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Maternal Serum Amyloid a Level as a Novel Marker of Primary Unexplained Recurrent Early Pregnancy Loss

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Abstract **Background:** Recurrent abortion affects 1-2% of woman. Serum Amyloid A belongs to a family of apolipo proteins produced in aresponse to cytokines released by activated monocytes and macrophages, isolated in the last 50 years. Our current study emphasizes human SAA protein as a sensitive biochemical marker for primary unexplained recurrent miscarriage. The aim of the current study is to examine the hypothesis that primary unexplained REPL might be associated with high maternal serum levels of SAA, which in turn could lead to defective trophoblastic invasion into the decidua, and subsequent pregnancy failure and miscarriage. **Patients and Method:** A prospective study (case control study) in Al-Zahraa Maternity Hospital, Najaf, Iraq, from first of January to the first of December of 2019. the study was conducted among 91 who were divided into two groups Group 1: women with missed miscarriage in the first trimester with at least two consecutive primary unexplained REPLs and no previous live births were enrolled. Group 2: A control group was formed of women with miscarriage no history of REPL who had at least one previous uneventful pregnancy with no adverse outcomes. Serum samples were collected to measure SAA levels. **Result:** The main outcome was the association between SAA and primary unexplained REPL. A total number of 91 participants. Mean SAA level was significantly higher among women with REPL than among women in the control group (P<0.001). The SAA level was dependent indicator of primary unexplained REPL, P<0.001 Elevated SAA levels found among women with primary unexplained REPL could represent biomarker for this complication of pregnancy. **Conclusions:** The present findings suggest that SAA is potentially a novel marker for primary unexplained REPL that warrants further investigation. For example, studies could be conducted to compare SAA levels among women with a history of primary unexplained REPL during the pregnant and non-pregnant states, as well as before and after mischarge. In future, such studies might guide the timing for initiation of new treatments, such as gene therapy or the use of immune-receptor antagonists.

Key Words abortion, cytokines, amyloid, pregnant

1. Introduction

Early recurrent pregnancy loss (RPL) was defined as the occurrence of at least two failed pregnancies before 10 weeks (documented by ultrasonographic or histopathologic examination). Recurrent early pregnancy loss (REPL) affects 1%-2% of all pregnant women [1], [2]. Recurrent early pregnancy loss (REPL) affects 1-2% of all pregnant women 1,2 Most RPL is not due to chance alone and should be investigated clinically [3]. RPL classification as Primary RPL is defined as pregnancy loss with no previous live births while secondary RPL refers to women with pregnancy loss and at least, one live birth which make up to no- 61% of all people with RPL [4]. Approximately 15% of all pregnancies that can be visualized on ultrasound end in pregnancy loss [5]. Three or more losses affect 1-2% of women of reproductive age and two or more losses affect around 5% [6]. Despite extensive investigation of women with three or more miscarriages, the cause of recurrent pregnancy loss remains unknown in the majority of cases [7].

Main causes of RPL were super-receptivity [8] ; Migration and Invasion of Trophoblastic [9]; Cellular Movement [10]; Karyotyping the Products of Conception [11]; and Subfertility and Super fertility [12]. Medical management of recurrent pregnancy loss typically includes diagnosis and treatment by a reproductive endocrinologist and/or a high-risk obstetrician/gynecologist [13]. Genetic counseling concerning the potential for successful pregnancy without treatment, in addition to a discussion of the uncertainties of diagnostic and treatment options and their safety and efficacy, may also be appropriate [14].

Serum amyloid A (SAA) Is an immunoregulatory protein involved in the acute- phase reaction [15]. Amyloids have been known to arise from many different proteins [16]. It is a pseudogene. Although SAA is predominantly synthesized in the liver extra-hepatic sources, including first-trimester trophoblasts, have been described. In these cells, SAA exerts immunoregulatory effects and key effects on trophoblastic migration, invasion, and differentiation [17].

2. Patients and Methods

A prospective study (case control) was conducted at Al-Zahraa Maternity Hospital, Najaf, Iraq, between first of January and first of December of 2019. A total number of 91 (30 patients (RPL) and 61 controls) were enrolled in the study.

A. The Inclusion Criteria

- Women with missed miscarriage in the first trimester of the present pregnancy(6-12weeks).
- Patients ahad at least 2 or more consecutive recurrent pregnancy loss (REPL).and maternal age was between (19-35) years old.
- The control group missed miscarriage include patient with no history of REPL and at least one previous uneventful pregnancy with no adverse outcomes were enrolled in the study.

B. The Exclusion Criteria

- Any acute or chronic inflammatory state, such as infection, inflammatory arthritis, trauma, systemic autoimmune or inflammatory diseases, lack of antiphospholipid antibodies (i.e. Lupus anticoagulant and anticardiolipin) and/or various neoplasms.
- Polycystic ovarian syndrome.
- Smoking.
- Multiple pregnancy.
- Diabetes mellitus.
- Hypertension.
- History of pre-eclampsia and/or intrauterine fetal growth restriction.

The objectives and techniques were explained to eligible patients, and written informed consent was subsequently obtained from all participants. Gestational age of the fetus was calculated at recruitment using the first day of last menstrual period, with confirmation by transvaginal ultrasonography. Missed miscarriage during the first trimester was characterized by negative cardiac pulsation within an intact intrauterine gestational sac at 6 weeks, an intrauterine gestational sac lacking a yolk sac (diameter >30 mm).

Participants in both groups underwent either surgical or medical termination of the failed pregnancy. After enrollment on diagnosis of the missed mischarge (but before any intervention), a 5-mL sample of venous blood was taken from each participant under aseptic conditions. Blood samples were centrifuged at 2500 g for 15 minutes at 4°C, separated into serum aliquots, and stored at -80°C until used for the serum amyloid A assay.

C. Statistical Analysis

Levels of serum amyloid A were assayed simultaneously for both groups using the same microtiter plates provided with the Human serum amyloid The data were analyzed using SPSS version 24.0 (IBM, Armonk, NY). Numerical data were tested for normal distribution using the Shapiro–Wilk test and Kolmogorov Smirnov test. Normally distributed data were presented as the mean ± standard deviation; between-group differences were assessed using the Student t test. Skewed numerical data were presented as the median and interquartile range (IQR); between-group differences were compared non-parametrically using theindepended Ttest. Qualitative data were presented as number (percentage); the predictor factor (OR)and CI 95%were used to compare the two groups, as appropriate. Linear-by- linear association was used to compare variables.

Receiver operating characteristic curve analysis was performed to examine the level of serum amyloid A required to discriminate between cases and controls.

- The area under this curve was estimated and the optimum cutoff value of serum amyloid A defined according to the highest Youden index (J).
- Multivariable logistic regression was used to determine independent indicators of primary unexplained REPL. All P values were two-tailed, with a value of less than 0.05 considered statistically significant

3. Results

A. Demographic Characteristic of Patients

Table 1 showed that there was highly significant difference ($p<0.05$) between studying groups regarding para1 delivery (P1D) and para 2 delivery (P2D) also, there was highly significant difference ($p<0.05$) between studying groups regarding history of two abortion and history of three abortion (P value <0.001) as well as no significant difference between studying groups regarding the residence in urban or rural area (P value >0.5). In addition, no significant difference between studying groups regarding the method of termination (P value >0.5). The statistical difference between patients and control regarding the age, BMI, duration of miscarriage (days) and gestational age (weeks), There were no statistically significant between-group differences (P value >0.5) regarding the age, duration of miscarriage (days) and gestational age (weeks) Table 2 while there was significant difference regarding BMI. Estimate size of effects for parameters in patient's group comparison with control group, Table 2.

B. The Serum Amyloid a Level Between Patients With Recurrent Pregnancy Loss Compared With Control

Mean of SAA was significantly higher among women with REPL than among control individuals (P<0.001). Table 3 showed that there was a significant difference in the size effects of SAA (ng/ml) (OR=1.378, 95% CI (1.121-1.695, sig. 0.002) in patient's group comparison with control group, not significant in others parameters Age, BMI, and gestational

GATEGORY		Study Group		Total	Chi- Square	Sig.
		Patients	Control			
No. Abortion para delivery	P1D	0	44	44	62.515	<0.001
		0.0%	72.1%	48.4%		
	P2D	0	15	15		
		0.0%	24.6%	16.5%		
Nulliparity history	2 abortions	12	2	14	83.243	<0.001*
		40.0%	3.3%	15.4%		
	3 abortions	18	0	18		
		60.0%	0.0%	19.8%		
Residence	Urban	26	55	81	0.251	0.616
		86.7%	90.2%	89.0%		
	Rural	4	6	10		
		13.3%	9.8%	11.0%		
Method of termination of pregnant	Medical	17	39	56	0.449	0.503
		56.7%	63.9%	61.5		
	Surgical	13	22	35		
		43.3%	36.1%	38.5		

Table 1: para1 delivery (P1D) and para 2 delivery (P2D)

Demographic	Study Group			Control			t-test	Sig.
	Range Min-Max	Mean	SD.	Range Min- Max	Mean	SD.		
Age	16 19-35	25.13	3.07	16 19-35	24.93	3.44	0.27 9	0.78 1
BMI	7 18-25	21-30*	2.23	8 17-25	20.20	2.08	2.26 7	0.02 *
Duration of misscarrge (days)	9 5-14	7.93	2.52	9 5-14	8.25	2.77	0.52 1	0.60 1
Gestational age (wk)	2 6-8	6.87	0.68	7 1-8	6.79	0.98	0.39 9	0.69 1

Table 2: Demographic characteristic of patients

age between study groups. The statistical difference between patients and control regarding the serum amyloid A.

Data regarding the ability of SAA level to discriminate between the cases of primary unexplained REPL and control individuals are presented in Figure 1. The area under the receiver operating characteristic curve indicated excellent discriminative value. Use of serum amyloid A level to discriminate between women with or without primary unexplained recurrent early pregnancy loss

The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, and negative predictive value of this cutoff level are outlined Table. Receiver operating characteristic curve to determine the ability of serum amyloid A level to discriminate between women with or without primary unexplained recurrent early pregnancy loss. The area under the curve was 0.978. The multivariable logistic regression model for determinants of primary unexplained REPL., the SAA level was found to be a dependent indicator of primary unexplained REPL. The model had good overall fit as evidenced by the -2 log likelihood test (P<0.001), the Hosmer–Leme show test (AUC=95% (0.923-0.997).

The correlation of serum amyloid A levels with age (years), BMI, and gestational age in patients and control groups. While there were no correlation (Pvalue>0.05) between serum amyloid A levels of the patients and the age of the patients as well as BMI and gestational age (wk) of control group tableThere were linear correlation (Pvalue<

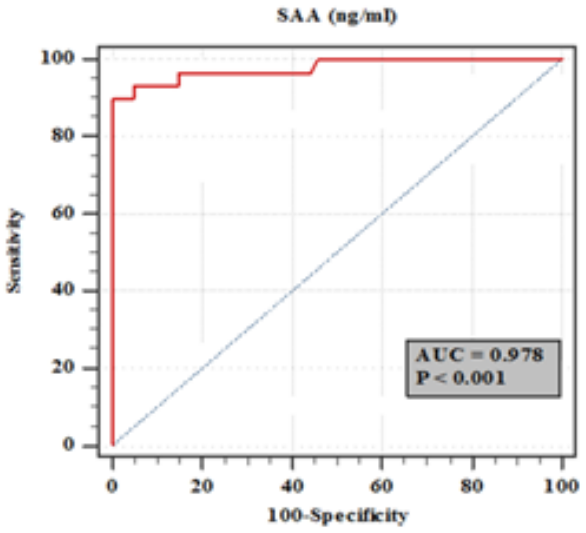

Figure 1: The ability of SAA level to discriminate between the cases of primary unexplained REPL

0.001) between serum amyloid A levels of the patients and the age of the patients as well as between serum amyloid A levels of the patients and the BMI of the patients and linear correlation (P value <0.05) between serum amyloid A levels of the patients and the gestational age (weeks) Figure 2.

Biomarker	Patients N=30			Control N=60			t-test	Sig.
	Range Min-Max	Mean	SD.	Range Min-Max	Mean	SD.		
SAA (ng/ml)	66.2 33.0-99.2	72.8*	13.59	45.0 11.2-56.2	32.918	12.1	14.18	<0.001

Table 3: The serum amyloid A level

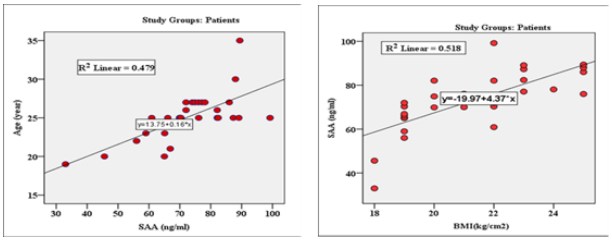

Figure 2: The correlation of serum amyloid A levels with age (years) for patients' group. The correlation of serum amyloid. A level with BMI for patients' group

4. Discussion

Early recurrent pregnancy loss is a challenging and frustrating condition for both patient and clinicians, so that its prediction and prevention and adequate management is significantly reduce the complications or unnecessary intervention [17]. We study the serum amyloid A as biochemical predictor of subclinical infection as several studies conclude that SAA is a significant acute phase reactant and an important inflammatory marker. Studies might guide the timing for initiation of new treatments [18]. Such as gene therapy or the use of immune-receptor antagonists [19].

In the our study, the difference was significantly high in serum amyloid A level between studied group ($p<0.001$), it was higher in women with missed misscarrige with REPL followed by group of missed misscarrige with no history of any misscarrge previously.the mean serum amyloid A 72.800mg/L ,32.918mg/L respectively. Women with primary unexplained REPL displayed levels of SAA that were substantially greater than those detected among control individuals. Multivariable logistic regression analysis identified SAA level as an in dependent indicator of this pregnancy complication after adjusting for maternal age and gestational age [20].

Sandri, et al., [21], At physiological levels, SAA modulates trophoblastic invasion into the decidua (a necessary step during the early stages of pregnancy stages) via activation of toll-like receptor, and maintains a functional balance between proinflammatory and anti-inflammatory cytokines for feto–maternal tolerance.19 By contrast, increased levels of SAA are associated with impaired trophoblastic invasion and syncytialization.

Invasion of trophoblasts is inhibited at high levels of SAA, indicating a reversed physiological effect of this protein that is possibly also active during the acute-phase reaction, when maternal serum levels of SAA can increase by up to 1000 times [22]. Failure of trophoblastic invasion can also occur via elevated levels of pro-inflammatory mediators such as TNF, thereby leading to early pregnancy failure and miss-carrige. Both unbalanced production of pro-inflammatory mediators and failed trophoblastic invasion are found in other placental disorders, notably pre-eclampsia and intrauterine growth restriction.13,14 The hypoxic state often associated with these placental disorders can also induce expression of SAA [19].

5. Recommendations

1) Further studies to clarify the relation between recurrent early pregnancy loss and serum amyloid level are required with large sample size.
2) Karyotyping of the coceptus should be available for further studies.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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