LIBYAN JOURNAL OF MEDICINE
2025, VOL. 20, 2472492
https://doi.org/10.1080/19932820.2025.2472492

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Investigating the predictive role of serum amyloid A and its association with immunological and coagulation biomarkers in recurrent pregnancy loss

Mahmoud Thabet[a], Kawkab Ali Hasan[b], Ismail A. Elhefnawy[c], Ghada Barakat[d], Dalia Moemen[d], Ahmed Ragab[a,e], Dalia Mahmoud Abdelmonem Elsherbini [f], Mohamed El-Sherbiny[g], Nagwan Ahmed Bahgat[a], Maged Ragheb Elshamy[h], Rayan G. Albarakati[i], Baisakhi Kar[b], Sara Izzeldin Hassan[b], Spogmai Arif[b], Saima Reshi[b], Abida Ikram[b], Rebecamma Ommen[b], Nayla Jamal Bushaqer[j], Mahmoud Mohamed Abdel-Razik[a] and Waleed Eldars[d,k]

[a]Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt; [b]Al Sharq's Obstetrics & Gynecology Clinic, Al Sharq Hospital, Fujairah, United Arab Emirates; [c]Obstetrics and Gynecology Damietta Specialized Hospital, Ministry of Health, Damietta, Egypt; [d]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt; [e]Clinical Medical Sciences Department, Fakeeh College for Medical Sciences, Al-Hamra'a, Jeddah, Saudi Arabia; [f]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia; [g]Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Riyadh, Saudi Arabia; [h]Department of Obstetrics and Gynecology, College of Medicine, Jouf University, Sakaka, Saudi Arabia; [i]Department of Clinical Medical Sciences, College of Medicine, AlMaarefa University, Riyadh, Saudi Arabia; [j]OBS & Gynecology Department, Bahrain Defence Force (BDF) Hospital, Riffa, Bahrain; [k]Department of Basic Medical Sciences, Faculty of Medicine, New Mansoura University, New Mansoura, Egypt

ABSTRACT

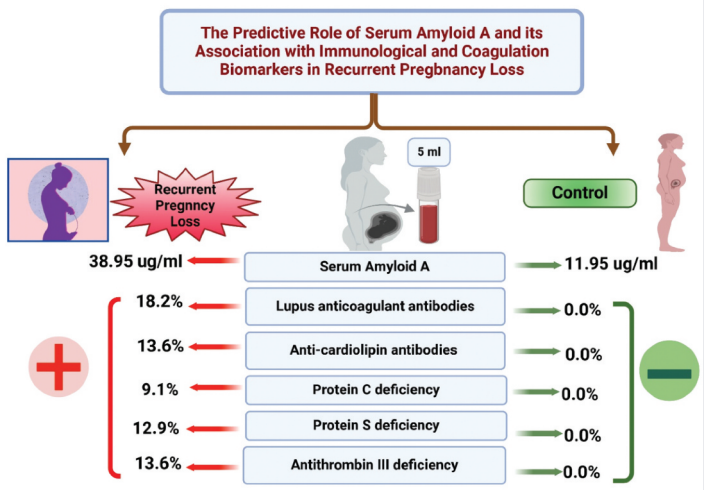
To evaluate the predictive role of serum amyloid A (SAA) levels and their association with antiphospholipid antibodies (APA) and coagulation markers such as lupus anticoagulants (LA), anti-cardiolipin (ACA), protein C (PC) deficiency, protein S (PS) deficiency, and antithrombin III (ATIII) deficiency in recurrent pregnancy loss (RPL). This prospective case-control study comprised two groups: the study group ($n = 88$) included women with recurrent pregnancy loss at Mansoura University Hospital between January 2019 and December 2020, and the control group ($n = 52$) included women without obstetric or medical complications. Demographic, clinical, and laboratory data, including serum samples collected at 10 weeks of gestation, were collected from all participants. The study measured SAA levels, lupus anticoagulants, anti-cardiolipin, protein C, protein S, and antithrombin III levels. The SAA level was significantly elevated in the recurrent pregnancy loss group compared to that in the control group. Lupus anticoagulant positive, anti-cardiolipin positive Immunoglobulin M (IgM), and deficiencies in protein C, protein S, and antithrombin III were significantly observed in patients with RPL ($p < 0.05$). The SAA levels were significantly elevated in both LA-positive and ACA-positive IgM patients. The receiver operating characteristic (ROC) curve analysis demonstrated that at SAA > 24.8 for the prediction of recurrent pregnancy loss, sensitivity was 98.86%, and specificity was 92.31%. Positive and negative predictive values were 95.6% and 98.0%, respectively. The area under the curve = 0.971 (0.927–0.992). SAA is associated with recurrent pregnancy loss and may therefore serve as a potential predictor of this condition. The observed elevation in SAA levels could be primary or secondary to the inflammatory response that promotes thrombotic activity in RPL patients at risk of APA, Protein S, Protein C, and ATIII deficiencies. Implementing SAA screening during pregnancy may facilitate the identification of individuals who could potentially benefit from novel treatment strategies.

ARTICLE HISTORY
Received 18 October 2024
Accepted 23 February 2025

KEYWORDS
Antiphospholipid syndrome; recurrent early pregnancy loss; serum amyloid A protein; protein C; protein S

CONTACT Dalia Mahmoud Abdelmonem Elsherbini dmelsherbini@ju.edu.sa Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, P.O.Box 2014, Sakaka 72388, Saudi Arabia

Supplemental data for this article can be accessed online at https://doi.org/10.1080/19932820.2025.2472492



Graphical abstract summarizing the predictive role of serum amyloid A and its association with immunological and coagulation biomarkers in recurrent pregnancy loss

1. Background

The terminology and definitions used for recurrent miscarriages exhibit considerable variation. The American Society for Reproductive Medicine (ASRM) has used the term recurrent pregnancy loss (RPL) and recommended clinical evaluation after two first-trimester clinical pregnancy losses (specifically, those documented using ultrasonography or histopathological examination). However, they recommended a threshold of three or more losses for epidemiological studies [1], Whereas the European Society of Human Reproduction and Embryology (ESHRE) from 2017 defined RPL as two or more pregnancies that do not have to be consecutive [2]. The World Health Organization (WHO) defines RPL as three or more consecutive pregnancy losses before the 20th week of pregnancy [3]. Worldwide, recurrent pregnancy loss affects 2–3% of pregnant women and is a prevalent reproductive concern [4]. Recurrent pregnancy loss is a complex childbearing issue related to paternal chromosomal abnormalities, autoimmune illnesses, fetal chromosomal reconfiguration, uterine abnormalities, endocrine impairments, maternal infections, coagulation, anti-sperm antibodies, and lifestyle variables [5]. However, the specific reason remains undetermined for approximately 50% of cases [6,7].

In mammals, serum amyloid A (SAA) plays a crucial role as an acute-phase protein. The human genome contains three SAA genes (*SAA1*, *SAA2*, and *SAA4*) along with a pseudogene (*SAA3*). SAA1 and SAA2 are responsible for producing the acute-phase SAA proteins. A-SAA protein has been attributed to four primary functions [8,9]: (i) Increased A-SAA levels are required to develop secondary reactive amyloidosis [10]; (ii) As acute-phase reactant concentrations can be associated with damaged tissue, A-SAA may monitor activity and treatment response in many inflammatory conditions [11]; (iii) A-SAA immediately interacts with high-density lipoproteins during the acute phase, limiting its anti-atherogenic effects [12]; (iv) At low concentrations, SAA mediates immunoregulatory function, trophoblast invasion, differentiation, and metalloprotease activity within the placenta [13], whereas, at high levels, disturbances in these functions occur [14].

Antiphospholipid syndrome (APS) mostly encompasses pregnancy issues such as pre-eclampsia, impaired placental function, stunted fetal development, and recurrent pregnancy loss [15]. Most investigations on antiphospholipid antibodies (APA) have primarily focused on anti-cardiolipin antibodies (ACA) and lupus anticoagulants (LA) [16]. Anti-cardiolipin antibodies (ACAs) cause thrombosis by binding to phospholipids and preventing the release of gonadotropins and placental anticoagulants. The precise mechanism by which these antibodies produce placental-associated thrombosis is not fully understood. However, existing data suggest that these antibodies can induce local acute inflammatory reactions and neutrophil infiltration, eventually resulting in fetal death [17].

Nevertheless, the presence of ACA and LA exhibits poor concordance [18], indicating that the two antibodies may identify separate epitopes. LA is significantly linked to both arterial and venous blood clot formation. However, it remains unclear whether the

presence of anti-cardiolipin antibodies (ACA) without LA is linked to blood clot formation [19]. Similarly, a study found that four serologically detected antiphospholipid antibodies, including anti-cardiolipin antibodies, did not alter thrombosis in lupus anticoagulant-positive patients [20]. In the majority of prospective studies, individuals who tested positive for ACA and LA were assembled together [16].

Multiple studies have shown that acquired or hereditary thrombophilia is associated with RPL [21–23]. Antithrombin III (ATIII), protein C (PC), and PS deficits are significant factors contributing to thrombophilia, a condition linked with an increased likelihood of venous thromboembolism during gestation [24]. Proteins C and S are essential plasma proteins that rely on vitamin K for their function. They play a vital role in the body's natural anticoagulant system by specifically deactivating factors Va and VIIIa. Deficiencies in proteins C and S are primarily caused by mutations in the respective genes. These deficiencies can be acquired through various mechanisms or through the use of certain medications [25]. This condition demands increased attention due to hypercoagulability during pregnancy. Normal pregnancy entails increased procoagulants, decreased fibrinolysis, and reduced anticoagulants to preserve placental hemostasis [26].

Previous studies have documented that serum amyloid A is theoretically a promising marker for RPL [27,28]. Researchers have suggested that the role of SAAs may be linked to the placenta, influencing the initial trophoblast invasion during early pregnancy and helping maintain equilibrium between pro-inflammatory and anti-inflammatory cytokines [27]. In addition, some studies have demonstrated the role of antiphospholipid antibodies and thrombophilia in increasing susceptibility to RPL [16,29–32]. No previous studies have examined the levels of lupus anticoagulant-positive, anti-cardiolipin-positive IgM patients positive, antithrombin, protein C, and protein S in females with RPL who showed high levels of SAA. Therefore, we aimed to explore the association between thrombotic and inflammatory consequences in females with RPL. This study explored the predictive role of serum amyloid and investigated the immunological and coagulation biomarkers associated with recurrent pregnancy loss at Mansoura University Hospital.

2. Subjects and methods

2.1. Study design and patients

This case-control study was conducted in the Obstetrics and Gynecology Department of Mansoura University Hospital. The study protocol was evaluated and approved by institutional review board (IRB) of Mansoura Faculty of Medicine, code number (R/18.10.317) on 27 November 2018 in accordance with the Declaration of Helsinki and a signed informed consent was obtained from all patients participating in the study.

2.1.1. Eligibility criteria

This study included women who presented with recurrent pregnancy loss (3 or more consecutive pregnancy losses prior to 20 weeks from the last menstrual period) between January 2019 and December 2020. The case group included women with RPL who had no history of substance addiction or other medical conditions. Females attending the outpatient clinic during the first trimester of pregnancy with no antenatal care medical or obstetrical issues were included as controls. Their age ranged from 20 to 38 years (mean ± 28 years). During the initial stage of screening, women who had previously experienced any renal, rheumatic, digestive, endocrine, or nutritional diseases, including malnutrition, as well as those who had undergone surgery, blood transfusion, or blood donation within the past six months, patients with systemic lupus erythematosus (SLE), pre-eclampsia, uterine abnormalities, blood group incompatibilities, and uncontrolled Diabetes Mellitus (DM)/gestational diabetes were excluded. Similarly, those with hypertension (systolic pressure ≥150 mmHg and/or diastolic pressure ≥95 mmHg) were also excluded from the study.

The data of the examined patients, including age, body mass index (BMI), parity, number of abortions, gestational age at delivery, and delivery style, were accurately documented.

2.1.2. Study sample

The necessary sample size was determined based on the lack of information regarding the level of serum Amyloid A in females with RPl. Thus, the prevalence of antithrombin III, PS, and PC deficiency in females with RPL was used for sample calculation.

$$n = p \times (1 - p) \times (z/e)^2$$

Where n is the sample size required for a large population; p is the proportion of cases with antithrombin III, PC, or PS deficiency; z is the confidence level, and e is the error margin. p is based on a previous study showing that the prevalence of antithrombin III, PC, and PS deficiency in females with RPL was 2.88%, 3.85%, and 5.77%, respectively, and 0.0%, 0.9%, and 0.9%, respectively, in the control group [24]. The value of z was specified as 1.96 for a 95% confidence level (α), and (e) was set at 5%. Based on these factors, the sample size was determined to be 43–84, which is sufficient for the present study. The study comprised 88 participants in the test group and 52 in the control group.

2.2. Laboratory data

In the hospital, 5 mL of blood was drawn from all cases by vein puncture at 10 weeks of gestational age. Sterile tubes were used to collect serum after a 10-minute centrifugation at 3400 rpm and the samples were then examined using the following parameters.

2.2.1. Detection of serum amyloid A

SAA levels were measured simultaneously in both groups using identical microtiter plates supplied with the Human SAA solid-phase sandwich enzyme-linked immunosorbent assay kit (BioSource Europe, Nivelles, Belgium) following the manufacturer's instructions. The coefficients of variance across and within assays were 7.4% and 6.1%, respectively, with a sensitivity of <0.004 µg/mL.

2.2.2. Detection of anticardiolipin anti bodies (ACA) and lupus anticoagulant

The levels of anti-cardiolipin antibodies were determined using an enzyme-linked immunoassay kit from Orgentec Diagnostika (Mainz, Germany). Results are standardized and quantified using the polyclonal 'Harris' standards [33], as described by Loizou et al. [34], Validated in-house lupus ratio (LR) procedures were used to detect lupus anticoagulant levels in fresh plasma samples. Two LR tests were performed: one using activated partial thromboplastin time (LR-APTT) and the other using Russell viper venom time (LR-RVVT). LR tests were conducted by combining the patient plasma and pooled normal plasma in a 1:1 ratio. Two coagulation times were measured for each LR test: one using a low-phospholipid reagent, and the other using a high-phospholipid reagent. The ratio between the coagulation times at low and high phospholipid concentrations was divided by the corresponding ratio obtained from the pooled normal plasma. Based on the method described earlier, the final ratio was determined as the LR of the patient's plasma [35].

2.2.3. Determining antithrombin III (ATIII), protein C (PC), or PS deficiencies

Antithrombin III activity was measured using the chromogenic substrate S-2765 (Chromogenix) in a microplate system [36]. The testing was conducted in accordance with the manufacturer's specifications [32]. The protein C (PC) or PS samples were fed into the coagulation enzyme-linked immunosorbent assay (ELISA) ASEKULISA analyzer. The AESKULISA utilizes microplates coated with a capture antibody that specifically binds to human protein C or S. The relative percent concentration of Protein C and S antigen in plasma may be calculated by using the standard curve derived from the Reference Plasma included in the kit in accordance with the manufacturer's guidelines [25].

2.3. Statistical analysis

Data were analyzed using SPSS (version 24.0; IBM, Armonk, NY, USA) and MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium). The Kolmogorov-Smirnov test was used to assess the normal distribution of the numerical data. A p-value greater than 0.050 indicates that the data are normally distributed. As the data were not normally distributed, the results were reported as the median and interquartile range (IQR) using the Mann-Whitney U test. Qualitative nominal data were expressed as frequency (%), and Fisher's exact tests were applied to compare two groups, as in the control group; more than 20% of cells had expected frequencies of <5. The receiver operating characteristic (ROC) curve was analyzed to assess the SAA threshold necessary to differentiate between cases and controls. The area under the curve was estimated, and the optimal cutoff value for SAA was determined based on the highest Youden index (J). The relative risk and 95% confidence interval were estimated separately for each parameter using GraphPad software (San Diego, CA, USA).

3. Results

The flow of participants through the present study is demonstrated in Figure 1. Data from a total of 140 participants (88 for the study group and 52 for the control group) were included in the final analysis. This study revealed that among women with recurrent pregnancy loss compared to the control group, parity and the number of first-trimester abortions were significant factors. However, no statistically significant differences were found between the groups regarding other demographic variables (Table 1). The median SAA was significantly higher in women with recurrent pregnancy loss than in the control group ($p < 0.001$), where the median (interquartile range) of SAA in the RPL group was 38.95(31.20–62.35), while in the control group was 11.95(10.40–13.48). Lupus anticoagulant, anti-cardiolipin positive IgM, protein C, protein S, and antithrombin III deficiencies were reported in patients with RPL, which was significant ($p < 0.05$) compared to the control group (Table 1 and Figure 2a). The risk of recurrent pregnancy loss is increased in patients with positive lupus anticoagulant, anti-cardiolipin-positive IgM, protein C, protein S, and antithrombin III deficiency, as shown in Table 2.

In the RPL group, SAA levels were significantly elevated in both lupus anticoagulant-positive and anti-cardiolipin-positive IgM patients ($p < 0.05$), as shown in Table 3 and Figures 2b and c, respectively. In contrast, SAA was elevated in RPL patients with protein C, protein S, or antithrombin III deficiency, which was not statistically significant ($p > 0.05$) (Table 3, Figures 2d–f). The ROC curve analysis linking SAA and recurrent pregnancy

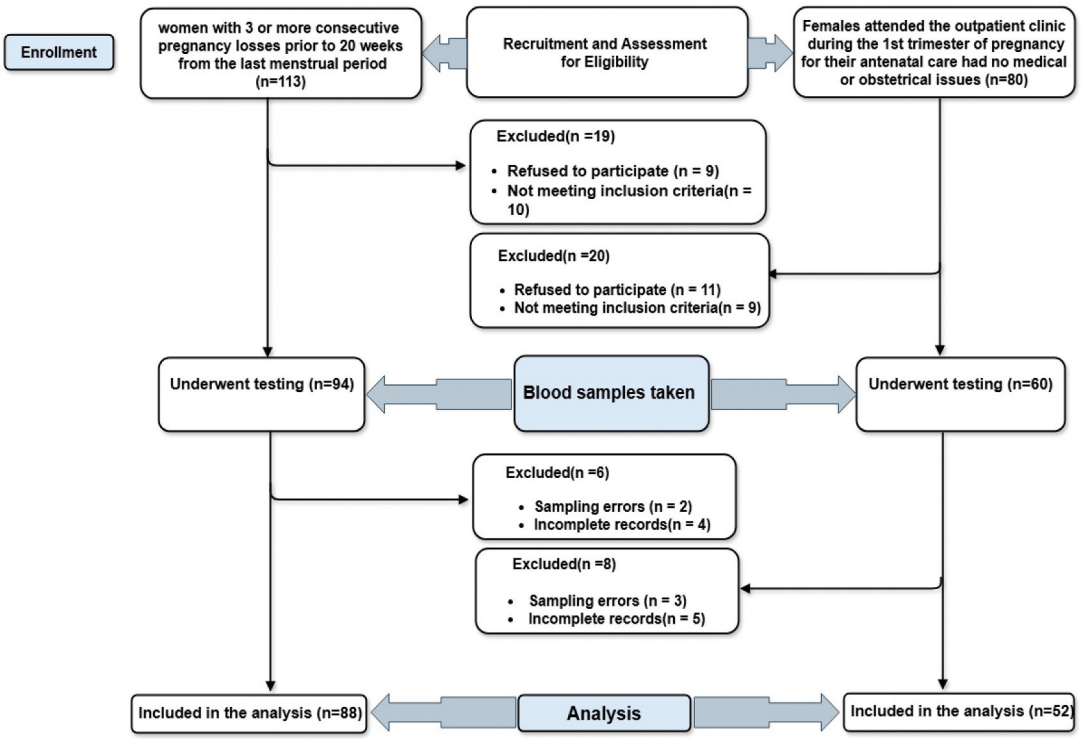


Figure 1. Flowchart of the study.

Table 1. Comparison of women with and without with or without recurrent pregnancy loss.

	Study group N = 88	Control group N = 52	*P* value
Age *Median (Interquartile range)*	28 (24.75–30.00) years	27 (21.75–34.75) years	0.91
No of pregnancy *Median (Interquartile range)*	5 (4.00–7.00)	2 (1.00–3.00)	<0.001***
No of delivery *Median (Interquartile range)*	1 (0–2.00)	1 (0–2.25)	0.34
No of first trimester abortions *Median (Interquartile range)*	3 (3–4)	0	<0.001***
Serum amyloid A (ug/ml) *Median (Interquartile range)*	38.95 (31.20–62.35)	11.95 (10.40–13.48)	<0.001***
Lupus anticoagulant antibodies	16 (18.2%)	0	0.001^^^
Anti-cardiolipin antibodies	12 (13.6%)	0	0.005^^
Protein C deficiency	8 (9.1%)	0	0.022^
Protein S deficiency	11 (12.5%)	0	0.007^^
Antithrombin III deficiency	12 (13.6%)	0	0.003^^

For numerical data, median and interquartile range (IQR) using the Mann-Whitney U test was applied, $p < 0.05$*, 0.01**,0.001*** is statistically significant.

The qualitative nominal data were expressed as frequency (%), and Fisher's exact tests were applied; $p < 0.05$^, 0.01^^,0.001^^^ is statistically significant.

loss (Table 4 and Figure 3) showed that at SAA > 24.8 for the prediction of recurrent pregnancy loss, the sensitivity was 98.86% (93.8–100.0%), and the specificity was 92.31% (81.5–97.9%). The positive predictive value was 95.6% (89.5–98.2%), while the negative predictive value was 98.0% (87.2–99.7%). The area under the curve = 0.971 (0.927–0.992).

4. Discussion

SAA is an acute-phase protein that is induced upon exposure to injury, inflammation, or infection [13]. Its main role depends on the migration of immune cells, induction of cytokines, and enhancement of phagocytosis at the site of inflammation [14]. This study investigated the relationship between serum amyloid A level and recurrent pregnancy loss. Another aim of our study was to explore the link between thrombotic and inflammatory consequences in females with RPL by detecting APA, Protein C, Protein S, and ATIII levels.

The current study revealed that serum amyloid A levels were significantly elevated in the recurrent pregnancy loss group compared to those in the control group. In a study conducted in Egypt, serum amyloid A levels were elevated in women with primary unexplained recurrent pregnancy loss [37]. Similarly, Ming et al. [38] reported that maternal SAA was considerably greater among patients with recurrent early miscarriages than among normal pregnancy controls. In addition, a relatively valuable study documented that SAA plays a role in preterm labor, as it reported that the level of SAA was elevated in animals with preterm delivery [39]. In agreement with these findings, some authors have found that excessive

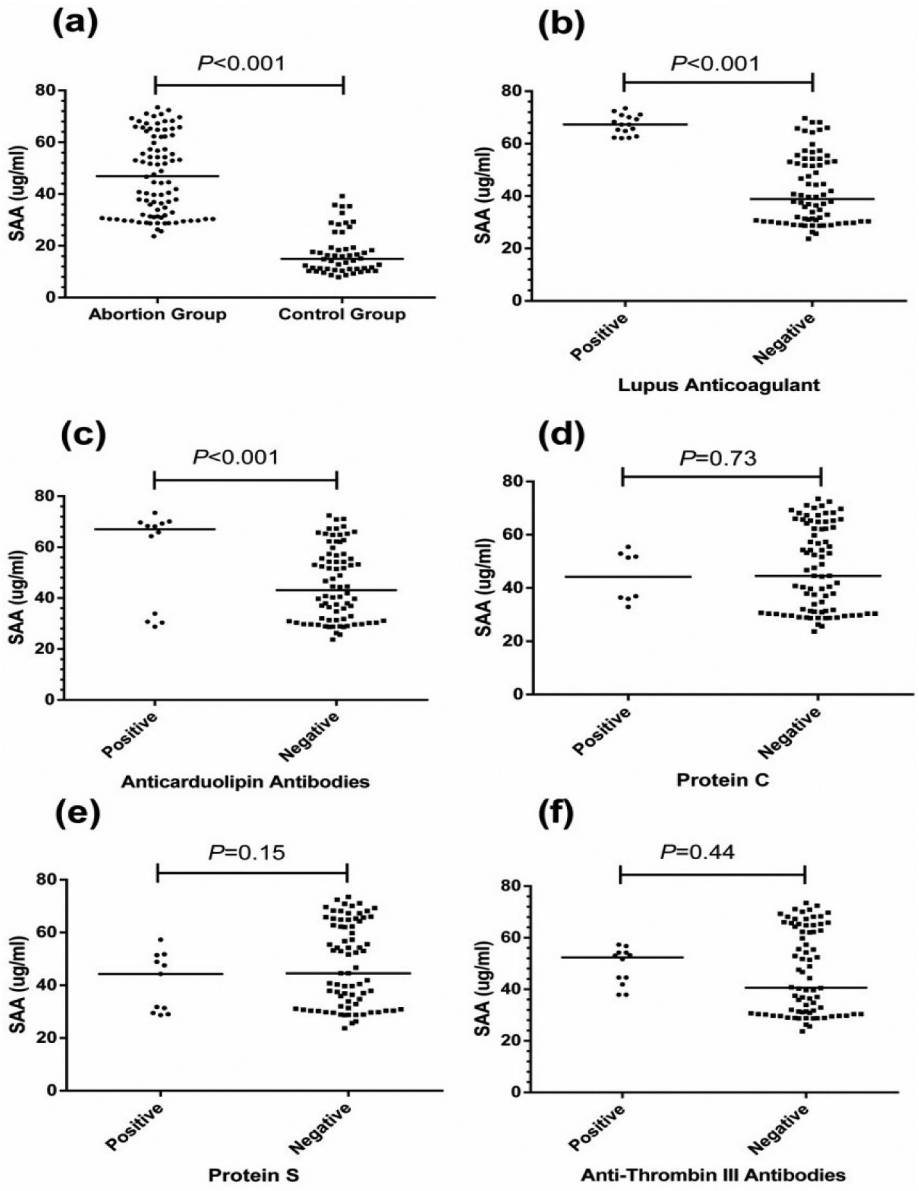


Figure 2. Serum Amyloid A (SAA) levels. a)saa in females with RPL (88 cases) and control group (52 cases); b) SAA in females with RPL (88 cases) with positive lupus anticoagulant (16 cases) and negative lupus anticoagulant (72 cases); c) SAA in females with RPL (88 cases) with positive anti-cardiolipin antibodies (12 cases) and negative (76 cases); d) SAA in females with RPL (88 cases) with protein C deficiency (8 cases) and negative (80 cases); e) SAA in females with RPL (88 cases) with protein S deficiency (11 cases) and negative (77 cases); f) SAA in females with RPL (88 cases) with antithrombin III deficiency (12 cases) and negative (76 cases).

Table 2. Relative risk &95% interval for immunological markers between women with or without recurrent pregnancy loss.

Risk Factor	Relative Risk (95% CI)	*p*-value
Lupus anticoagulant antibodies	1.72 (1.37–2.93)	0.0005***
Anti-cardiolipin antibodies	1.68 (1.26–3.37)	0.0037**
Protein C deficiency	1.65 (1.10–3.79)	0.0257*
Protein S deficiency	1.68 (1.23–3.86)	0.0070**
Antithrombin III deficiency	1.68 (1.26–3.37)	0.0037**

$p < 0.05$*, 0.01**, 0.001*** is statistically significant.

amounts of SAA in the umbilical cord blood are linked to the development of very early neonatal infections among preterm infants [40,41]. Some studies have reported that serum amyloid A is produced by human fetal membranes. These studies also concluded that the factors responsible for the initiation of parturition induce SAA production [42,43]. Fetal membrane rupture is evoked by pro-inflammatory cytokines that are also responsible for the production of prostaglandin E2 (PGE2), interleukin 6 (IL-6), and interleukin 1β (IL-1β) [44]. Li et al. study stated that serum amyloid A was detected by the

Table 3. Serum Amyloid A in women with recurrent pregnancy loss.

	Serum amyloid A (ug/ml) *Median (Interquartile range)*		
	Positive finding	Negative finding	*P* value
Lupus anticoagulant antibodies	65.4 (64.30–68.73)	34.35 (29.88–39.93)	<0.001***
Anti-cardiolipin antibodies	64.95 (37.68–66.28)	35.50 (30.13–53.65)	<0.001***
Protein C deficiency	44.60 (38.20–52.03)	36.00 (30.20–64.20)	0.73 (ns)
Protein S deficiency	45.50 (36.70–56.70)	35.60 (30.15–64.25)	0.15 (ns)
Antithrombin III deficiency	45.70 (42.28–56.20)	37.50 (30.50–64.28)	0.44 (ns)

$p < 0.001$*** is statistically significant; ns: non-significant.

Table 4. Serum Amyloid A level is used to distinguish between women with or without recurrent pregnancy loss.

	Estimate	95%
Category	140	–
Patients (Positive group)	88(62.86%)	–
Patients (Negative group)	52(37.14%)	–
Area under the ROC curve (AUC)	0.971	0.927–0.992
Youden index J	0.91	–
Standard Error	0.015	–
Optimum cutoff level of SAA (ng/ml)	>24.8	–
Sensitivity	98.86	93.8–100.0
Specificity	92.31	81.5–97.9
+PV	95.6	89.5–98.2
-PV	98.0	87.2–99.7
+LR	12.85	5.0–33.0
-LR	0.012	0.002–0.09
Significance level P (Area = 0.5)	<0.0001	-

+PV: Positive predictive value; -PV: Negative predictive value; +LR: Positive likelihood ratio; -LR:Negative likelihood ratio; ROC curve: Receiver operating characteristic curve; AUC: Area Under Curve; SAA: Serum amyloid A.

immunohistochemical staining in the amnion epithelial, chorionic trophoblast, and fibroblast cells. They demonstrated its role in mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) pathway activation [42]. Extravillous cytotrophoblast invasion into the decidua, myometrium, and spiral arteriole remodelling is crucial for healthy pregnancy. This process of invasion is mediated by metalloproteases that are modulated by different cell types of SAA [45,46].

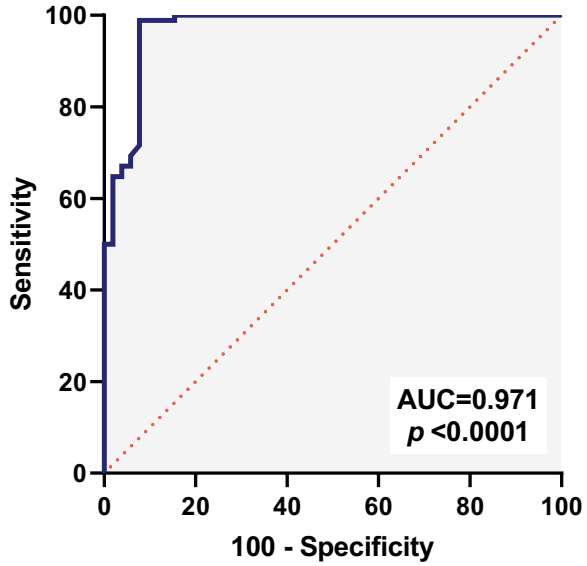


Figure 3. Receiver operating characteristic curve to determine the ability of serum amyloid a level to discriminate between women with and without recurrent pregnancy loss. AUC; area under curve.

Similar to our findings, Sandri et al. indicated that moderate levels of SAA have a positive impact on placental formation and function, while excessive amounts of SAA have a negative impact and disturb placentation. They found that by using extravillous trophoblastic (EVT) cells, which were isolated from the human placenta, SAA at a level of 1 to 10 mg/mL caused duplication of EVT invasive power in a toll-like receptor 4(TLR4)-related way; however, at 20 mg/mL, it resulted in EVT cell invasive process inhibition [14]. Another crucial role of SAA in achieving successful pregnancy, besides trophoblastic invasion, is maintaining the balance of anti-and pro-inflammatory cytokines necessary for preserving fetomaternal tolerance and progression of pregnancy. Lin et al. reported that in pregnancy-related complications like gestational DM, pre-eclampsia, recurrent early pregnancy loss, and chorioamnionitis-related preterm labour, there is a non-specific elevation of maternal plasma SAA attributed to the inflammatory aspect of these

comorbidities. They concluded that SAA may not be considered an optimal biomarker for certain gestational disorders; however, the different levels of increase in SAA may indicate the extent of these comorbidities [47]. SAA may be responsible for the induction of abortion by impeding syncytialization and trophoblastic invasion into the decidua. Similarly, it elicits other pro-inflammatory mediators such as interleukin-6 and Tumor Necrosis Factor (TNF) [28,48]. Additionally, it has been demonstrated that intrauterine growth retardation and pre-eclampsia both cause failed trophoblastic invasion and induction of pro-inflammatory mediators [49,50].

Serum amyloid A levels were measured against other factors that may share the etiology of recurrent pregnancy loss. In the RPL group, SAA levels were significantly elevated in both lupus anticoagulant-positive and anti-cardiolipin-positive IgM patients. The presence of ACAs and LA, which are two well-defined antiphospholipid antibodies, has been associated with repeated miscarriages during pregnancy [31,51–53]. The results of our study align closely with these findings since we observed a considerably greater level of lupus anticoagulant and anti-cardiolipin in the RPL group than in the control group. Anticardiolipin antibodies cause thrombosis by adhering to phospholipids and preventing the release of placental anticoagulant proteins and gonadotropins [54,55]. The precise mechanism of action remains uncertain; however, it is hypothesized that these antibodies may induce localized inflammatory reactions and neutrophil infiltration, eventually resulting in fetal miscarriage [17]. Research conducted on animals has demonstrated that passive transmission of pure anticardiolipin immunoglobulin G (IgG) may lead to unexpected fetal loss [56]; normalization of the levels of these antibodies during pregnancy reduces the chances of miscarriage and improves fetal survival rates [57]. While the prevalence of APS in the general population is estimated to be between 0.2% and 2% among pregnant women, the likelihood of subsequent miscarriages in these individuals is estimated to be approximately 10% [58,59]. The primary cause of this issue is the presence of antiphospholipid antibodies (APAs), acquired autoantibodies that specifically attack negatively charged phospholipids and phospholipid-binding proteins in these patients [60,61]. Furthermore, along with ACAs, lupus anticoagulants have exhibited obstetric difficulties in 15–20% of patients, with a considerably greater rate of RPL, roughly 50–75%, owing to severe pre-eclampsia and unexplained intrauterine fetal mortality [53,62]. Clinical experience has revealed that ACAs and LA are the most prevalent APA antibodies. Experiments have identified additional target antigens, including prothrombin, Beta-2 glycoprotein 1(β2 GP1), annexin V, protein S, and protein C [63–65], implying that the main targets of APAs are not phospholipids but rather phospholipid-binding proteins and/or phospholipid-protein complexes. Evidence has shown that Elevated levels of APAs are correlated with spontaneous RPL along with multiple thromboses and thrombocytopenia [66].

Our research revealed that SAA was elevated in patients with RPL and protein C, protein S, and antithrombin III deficiency, which was insignificant. Regarding the prevalence of protein C, S, and ATIII deficiencies in the RPL group, our results are consistent with those of previous studies [24–26]. A considerable thrombotic propensity has long been linked to ATIII deficiency during pregnancy. However, the link between miscarriages and stillbirths and genetic ATIII deficiency has recently been established. Women with ATIII deficiency and have PC, PS, and PC resistance are at risk [67]. The term 'familial thrombophilia' refers to inherited thrombotic diseases that arise from particular genetic abnormalities related to plasma coagulation proteins implicated in the protein C and heparin-antithrombin III anticoagulant pathways. These uncommon hereditary conditions are often inherited as autosomal dominant diseases [32]. The link between higher fetal loss and familial thrombophilia was shown by Preston et al. [68]. A deficit in protein S may result in thromboembolism and subsequent recurrent abortion [32]. McNamee et al. evaluated 37 expectant mothers with protein C, protein S, and antithrombin deficiencies. Vitamin K antagonists and low molecular weight heparin were used to treat them. There were no miscarriages in the 26 treated women. On the other hand, 5 out of 11 women, or 45% of the group who did not receive therapy, had fetal loss [69].

In our study, we hypothesized that increased levels of SAA in lupus anticoagulant-positive, anticardiolipin-positive IgM patients positive, and antithrombin, protein C, and protein S deficiencies could be attributed to the link between thrombotic and inflammatory consequences in the RPL group. TNF-α, IL-1β, and IL-6, three pro-inflammatory mediators, are primarily responsible for controlling SAA expression and induction in the liver [70]. Ames et al.'s earlier research [71] showed that a significant number of thrombotic patients with primary antiphospholipid syndrome (PAPS) have low-grade inflammation, which the illness itself must bring on. The 'two hit hypothesis' states that while the Antibodies against phospholipid (aPL) (the first hit) generate a thrombophilic state, clotting only occurs when a second thrombophilic condition (the second hit) is present [72]. Antiphospholipid antibodies cause placental tissues and recruit neutrophils to produce pro-inflammatory cytokines and tissue factors in obstetrical acute phase syndrome. Antiphospholipid antibodies further stimulate the complement system, which in turn causes a positive

feedback loop that draws neutrophils and activates the placenta. Myometrial contractions and cervical ripening occur when these cells are activated, leading to labor induction [73]. According to Ames et al. [71], patients SAA levels are higher, especially when many thrombotic episodes occur. Patients with APS had greater plasma SAA levels than any of the control groups [74,75].

The relationship between elevated SAA levels and previous occlusive events remains unclear, as it is challenging to establish whether these increased levels are the cause or consequence of such events. This uncertainty arises from the fact that SAA plays a role in promoting tissue factor expression on both monocytes and endothelial cells [76,77]. Consequently, assessing their efficacy as indicators of thrombotic vulnerability in PAPS can only be performed in relation to re-thrombosis, as the majority of PAPS subjects will be taking oral anticoagulants following their first occlusive episode [71].

4.1. Clinical implementation

The link between maternal SAA levels and APA, Protein S, Protein C, and ATIII deficiency in patients with RPL has not been thoroughly investigated. The correlation between elevated maternal blood SAA levels and RPL may prompt more investigation into the potential use of detecting this protein as a biomarker for recurrent spontaneous miscarriage, especially if the patient proved to have positive associated thrombotic or APA markers. The implementation of SAA screening during pregnancy would allow for the identification of individuals who might potentially benefit from novel treatment strategies such as gene therapy and antagonists of immune receptors.

4.1.1. Limitation of the study

Due to the cross-sectional design of the research, it is not possible to determine whether higher SAA levels are the cause or result of past occlusive events. One of the limitations of our study is that we did not perform SAA in females with RPL in the non-pregnant state. Further studies are needed to compare SAA levels among females with a history of primary unexplained RPL during pregnancy and non-pregnancy states, as well as before and after spontaneous abortion.

5. Conclusion

The current study observed elevated SAA levels in females with RPL. This might be either a main effect or a consequence of the inflammatory response that promotes thrombotic activity in patients with RPL. In addition, we observed increased levels of SAA in RPL females with positive APA or with Protein S, Protein C, and ATIII deficiency, suggesting that the increased level of SAA in these patients could be attributed to the link between thrombotic and inflammatory consequences. Further studies are required to confirm this relationship and investigate its significance.

Acknowledgments

We would like to pay our gratitude and our respects to our colleague Dr. Omyma. A. Elsayed after helping in initial conceptualization of our research. Dr. Omyma. A. Elsayed passed away on April of 2020. She was a dedicated associate professor in Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The Authors would like to thank Researchers Supporting Project number [MHIRSP2025006], AlMaarefa University, Riyadh, Saudi Arabia, which graciously supported this work.

Institutional review board statement

The research protocol was evaluated and approved by institutional review board (IRB) of Mansoura Faculty of Medicine, code number (R/18.10.317) on 27 November 2018, in line with the Declaration of Helsinki. Additionally, signed informed permission was collected from all patients participating in the study.

ORCID

Dalia Mahmoud Abdelmonem Elsherbini http://orcid.org/0000-0001-5262-6134

References

[1] Regan L, Rai R, Saravelos S, et al. Recurrent MiscarriageGreen-top guideline no. 17. BJOG: Int J Obstet & Gynaecol. 2023;130(12). doi: 10.1111/1471-0528.17515

[2] RPL, E.G.G.o., Bender Atik R, Christiansen OB, et al. ESHRE guideline: recurrent pregnancy loss: an update in 2022. Hum Reprod Open. 2023;2023(1):hoad002. doi: 10.1093/hropen/hoad002

[3] Vomstein K, Feil K, Strobel L, et al. Immunological risk factors in recurrent pregnancy loss: guidelines versus current state of the art. J Clin Med. 2021;10(4):869. doi: 10.3390/jcm10040869

[4] El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. Int J Women's Health. 2017;9:331–345. doi: 10.2147/IJWH.S100817

[5] Al-Fahham AA. Etiology of antisperm antibodies in the serum of virgins. Open J Obstet Gynecology. 2018;8(3):236–245. doi: 10.4236/ojog.2018.83025

[6] Ghaebi M, Nouri M, Ghasemzadeh A, et al. Immune regulatory network in successful pregnancy and

reproductive failures. Biomed & Pharmacother. 2017;88:61–73. doi: 10.1016/j.biopha.2017.01.016
[7] Gupta M. Immunology and pregnancy loss. In: Mehta S, Gupta B, editors. Recurrent Pregnancy Loss. 2018:53–65.
[8] Malle E, Steinmetz A, Raynes JG. Serum amyloid a (SAA): an acute phase protein and apolipoprotein. Atherosclerosis. 1993;102(2):131–146. doi: 10.1016/0021-9150(93)90155-N
[9] Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem. 1999;265(2):501–523. doi: 10.1046/j.1432-1327.1999.00657.x
[10] Husebekk A, Skogen B, Husby G, et al. Transformation of amyloid precursor SAA to protein AA and Incorporation in amyloid fibrils in vivo. Scand J Immunol. 1985;21(3):283–287. doi: 10.1111/j.1365-3083.1985.tb01431.x
[11] Malle E, De Beer FC. Human serum amyloid a (SAA) protein: a prominent acute-phase reactant for clinical practice. Eur J Clin Invest. 1996;26(6):427–435. doi: 10.1046/j.1365-2362.1996.159291.x
[12] Artl A, Marsche G, Lestavel S, et al. Role of serum amyloid a during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol. 2000;20(3):763–772. doi: 10.1161/01.ATV.20.3.763
[13] Eklund KK, Niemi K, Kovanen PT. Immune functions of serum amyloid A. Crit Rev Immunol. 2012;32(4):335–348. doi: 10.1615/CritRevImmunol.v32.i4.40
[14] Sandri S, Urban Borbely A, Fernandes I, et al. Serum amyloid a in the placenta and its role in trophoblast invasion. PLOS ONE. 2014;9(3):e90881–e90881. doi: 10.1371/journal.pone.0090881
[15] Xu J, Chen D, Duan X, et al. The association between antiphospholipid antibodies and late fetal loss: a systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2019;98(12):1523–1533. doi: 10.1111/aogs.13665
[16] Nielsen HS, Christiansen OB. Prognostic impact of anticardiolipin antibodies in women with recurrent miscarriage negative for the lupus anticoagulant. Hum Reprod. 2005;20(6):1720–1728. doi: 10.1093/humrep/deh790
[17] Bu C, Zhang C, Li Z, et al. Autoantibodies to plasminogen and tissue plasminogen activator in women with recurrent pregnancy loss. Clin Exp Immunol. 2007;149(1):31–39. doi: 10.1111/j.1365-2249.2007.03382.x
[18] Rote NS, Johnson DD, Branch DW. Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin. Am J Obstet Gynecol. 1990;163(2):575–584. doi: 10.1016/0002-9378(90)91201-M
[19] Galli M, Luciani D, Bertolini G, et al. Anti–β2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. Blood. 2003;102(8):2717–2723. doi: 10.1182/blood-2002-11-3334
[20] Triplett DA, Brandt JT, Musgrave KA, et al. The relationship between lupus anticoagulants and antibodies to phospholipid. JAMA. 1988;259(4):550–554. doi: 10.1001/jama.1988.03720040042024
[21] Bates SM. Management of pregnant women with thrombophilia or a history of venous thromboembolism. ASH Educ Program Book. 2007;2007(1):143–150. doi: 10.1182/asheducation-2007.1.143
[22] Kupferminc MJ. Thrombophilia and pregnancy. Reprod Biol Endocrinol. 2003;1(1):1–22. doi: 10.1186/1477-7827-1-111
[23] Robertson L, Wu O, Langhorne P, et al. Thrombophilia in pregnancy: a systematic review. Br J Haematol. 2006;132(2):171–196. doi: 10.1111/j.1365-2141.2005.05847.x
[24] Mekaj Y, Lulaj S, Daci F, et al. Prevalence and role of antithrombin III, protein C and protein S deficiencies and activated protein C resistance in Kosovo women with recurrent pregnancy loss during the first trimester of pregnancy. J Hum Reprod Sci. 2015;8(4):224–229. doi: 10.4103/0974-1208.170407
[25] Mukhtar B, Garg R, Ibrahim G, et al. Investigating protein C and S levels in pregnant women with recurrent early pregnancy loss versus normal pregnancy. J Med Life. 2023;16(1):160. doi: 10.25122/jml-2022-0267
[26] Alshammary HN, Almosawi HM, Hadi FS. Deficiency of protein C and protein S in recurrent pregnancy loss. Med J Babylon. 2015;12:348–356.
[27] AL-Turiahi AM. Maternal serum amyloid a level as a marker of primary unexplained recurrent missed miscarriages. Medico-Legal Update. 2021;21(2):223–229. doi: 10.37506/mlu.v21i2.2677
[28] Ibrahim MI, Ramy AR, Abdelhamid AS, et al. Retracted: maternal serum amyloid a level as a novel marker of primary unexplained recurrent early pregnancy loss. Int J Gynecology & Obstet. 2017;136(3):298–303. doi: 10.1002/ijgo.12076
[29] Daboubi M. Anti-cardiolipin antibodies in women with recurrent abortion. EMHJ-East Mediterranean Health J. 2001;7(1–2):95–99. doi: 10.26719/2001.7.1-2.95
[30] Cohn D, Goddijn M, Middeldorp S, et al. Recurrent miscarriage and antiphospholipid antibodies: prognosis of subsequent pregnancy. J Thromb Haemostasis. 2010;8(10):2208–2213. doi: 10.1111/j.1538-7836.2010.04015.x
[31] Shaikhomar OA, Ali ST. A comparative analysis of anticardiolipin, anti-β2-glycoprotein-1, and lupus anticoagulants in Saudi women with recurrent spontaneous abortions. J Pers Med. 2022;13(1):2. doi: 10.3390/jpm13010002
[32] Coumans A, Huijgens P, Jakobs C, et al. Haemostatic and metabolic abnormalities in women with unexplained recurrent abortion. Hum Reprod. 1999;14(1):211–214. doi: 10.1093/humrep/14.1.211
[33] Harris E, Gharavi A, Patel S, et al. Evaluation of the anticardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol. 1987;68(1):215.
[34] Loizou S, McCrea J, Rudge A, et al. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. Clin Exp Immunol. 1985;62:738.
[35] Jacobsen E, Barna-Cler L, Taylor J, et al. The Lupus Ratio test–an interlaboratory study on the detection of lupus anticoagulants by an APTT-based, integrated, and semi-quantitative test. Thromb Haemost. 2000;83(5):704–708. doi: 10.1055/s-0037-1613896
[36] Tollefsen DM. Laboratory diagnosis of antithrombin and heparin cofactor II deficiency. Semin Thromb Hemostasis. 1990;16(2):162–168. Copyright© 1990 by Thieme Medical Publishers, Inc. doi: 10.1055/s-2007-1002664
[37] Li W, Wang W, Zuo R, et al. Induction of pro-inflammatory genes by serum amyloid A1 in human amnion fibroblasts. Sci Rep. 2017;7(1):693–693. doi: 10.1038/s41598-017-00782-9
[38] Ming N, Liu Z, Wu H, et al. Expression and significance of serum interferon-g, serum amyloid a protein and

soluble fms-like tyrosine kinase-1 in patients with unexplained recurrent spontaneous abortion. J Clin Med Pract. 2022;26:25–28.
[39] Yang Q, Whitin JC, Ling XB, et al. Plasma biomarkers in a mouse model of preterm labor. Pediatr Res. 2009;66 (1):11–16. doi: 10.1203/PDR.0b013e3181a207e3
[40] Mithal LB, Palac HL, Yogev R, et al. Cord blood acute phase reactants predict early onset neonatal sepsis in preterm infants. PLOS ONE. 2017;12(1):e0168677–e0168677. doi: 10.1371/journal.pone.0168677
[41] Sack GH Jr. Serum amyloid a – a review. Mol Med. 2018;24(1):46–46. doi: 10.1186/s10020-018-0047-0
[42] Passey SL, Bozinovski S, Vlahos R, et al. Serum amyloid a induces toll-like receptor 2-dependent inflammatory cytokine expression and atrophy in C2C12 skeletal muscle myotubes. PLOS ONE. 2016;11(1):e0146882–e0146882. doi: 10.1371/journal.pone.0146882
[43] Sun L, Ye RD. Serum amyloid A1: structure, function and gene polymorphism. Gene. 2016;583(1):48–57. doi: 10.1016/j.gene.2016.02.044
[44] Li XQ, Zhu P, Myatt L, et al. Roles of glucocorticoids in human parturition: a controversial fact? Placenta. 2014;35 (5):291–296. doi: 10.1016/j.placenta.2014.03.005
[45] O'Hara R, Murphy EP, Whitehead AS, et al. Local expression of the serum amyloid a and formyl peptide receptor–like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. Arthritis & Rheumatism. 2004;50 (6):1788–1799. doi: 10.1002/art.20301
[46] Lee HY, Kim M-K, Park KS, et al. Serum amyloid a stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochem Biophys Res Commun. 2005;330(3):989–998. doi: 10.1016/j.bbrc.2005.03.069
[47] Lin Y-K, Zhu P, Wang W-S, et al. Serum amyloid A, a host-derived DAMP in pregnancy? Front Immunol. 2022;13:978929–978929. doi: 10.3389/fimmu.2022.978929
[48] Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760–765. doi: 10.1126/science.1251816
[49] Farina L, Winkelman C. A review of the role of pro-inflammatory cytokines in labor and noninfectious preterm labor. Biol Res Nurs. 2005;6(3):230–238. doi: 10.1177/1099800404271900
[50] Keelan JA, Blumenstein M, Helliwell RJA, et al. Cytokines, prostaglandins and parturition—A review. Placenta. 2003;24:S33–S46. doi: 10.1053/plac.2002.0948
[51] Branch DW, Silver R, Pierangeli S, et al. Antiphospholipid antibodies other than lupus anticoagulant and anti-cardiolipin antibodies in women with recurrent pregnancy loss, fertile controls, and antiphospholipid syndrome. Obstet & Gynecology. 1997;89 (4):549–555. doi: 10.1016/S0029-7844(97)00065-3
[52] Mankee A, Petri M, Magder LS. Lupus anticoagulant, disease activity and low complement in the first trimester are predictive of pregnancy loss. Lupus Sci & Med. 2015;2(1):e000095. doi: 10.1136/lupus-2015-000095
[53] Kanwal A, Nayyar A, Noureen F, et al. Estimation of lupus anticoagulant in women with recurrent miscarriages. Prof Med J. 2024;31(6):887–891. doi: 10.29309/TPMJ/2024.31.06.8147
[54] Di Simone N, Meroni PL, de Papa N, et al. Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered β2-glycoprotein I. Arthritis Rheum. 2000;43(1):140–150. doi: 10.1002/1529-0131(200001)43:1<140::AID-ANR18>3.0.CO;2-P
[55] Sammaritano LR, Gharavi AE, Soberano C, et al. Phospholipid binding of antiphospholipid antibodies and placental anticoagulant protein. J Clin Immunol. 1992;12(1):27–35. doi: 10.1007/BF00918270
[56] Bakimer R, Fishman P, Blank M, et al. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). J Clin Invest. 1992;89(5):1558–1563. doi: 10.1172/JCI115749
[57] Salazar-Páramo M, Jara LJ, Ramos A, et al. Longitudinal study of antinuclear and anti-cardiolipin antibodies in pregnant women with systemic lupus erythematosus and antiphospholipid syndrome. Rheumatol Int. 2002;22(4):142–147. doi: 10.1007/s00296-002-0207-x
[58] Silver RM. Catastrophic antiphospholipid syndrome and pregnancy. Semin Perinatol. 2018;42(1):26–32. doi: 10.1053/j.semperi.2017.11.006
[59] Andreea R, Dudu SC, Ciobanu A, et al. Pregnancy management in women with antiphospholidic syndrome. Maedica. 2019;14(2):148. doi: 10.26574/maedica.2019.14.2.148
[60] Galli M, Barbui T. Antiphospholipid antibodies and thrombosis: strength of association. Hematol J. 2003;4 (3):180–186. doi: 10.1038/sj.thj.6200206
[61] Hanly JG. Antiphospholipid syndrome: an overview. CMAJ. 2003;168:1675–1682.
[62] Jerrold SL, Branch W, Munther A. The antiphospholipid syndrome. N Engl J Med. 2002;346(10):752–763. doi: 10.1056/NEJMra002974
[63] Bevers EM, Galli M, Barbui T, et al. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. Thromb Haemost. 1991;66(6):629–632. doi: 10.1055/s-0038-1646476
[64] Oosting JD, Derksen RH, Bobbink IW, et al. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanism? [see comments]. Blood. 1993;81 (10):2618–2625. doi: 10.1182/blood.V81.10.2618.2618
[65] Schousboe I. Beta 2-glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. Blood. 1985;66(5):1086–1091. doi: 10.1182/blood.V66.5.1086.1086
[66] Alijotas-Reig J, Esteve-Valverde E, Anunciación-Llunell A, et al. Pathogenesis, diagnosis and management of obstetric antiphospholipid syndrome: a comprehensive review. J Clin Med. 2022;11(3):675. doi: 10.3390/jcm11030675
[67] Ogasawara MS, Aoki K, Katano K, et al. Factor XII but not protein C, protein S, antithrombin III, or factor XIII is a predictor of recurrent miscarriage. Fertil Steril. 2001;75 (5):916–919. doi: 10.1016/S0015-0282(01)01688-0
[68] Preston F, Rosendaal F, Walker I, et al. Increased fetal loss in women with heritable thrombophilia. Lancet. 1996;348(9032):913–916. doi: 10.1016/S0140-6736(96)04125-6
[69] McNamee K, Dawood F, Farquharson RG. Thrombophilia and early pregnancy loss. Best Pract & Res Clin Obstet & Gynaecol. 2012;26(1):91–102. doi: 10.1016/j.bpobgyn.2011.10.002
[70] Zhang N, Ahsan MH, Purchio AF, et al. Serum amyloid A-luciferase transgenic mice: response to sepsis, acute arthritis, and contact hypersensitivity and the effects of proteasome inhibition. J Immunol. 2005;174(12):8125–8134. doi: 10.4049/jimmunol.174.12.8125

[71] Ames P, Antinolfi I, Ciampa A, et al. Primary antiphospholipid syndrome: a low-grade auto-inflammatory disease? Rheumatology. 2008;47(12):1832–1837. doi: 10.1093/rheumatology/ken382

[72] Meroni PL, Borghi MO, Raschi E, et al. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol. 2011;7(6):330–339. doi: 10.1038/nrrheum.2011.52

[73] Arreola-Diaz R, Majluf-Cruz A, Sanchez-Torres L, et al. The pathophysiology of the antiphospholipid syndrome: a perspective from the blood coagulation system. Clin Appl Thromb/Hemostasis. 2022;28:10760296221088576. doi: 10.1177/10760296221088576

[74] Sciascia S, Roccatello D, Bertero MT, et al. 8-isoprostane, prostaglandin E2, C-reactive protein and serum amyloid a as markers of inflammation and oxidative stress in antiphospholipid syndrome: a pilot study. Inflammation Res. 2012;61(8):809–816. doi: 10.1007/s00011-012-0468-0

[75] Sodin-Šemrl S, Žigon P, Čučnik S, et al. Serum amyloid a in autoimmune thrombosis. Autoimmun Rev. 2006;6(1):21–27. doi: 10.1016/j.autrev.2006.03.006

[76] Cai H, Song C, Endoh I, et al. Serum amyloid a induces monocyte tissue factor. J Immunol. 2007;178(3):1852–1860. doi: 10.4049/jimmunol.178.3.1852

[77] Cai H, Song C, San Lim IG, et al. Importance of C-reactive protein in regulating monocyte tissue factor expression in patients with inflammatory rheumatic diseases. J Rheumatol. 2005;32:1224–1231.