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Maternal serum amyloid a as a potential biomarker for primary unexplained recurrent early pregnancy loss: a case-control study in Iraq

Serum amyloid in a recurrent early pregnancy loss

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ABSTRACT

Objective. Recurrent pregnancy loss (RPL) is a multifactorial condition where many of the proposed risk factors lack an etiologic explanation, especially in primary unexplained recurrent early pregnancy loss (REPL). Herein, the association between maternal serum amyloid A (SAA) levels and primary unexplained REPL is examined among women who presented with first-trimester miscarriage.

Materials and Methods. A cross-sectional study was conducted at the Al-Yarmook Teaching Hospital from June 2023 to June 2024. It included 140 women who were diagnosed with a case of missed miscarriage and were divided into two groups. The study cohort consisted of 70 women with more than two consecutive REPLs without previous live birth deliveries. The control group included 70 women who had delivered at least once and never experienced REPL. Complete demographic data were taken from all subjects. SAA levels in both groups were estimated using ELISA kits.

Results. Significant differences were noted in the marriage duration, parity and history of spontaneous miscarriage between cases with REPL. SAA was very highly elevated in this study group 53.52 ± 10.65 micrograms/ml as compared to the control group 11.33 ± 3.27 micrograms/ml, $p < 0.0001$. Moreover, SAA showed a positive significant correlation with body mass index, $p = 0.041$, while showing negative association with marriage duration, $p = 0.921$ and age, $p = 0.184$.

Conclusions: Elevated SAA levels in patients with primary indeterminate REPL may be associated with adverse outcomes during the first trimester. Further studies are required to establish SAA level utility in identifying women who have an increased risk of REPL, thereby further informing counseling services and guiding therapeutic interventions.

Key words

Primary indeterminate recurrent pregnancy loss; trophoblastic invasion; serum amyloid A.

Introduction

Recurrent pregnancy loss (RPL) has been medically defined as having two or more consecutive pregnancy losses, confirmed by ultrasound or histopathology, occurring before 24 weeks of gestation. It affects approximately 2%–5% of women of reproductive age worldwide [1,2]. A wide etiological spectrum surrounds RPL, including chromosomal abnormalities, uterine anomalies, endocrine dysfunctions, thrombophilic conditions, antiphospholipid syndrome, and advanced maternal age [3]. Yet, in almost 50% of cases, no certain cause is able to be determined—a condition that results in a big challenge to both clinicians and the couples themselves [4]. RPL is further classified into primary, where pregnancy losses take place in the absence of any prior live birth, and secondary, where the woman has had at least one previous viable pregnancy [5]. Primary unspecified recurrent early pregnancy loss, where no known anatomical, genetic, or immunological abnormalities explain the pregnancy losses, is a particularly perplexing subset. However, over the last few years, evidence has started showing that dysregulation of the maternal-fetal immunological interactions in unexplained cases may take a central place.

Serum amyloid A (SAA) is a small apolipoprotein acting as an acute-phase protein. It consists of 104 amino acids and has been recognized for its major role in inflammation and immune response modulation. In physiological conditions, SAA is present in low concentration; however, SAA levels increase more than 1000-fold in acute-phase reactions, infections, and immune activation [7]. SAA affects the behavior of endothelial and immune cells, including the inhibition of lymphocyte proliferation, modulation of platelet aggregation, the stimulation of prostaglandin and metalloproteinase production, and the facilitation of phagocytic activity [7, 8]. There is recent evidence that SAA may influence early placentation. At low concentrations, it could support trophoblastic invasion and placentation by influencing metalloproteinases activity. On the other hand, high concentrations of SAA had been hypothetically proposed to disrupt cytotrophoblast migration and invasion in their interaction with the decidua, thus leading to early pregnancy loss. This may indicate the double role of SAA as a possible biomarker of the immunological disturbances associated with REPL [9-11].

Given the high burden and unclear pathogenesis of primary indeterminate REPL, this study aims to investigate the association between maternal serum amyloid A levels and recurrent early pregnancy loss in a cohort of Iraqi women. We hypothesize that elevated SAA levels reflect an underlying subclinical inflammatory or immunological state that impairs normal trophoblastic function, thereby contributing to early miscarriage.

Materials and Methods

Study Design and Setting

This research has been designed as a cross-sectional case-control study and performed at Al-Yarmook university-affiliated teaching hospital in Baghdad, Iraq, from June 2023 to June 2024. The Hospital is one of the largest tertiary referral centers in Baghdad with a very high obstetrics and gynecology patient load and receives a substantial number of miscarriage cases on a weekly basis. The study followed the principles outlined in the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement [12].

Study Population

The research involved 140 women diagnosed with first-trimester missed miscarriage who visited the hospital during the study period. Participants were recruited consecutively after meeting the inclusion criteria and providing informed consent. Based on their reproductive history, the women were allocated into two groups: the study group, comprising 70 women who have experienced more than two consecutive early pregnancy losses and no prior viable deliveries (defined as primary indeterminate recurrent early pregnancy loss, or REPL), and the control group, comprising 70 women, each having at least one prior successful live birth. The control group was intentionally selected from women presenting with missed miscarriage but without a history of recurrent loss, rather than healthy ongoing pregnancies, because the aim of this study was to compare SAA levels within a clinically homogeneous condition.

Sample Size Justification

This retrospective study included all eligible women who presented during the defined study period; therefore, the sample size was determined by consecutive case availability rather than by a formal a priori power calculation, as the study was exploratory in nature.

The sample size was calculated according to the following equation for cross-sectional study with quantitative variables : $\text{Sample size} = (Z_{1-\alpha/2})^2 \text{SD}^2/d^2$, $Z_{1-\alpha/2}$ is a standard normal variate = 1.96. SD= standard deviation of the variable, d= absolute error or precision which assessed by the researcher. $\text{Sample size} = (1.96)^2 (0.4)^2 / (0.1)^2 = (3.84 * 0.16) / 0.01 = 61$ patients.

So the sample size is 61 patients; our study involved 140 patients.

Eligibility Criteria

Women were eligible for inclusion if they had a confirmed diagnosis of first-trimester missed miscarriage between six and twelve weeks of gestation, assessed by transvaginal ultrasonography. Participants in the study group were required to have experienced more than two consecutive unexplained early miscarriages with no prior live birth. Control group participants were required to have no history of miscarriage or adverse pregnancy events, but they have previous one or two live birth (parous women)..

To minimize confounding factors, several exclusion criteria were rigorously applied. Women were excluded if they had known antiphospholipid syndrome (including positive anticardiolipin antibodies or lupus anticoagulant), multifetal gestation, pre-existing medical conditions such as diabetes mellitus, hypertensive disorders, pre-eclampsia, or fetal growth restriction, and to be correlated with the definition of primary unspecified recurrent early pregnancy loss (REPL), where no known anatomical, genetic, or immunological abnormalities can explain the losses. Pre-eclampsia and fetal growth restriction were excluded by reviewing clinical records, blood pressure measurements, and antenatal ultrasound reports. Cases with any documented hypertensive disorder, proteinuria, or evidence of abnormal fetal growth or placental insufficiency were not included.

Cases of inevitable, incomplete, or septic miscarriage were excluded to ensure clinical homogeneity, as these conditions involve different inflammatory profiles and patterns of tissue expulsion that could potentially alter SAA levels. Missed miscarriage was defined as a non-viable intrauterine pregnancy without clinical signs of expulsion and confirmed sonographically.

Additionally, women with active or chronic infections, inflammatory diseases, autoimmune disorders, neoplasms, or a history of polycystic ovarian syndrome were excluded. Patients with documented aneuploidy in previous miscarriages, as well as those who are smokers, were also excluded. Autoimmune conditions were also excluded with standard auto-immune workup, which included thyroid function, autoantibodies, antiphospholipid antibody screen, and inflammatory markers (ESR/CRP). Patients who had abnormal results on these tests were excluded.

Clinical Evaluation and Diagnosis

All participants underwent a thorough clinical and obstetric evaluation, including detailed history-taking through a standardized questionnaire addressing demographic data, past obstetric outcomes, lifestyle factors, and comorbidities. Gestational age was calculated based on the first day of the last menstrual period and verified through transvaginal ultrasound. All participants underwent transvaginal ultrasound between 6 and 10 weeks of gestation to verify embryonic or fetal viability and crown-rump length.

Missed miscarriage was diagnosed based on standard ultrasonographic criteria, including the absence of fetal cardiac activity in an embryo with a crown-rump length (CRL) ≥ 7 mm, or the absence of an embryo (and hence a yolk sac or fetal pole) in a gestational sac with mean sac diameter (MSD) ≥ 25 mm, in accordance with international early pregnancy guidelines [13].

Blood Sample Collection and Biochemical Assay

Before any medical or surgical intervention to terminate the non-viable pregnancy, five milliliters of peripheral venous blood were collected from each woman under sterile conditions. The samples were quickly processed centrifuged, with serum separated and stored at -80°C until analysis. Maternal serum SAA levels were determined using a commercial Human SAA ELISA Kit (Casabio), designed for the quantitative measurement of Human SAA1 protein in serum, urine. Cat.No : YHB2679Hu, Species; Homo sapiens (Human), detection Range

156 ng/mL-10000 ng/mL. sensitivity 39 ng/mL, Assay Time 1-5h, Sample Volume 50-100ul

Detection Wavelength 450 nm CV(%) = $\text{SD}/\text{mean} \times 100$, Intra-Assay: CV<10% , Inter-Assay: CV<12%, Store at $2-8^{\circ}\text{C}$ trying to avoid freeze/thaw cycles, utilizes biotin double-antibody sandwich technology for enhanced sensitivity and specificity. Assess the linearity of the assay, samples were spiked with high concentrations of human SAA in various matrices and diluted with the Sample Diluent to produce samples with values within the dynamic range of the assay. All samples were processed in duplicate, and laboratory personnel were blinded to the clinical grouping of the patients to reduce bias.

Statistical Analysis

Data analysis was conducted with IBM SPSS Statistics, Version 25.0. Continuous variables such as maternal age, BMI, gestational age, and SAA levels were summarized as mean \pm SD and compared between groups using the Student's t-test or Mann-Whitney U test. Categorical variables were analyzed with either the Chi-square (χ^2) test or Fisher's exact test. Pearson's r measures the correlations between SAA level and clinical parameters. Multivariable regression analysis identified independent predictors of high SAA levels. ROC curve analysis assessed the diagnostic performance of SAA. Unless stated otherwise, $P < 0.05$ was considered statistically significant.

Ethical Statement

The study design was adhered to the ethical standards of the 1964 Helsinki Declaration. Ethical approval was granted by the university's scientific and ethical review board (Approval ID: MOG 89, dated March 26, 2023). All participants were informed about the study's aims and procedures and gave both verbal and written informed consent before participating.

Results

Demographic and Clinical Characteristics

A total of 140 women were enrolled in this study: 70 women with primary indeterminate REPL and 70 women in the control group with at least one previous live birth. All participants had a confirmed diagnosis of first-trimester missed miscarriage.

As shown in **Table 1**, there were no statistically significant differences between the REPL and control groups regarding maternal age, BMI, gestational age at sampling, miscarriage duration,

method used for miscarriage induction, socioeconomic status, or passive smoking exposure ($P > 0.05$). However, significant differences were found in parity and history of spontaneous miscarriage between the groups ($P < 0.001$).

Serum Amyloid A Levels

Table 2 illustrates the comparison of mean SAA levels between the two study groups. SAA concentrations were significantly higher among women with REPL ($53.52 \pm 10.65 \mu\text{g/mL}$) in comparison to the control group ($11.33 \pm 3.27 \mu\text{g/mL}$), with a highly significant difference ($P < 0.0001$).

Correlation Analysis

Table 3 presents the correlation between SAA levels and various clinical parameters. A statistically significant positive correlation was found between SAA and BMI ($P = 0.041$). Additionally, SAA levels showed positive associations with parity, prior miscarriage history, and gestational age at sampling. In contrast, SAA exhibited no significant correlation with gestational age ($r = -0.012$, $P = 0.921$) or maternal age ($r = -0.160$, $P = 0.184$).

Diagnostic Performance of SAA

To assess the discriminative ability of SAA levels between REPL and control groups, a receiver operating characteristic (ROC) curve was plotted (**Figure 1**). The area under curve determine good discriminative value, it is 0.81; The optimal cut off value in order to use SAA as a determinant predictor for REPL at level above than 20 microgram/ ml. , with sensitivity , specificity, positive predictive value, and negative predictive value illustrated in table 5.

Despite the statistical elevation of SAA in the REPL group, though it may serve as an adjunctive marker in identifying women at increased risk for early pregnancy failure.

Multivariate Analysis

the multivariable logistic regression analysis in **Table 4** shows that the SAA was found to be indeterminant for REPL, as well as the previous miscarriage evidenced by p value of less than 0.001 compared to other predictors in multivariate analysis.

Discussion

We found a statistically significant elevation of maternal serum SAA levels in women with REPL compared to controls ($P < 0.0001$), reinforcing its potential as an inflammatory biomarker in this context. Our results are consistent with previous studies, which reported a significant increase in SAA level in women with early pregnancy loss. The biological plausibility of our findings is offered by the bimodal role of SAA. Physiological levels of SAA are involved in cell migration, differentiation, including trophoblastic cells, while high levels of SAA could affect trophoblastic invasion. This hypotheses has been proven by Sandri et al., who showed that high SAA concentrations above $20 \mu\text{g/mL}$ in the first trimester of pregnancy result in a reduction of extravillous trophoblast migration via toll receptors, resulting in pregnancy loss, supporting our

hypothesis from our research [14]. In contrast, Li et al. suggested that SAA is physiologically produced in the fetal membrane, which is implicated in parturition via activation of toll receptors, which is a time-dependent process in pregnancy [15].

Immunological factors continue to be a critical area of exploration in unexplained REPL. Cavalcante et al. conducted a review showing elevated antinuclear antibody (ANA) prevalence in women with recurrent miscarriage, suggesting an autoimmune component even in idiopathic cases [16]. Likewise, Peng et al. identified an association of the IL-10 -1082A/G polymorphism with idiopathic recurrent miscarriage, implicating the role of anti-inflammatory cytokines in miscarriage [17]. The role of immune system biomarkers has been further elaborated by Kolanska et al. in predicting the prognosis and treatment of miscarriage. In the patient population with recurrent miscarriages, the use of immunotherapy (aspirin, low-dose heparin, and other medications) significantly increased the rate of live births, particularly when anti-ANA positivity was ascertained [18]. These findings, taken together with our results, emphasize the potential of immune-related biomarkers such as SAA to inform prognosis and guide personalized interventions.

Although BMI did not show a significant difference between the REPL group and control group, there is variation within the REPL group regarding BMI. This is a sign of variability within the group, not necessarily a comparison effect. Since BMI is known to be high in women with recurrent pregnancy loss in certain communities, such variability might be an indication of susceptibility to the condition, although not significant enough to cause a significant difference when compared with the control group [19].

The observed difference in gestational age at the time of sampling in the study group is expected, as the timing of missed miscarriage varies naturally among women with recurrent loss. Women with REPL often present earlier due to heightened vigilance, prior obstetric experience, and earlier detection of pregnancy complications [20]. Importantly, the difference in gestational age was small and remained within the first trimester window for all participant.

The screening of high SAA levels could, therefore, be a helpful approach in the identification of individuals at a higher risk of first-trimester loss. Given the influence of SAA on trophoblast proliferation and migration, it may serve as both a marker of ongoing subclinical inflammation and a pathophysiological contributor to pregnancy failure. Our findings support the hypothesis that elevated maternal serum SAA is associated with primary indeterminate REPL and may serve as a predictive marker of poor first-trimester outcomes. Confirmation of SAA as a dependable biomarker will require additional studies with larger cohorts and long-term follow-up, and its potential as a therapeutic target in immune-mediated pregnancy loss should be investigated.

Limitations

The study has several limitations that need to be acknowledged. First, the sample size was relatively small and drawn from a single tertiary center in Baghdad, which may limit generalizability. Second, the cross-sectional observational design does not permit causality between elevated SAA levels and recurrent early pregnancy loss to be established. Third,

although major known causes were excluded, several important evaluations, including partner karyotyping, detailed uterine anomaly assessment, and analysis of products of conception for aneuploidy, were not available within the scope of this study and may account for potential confounders. Fourth, the clinical data were limited to what was documented in the medical records, which may introduce information bias. This design introduces potential selection bias, since SAA levels may vary according to miscarriage subtype and underlying inflammatory background. Finally, the ROC analysis showed only limited discriminatory performance of SAA as a standalone marker, suggesting that its clinical utility may be enhanced when used in combination with other inflammatory or immunological biomarkers.

Conclusions

The results of the research revealed that SAA in the maternal sera of women with primary indeterminate REPL is significantly higher when compared to the controls. Although the result indicates a potential correlation between high SAA and previous early miscarriages, there is still a need for further research to validate the usefulness of SAA when used as part of a set of biomarkers in predicting the risks of early pregnancy.

Compliance with Ethical Standards

Authors' Contributions: A.I.A.: Conceptualization, study design, data collection, and drafting of the manuscript. Z.A.A.J.: Data analysis, interpretation of results, and critical revision of the manuscript. R.Z.O.: Supervision, methodology guidance, and final approval of the manuscript.

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Study Registration: None.

Conflict of Interest: None

Ethical Statement: The study design conformed to the ethical guidelines of the 1964 Helsinki Declaration. Ethical clearance was obtained from the university's scientific and ethical review board, Approval ID: MOG 89, dated March 26, 2023.

Consent to participate: Informed consent about the purpose and process of the study was obtained from all participants in writing on the consent form, supported by verbal consent.

Availability of data and material: The datasets used and/or analyzed are available from the corresponding author on reasonable request.

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Table 1: Comparison of Demographic and Clinical Characteristics Between Women With Primary Indeterminate REPL and Controls.

Variable	REPL Group (n = 70)	Control Group (n = 70)	P-value
Age (years)	29.3 ± 2.5 (23–34)	28.4 ± 2.5 (23–33)	0.137
BMI (kg/m ²)	26.5 ± 1.4 (24–29.1)	26.3 ± 1.3 (23–29.2)	0.520
Gestational age (weeks)	3.3 ± 0.7 (2–4.5)	3.4 ± 0.6 (2–4.7)	0.809
Parity	nulliparity	para one or two	< 0.001
Hx. of abortion	3 (2–4)	1 (1–1)	< 0.001
Gestational age at the date of sampling (weeks)	8.1 ± 0.4 (7–8.6)	8.2 ± 0.3 (7–8.7)	0.805
Method used for miscarriage induction			0.835
– Medical	15 (21.4%)	14 (20.0%)	
– Surgical	55 (78.6%)	56 (80.0%)	
Socioeconomic status			0.805
– Low SES	61 (87.1%)	60 (85.7%)	
– High SES	9 (12.9%)	10 (14.3%)	
Passive smoking status			0.227
– Yes	13 (18.6%)	19 (27.1%)	
– No	57 (81.4%)	51 (72.9%)	

Table 2: Comparison of SAA Levels Between the two groups.

Serum Amyloid A ($\mu\text{g/mL}$)	REPL Group (n = 70)	Control Group (n = 70)
Mean \pm SD	53.52 \pm 10.65	11.33 \pm 3.27
median	45.34 \pm 21.67	13.09 \pm 3.34
Standard Error of Mean	1.27	0.39
Range	27.12 – 69.90	6.0 – 17.21
Percentiles		
– 5th	29.10	6.31
– 25th	48.60	8.40
– 50th (Median)	55.21	11.70
– 75th	59.20	13.90
– 95th	68.80	16.40
– 99th	69.90	17.21
P-value*	< 0.0001	

*P-value calculated using the Mann–Whitney U test.

Table 3: The Correlation results Between SAA Levels and Clinical Parameters in Women With Primary Indeterminate REPL and Controls

Variable	REPL Group (n = 70)	Control Group (n = 70)
Age (years)	r = -0.160, P = 0.184	r = -0.268*, P = 0.025
BMI (kg/m ²)	r = 0.245, P = 0.041	r = -0.110, P = 0.365
Gestational age (days)	r = -0.012, P = 0.921	r = 0.114, P = 0.346
Parity	Not assessed	r = 0.069, P = 0.569
Hx. of miscarriage	r = -0.101, P = 0.404	Not assessed
Gestational age at time of sampling	r = 0.057, P = 0.642	r = -0.092, P = 0.446

* Correlation coefficients and P-values were calculated using Spearman's rank correlation test.

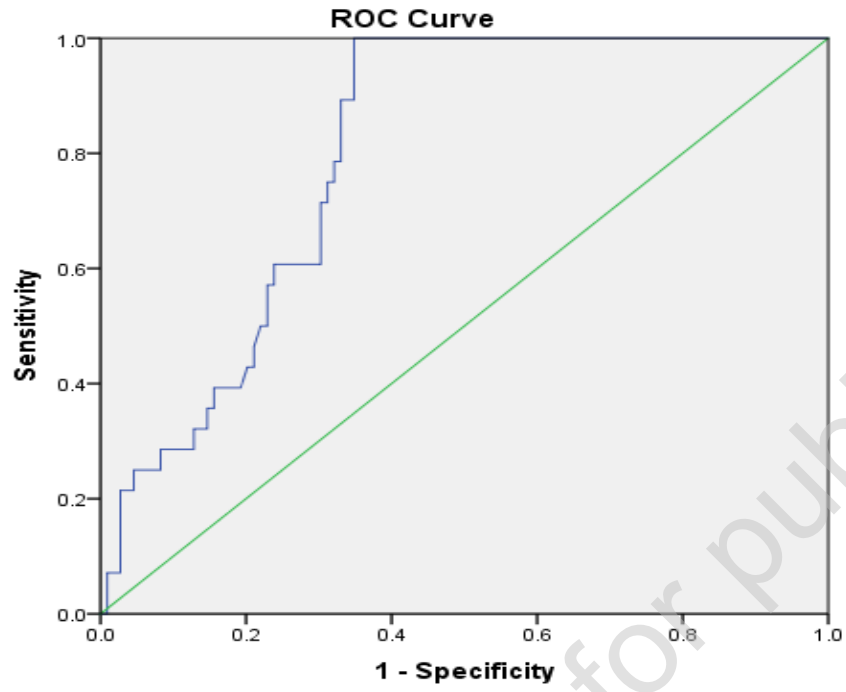
Table 4: Multivariate Linear Regression Model to detect the determinant of Primary Indeterminate REPL

Predictor	Standard Error	Odds ratio (95% confidence interval)	p-value
SAA (µg/mL)	0.00326	1.23(1.08–1.20)	< 0.001
Age (years)	-0.01611	0.87 (0.65–1.03)	0.819
Body Mass Index (kg/m ²)	-0.02647	0.64(0.58-1.06)	0.894
Duration of Marriage (years)	0.05947	0.34(0.57-1.19)	0.206
Gestational Age at Sampling (weeks)	0.10892	0.60(0.37–0.97)	0.499
Hx. of Spontaneous miscarriage	0.07548	1.23(1.08–1.20)	< 0.001

21.

Table 5 SAA to differentiate between REPL and control groups.

Assessment	value	95% confidence interval
Area under the receiver operating Characteristic curve	0.813	0.88-0.98
Youden index, ideal cutoff level of serum Amyloid A, µg/mL	0.7 >20	- -
Sensitivity,%	87.8	76.1-98.3
Specificity,%	84.2	72.4-94.5
Positive predictive value, %	88.4	68.4-87.8
Negative predictive value,%	83.1	75.3-79.9



Diagonal segments are produced by ties.

Figure 1: ROC curve demonstrating SAA's ability to discriminate between women with primary indeterminate REPL and controls. The AUC was 0.81, indicating good discriminative value.