DPI than women in the control group, suggesting that women with RPL have poorer vascular function than control women even though it is subclinical level. DPI is a measure of the degree of atherosclerosis and changes in vascular elasticity, these indicators may provide information about health status in assessing atherosclerosis, vascular function, and hemodynamics, and are important to detect and prevent cardiovascular diseases such as cardiovascular disorders, myocardial infarction, and cerebral infarction.[28,29]

It has been reported that women with RPL had vascular dysfunction. We have reported elevated uterine artery flow resistance in women with unexplained RPL and increasing systemic arterial stiffness.[9] In our previous study, impaired uterine perfusion was observed in a subgroup of women with RPL, and unknown factors were associated with pregnancy loss.[11]

Adrenomedullin is a cyclic peptide with cAMP-mediated vasodilatory properties, which is released when the vascular endothelium is damaged, and elevated adrenomedullin in the blood indicates vascular injury. In our previous study, RPL women had high plasma adrenomedullin levels in the mid-luteal phase of the non-pregnant cycle. Furthermore, plasma adrenomedullin concentrations are positively correlated with uterine artery PI and significantly higher compared with control women.[10]

Related research has shown that women who experienced RPL had a substantially increased risk of vascular dysfunction.[8] These are consistent with the fact that the RPL women in this study had vascular dysfunction compared with the control women.

Women with at least one aPL antibody had a lower DPI compared to the women without any antibodies. Those with aβ_2GPI IgG antibodies had lower DPI compared to the group without aβ_2GPI IgG antibodies. In multiple regression analysis, DPI was negatively correlated with BMI and aβ_2GPI IgG. This study demonstrates that women with RPL have vascular dysfunction and are associated with aPL.

Impaired uterine perfusion may be associated with pregnancy loss and/or FGR and hypertensive disorders of pregnancy (HDP) while those are frequently observed in women with aPL.[8] Previous studies have reported that HDP and/or FGR are linked to trophoblastic

TABLE 4 Various types of antiphospholipid antibodies and APG indices in women with RPL.

		DPI	*p* value	SP	*p* value	RBV	*p* value	BVT	*p* value
LAC	+	116.5 ± 3.4	0.612	−76.7 ± 23.4	0.688	−20.6 ± 16.4	0.314	−33.9 ± 14.1	0.309
	−	115.7 ± 3.9		−71.7 ± 17.6		−25.4 ± 17.0		−39.7 ± 16.4	
aCL IgG	+	115.6 ± 3.4	0.702	−67.9 ± 20.7	0.302	−23.9 ± 22.0	0.752	−40.4 ± 19.2	0.924
	−	115.7 ± 4.1		−71.9 ± 17.5		−25.7 ± 17.2		−40.3 ± 16.2	
aCL IgM	+	116.2 ± 2.9	0.521	−68.9 ± 15.6	0.337	−28.0 ± 16.9	0.323	−41.4 ± 17.5	0.737
	−	115.5 ± 3.7		−71.6 ± 17.7		−25.8 ± 17.7		−40.4 ± 16.0	
aβ_2GPI IgG	+	112.8 ± 8.0	**0.002**	−70.7 ± 22.8	0.917	−17.5 ± 23.6	**0.006**	−36.6 ± 18.0	0.143
	−	116.5 ± 2.8		−72.4 ± 15.2		−27.9 ± 16.9		−39.7 ± 14.8	
K(+)aPE IgG	+	117.1 ± 2.8	**0.017**	−72.5 ± 15.7	0.600	−24.0 ± 14.1	0.513	−35.6 ± 15.8	0.127
	−	115.6 ± 3.7		−71.8 ± 17.6		−25.6 ± 17.6		−40.2 ± 16.1	
K(−)aPE IgG	+	115.2 ± 2.2	0.555	−67.8 ± 2.3	0.360	−27.8 ± 8.6	0.811.	−33.0 ± 10.7	0.307
	−	115.7 ± 4.0		−71.2 ± 18.1		−25.1 ± 17.6		−40.6 ± 16.7	
K(+)aPE IgM	+	114.9 ± 3.5	0.239	−76.2 ± 19.3	0.562	−25.0 ± 22.4	0.685	−44.1 ± 15.4	0.326
	−	115.6 ± 3.7		−71.5 ± 17.5		−25.7 ± 17.7		−40.2 ± 16.1	
K(−)aPE IgM	+	114.4 ± 3.0	**0.010**	−70.6 ± 19.8	0.986	−24.5 ± 19.2	0.913	−42.1 ± 14.8	0.614
	−	115.7 ± 3.7		−71.7 ± 17.5		−25.9 ± 17.5		−40.3 ± 16.2	
aPS IgG	+	116.0 ± 4.0	0.420	−72.0 ± 19.3	0.536	−31.6 ± 16.6	0.087	−41.4 ± 15.8	0.484
	−	115.5 ± 4.4		−70.0 ± 16.7		−27.1 ± 16.6		−40.5 ± 16.2	
aPS IgM	+	115.9 ± 3.7	0.572	−69.7 ± 14.5	0.791	−28.6 ± 18.0	0.460	−41.9 ± 15.3	0.418
	−	115.4 ± 4.5		−70.4 ± 17.2		−27.4 ± 16.5		−40.5 ± 16.2	
With at least one aPL[a]		114.9 ± 5.0	a versus b: **0.013** a versus c: <**0.001** b versus c: 0.055	−71.0 ± 19.1	a versus b: 0.081 a versus c: 0.975 b versus c: 0.885	−24.5 ± 19.8	a versus b:0.723 a versus c:0.366 b versus c:0.408	−40.2 ± 16.6	a versus b: 0.760 a versus c: 0.689 b versus c: 0.823
Without any aPL[b]		116.0 ± 3.4		−71.4 ± 16.9		−26.2 ± 16.3		−40.8 ± 16.3	
Control[c]		117 ± 2.3		−70.5 ± 13.9		−28.1 ± 13.6		−41.0 ± 14.1	

Note: Mean ± S.D.[range]. Superscript a-c indicates the respective groups. Bold values indicates statistically significant of $p < 0.05$.

Abbreviations: aβ2GPI, anti-β2GPI antibodies; aCL, anticardiolipin antibodies; aPL, antiphospholipid antibody; APG, acceleration photoplethysmogram; aPS, antiphosphatidylserine antibodies; BVT, blood vessel tension; DPV, differential pulse wave index K(+)aPE, kininogen dependent antiphosphatidylethanolamine antibody; K(−)aPE, kininogen independent antiphosphatidylethanolamine antibodies; LAC, lupus anticoagulant; RBV, relative blood volume; RPL, recurrent pregnancy loss.

invasion defects. aPL disrupt early trophoblast sensitization, causing issues like vasculopathy, thrombosis, and placental infarction later in pregnancy. Yet, the elevated uterine artery PI in previous studies likely reflects vascular dysfunction more than altered trophoblast entry.[31] Prior research noted uterine artery vascular dysfunction in pregnant women with aPL, although predicting adverse pregnancy outcomes remained inconclusive.[2,11,32] Our study strengthens the notion of vascular dysfunction in aPL associated RPL.

The present study revealed that aβ_2GPI IgG was correlated with DPI. In our study, the DPI in the group with aβ_2GPI IgG was significantly lower than that of the group without aβ_2GPI IgG. The involvement of immune complexes containing oxLDL, β_2GPI, and/or CRP in atherosclerosis is well-known.[33–35] Autoantibodies directed against oxLDL/β_2GPI complexes have been detected in patients with SLE and APS and have been shown to be strongly associated with atherothrombosis.[36] This evidence suggests that high levels of aβ_2GPI IgG may be a risk factor for atherosclerosis.

Under normal conditions, vascular endothelial cells regulate blood flow by releasing a number of biologically active substances, such as nitric oxide (NO), to promote vasodilation. However, the presence of aβ_2GPI antibody may interfere with the production and release of these normal bioactive substances, affecting the ability of blood vessels to dilate.[37] This may lead to endothelial dysfunction, which may affect the elasticity and dilatability of blood vessels. Thus, aβ_2GPI antibodies may have an effect on vascular exaggeration, which may lead to abnormal vascular function, affect blood flow regulation, and may even increase the risk of thrombosis and vascular problems.[38] Our study suggests that aβ_2GPI affects vascular health in women with RPL, in consistent with the above research.

Prior studies have demonstrated altered uterine artery blood flow and elevated markers of cardiovascular disease risk, suggesting that early systemic blood vessel changes may be progressing in women with RPL.[13] These changes result in atherosclerosis and arterial thrombosis, and later lead to coronary artery disease and/or stroke.[15,39]

In our multiple regression analysis, DPI was negatively correlated with presence of aβ_2GPI IgG and BMI, SP was positively correlated with age and BMI, RBV was negatively correlated with LDL cholesterol, and BVT was negatively correlated with age, BMI, and sBP. The main risk factors for heart disease and stroke are known to be high blood pressure, LDL cholesterol, diabetes, smoking and second-hand smoke exposure, obesity, unhealthy diet and lack of exercise.[40] This is consistent with our study in which vascular dysfunction in women with RPL was associated with age, BMI, sBP, and LDL-C. In practice, improvement of lifestyle such as proper exercise and a nutritionally balanced diet can help individuals improving weight status, blood pressure, and lipid metabolism, thereby improving vascular function and overall health.

We suggest that this non-invasive APG measurement has the potential to be widely used for vascular testing in women with RPL at the clinical level in the future. By measuring APG, it is also possible to predict which cardiovascular events will occur in women with RPL later in life, and this data can be effective in motivating them to improve their lifestyles and reduce the likelihood of vascular dysfunction. Further studies will shed more light on the mechanisms of RPL and provide more information on the link between RPL and vascular dysfunction, leading to more information on effective treatment for women with PRL.

AUTHOR CONTRIBUTIONS

Titi Yang: Conceptualization, writing—original draft. **Emi Okada** and **Maho Todoroki**: Data curation. **Siyu Liu**: Investigation. **Rukmali Athurupana**: Writing—review and editing. **Kumie Kataoka**, **Chiaki Kashino**, **Takashi Mitsui**, **Toru Hasegawa** and **Yasuhiko Kamada**: Resources. **Hisashi Masuyama**: Methodology. **Mikiya Nakatsuka**: Supervision and correspondence. All authors reviewed and approved the final manuscript and are responsible for all aspects of the work to ensure accuracy and completeness of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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