Maternal serum amyloid A level as a marker of primary unexplained recurrent missed miscarriages

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Abstract

Objective: To assess maternal serum amyloid A (SAA) levels among women with primary unexplained recurrent missed miscarriages.

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:

1. **Group 1:** Patient with miscarriage in the early trimester with at least two consecutive primary unexplained REPLs and no previous live births were enrolled.
2. **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. The main outcome was the association between SAA and primary unexplained REPL. A total number of 91 participants. Mean SAA level increased in women with early missed abortion than those women in the control group ($p<0.001$). The level of SAA was dependent indicator of primary unexplained REPL, ($p<o.oo1$). found that serum amyloid Alevel in women with missed miscarriages represent as biomarker of this complication of pregnancy.

Conclusions: Serum amyloid A theoretically a promising marker that prompts further study for primary unexplained missed miscarriage. in the Studies may be performed, for example, measurement of SAA level in women with history of early recurrent missed abortion during pregnancy and before and also compare SAA levels in women with non-pregnant state Such studies may direct the timing for initiating new therapies the future.

Keywords: *Serum amyloid A, maternal, Recurrent early pregnancy loss.*

Introduction

Recurrent early pregnancy loss (RPL) is the incidence of minimally two failed-pregnancies before ten week-gestation (confirmed by ultrasound or histological examination). Recurrent early pregnancy loss (REPL) affects 1-2% of all pregnant women[1]. Two or more clinical pregnancies documented by ultrasound or confirmed by histopathology end pregnancy loss[2] in approximately 15% of all known clinical cases while

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three or more losses affect almost 1-2%, and two or more losses affect 5% of all clinical cases this indicates that most RPLs are not occur by chance and therefore, require a clinical investigations and assessment[3,4]. There are many risk factors could contribute to pregnancy loss such as advancing maternal age, paternal age over 40 years, frequent previous miscarriages [5,6]. RPL could be primary when women experienced RPL without giving any live birth, secondary RPL when woman experienced RPL.

Serum amyloid A (SAA) is an immunoregulation protein involved in the acute phase response. It is known that amyloid is arise from a variety of proteins[7]. SAA has a modulating effect on the immune system, and effect on migration, invasion and differentiation of trophoblasts.

At low concentrations, SAA regulates trophoblastic attack and mineral protease activity in the placental microenvironment, which is important for placental homeostasis. However, it is clear that this chain of events is hampered by the high level of the SAA. Some researchers have found that SAA reduces the secretion of hCG[(8)]. This indicates a possible impairment of SAA-induced synthesis, which in the trophoblast is stimulated by another inflammatory mediator, TNF-α [(9)]. The role of SAA may be related to the placenta, regulating the primary trophoblastic attack in early pregnancy and maintaining the balance between pro-inflammatory and anti-inflammatory cytokines. Therefore, the current study tried to investigate the hypothesis that unexplained early RPL may be related to maternal higher levels of serum SAA

Patients and Method

A future study (case control) was Continued at Al-Zahraa teaching Hospital, Najaf, Iraq, from first of January to the first of December of 2019.

A total number of 91 [30 patients (RPL) and 61 controls] were enrolled in the study.

Women were involved missed after diagnosis women have two or more successive unspoken key (MM) and they didn't have a prior birth.

Inclusion criteria included: In the first trimester of current pregnancy (6-12 weeks), patients with miscarriage had at least 2 or more consecutive repeated pregnancy losses and maternal age ranged from (19-35) years.

The control group includes a patienthave no history of (MM) and Only one prior non-adverse pregnancy with no side effect in the study.

Exclusion criteria; women were excluded if they had one or more of the following: acute or chronic inflammatory condition, poly cysticovariansyndrome, smoking, multiple gestation, diabetic mellitus, hypertension, irregular hysteroscopy, irregular HSG, history of pre-, eclampsia and/or, thyroid disorder or abnormal karyotype.

The protocol was approved and research was carried out at AL-ZAHRAA Maternity Hospital, Najaf, Iraq. The goals and method were clarified to qualified patients, and all participants subsequently received written informed consent in aseptic conditions aparticipant was given a 5-mL sample of venous blood and used for the Amyloid A serum assay.

The sample size was analyzed by using of SPSS version 24.0 and MedCalc version 12.5 the data was analyzed. for the normal distribution using the Shapiro–Wilk testandkolmogorov-smirnov test. Mean and stander deviation was used for the distributional data. Student t test had been using for comparing between two groups.

Qualitative information was presented as number (percentage); Pearson s R. compare between two groups, as appropriate... Analysis of the recipient. Service characteristic Curve executed to analyze the serum amyloid A degree needed to differentiate among two groups.

The region under this curve was calculated and serum amyloid A 's Optimum value for the cut off was defined according to the highest Youden index (J)... Logistic multi-variable regression (OR and CI 95%) was used to evaluate SSA dependent indicators of recurrent missed abortion All P values were double queued with a value deemed statistically important by less than 0.05.

Results

No statistically relevant variations between groups differences regarding the age, duration of miscarriage (days) and gestational age (weeks) table (1) while there was significant difference regarding BMI.

Table (1): The statistical difference between patients and control regarding the age, BMI, duration of miscarriage (days) and gestational age (weeks)

Study groups / Demographic	Patients			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	0.279	0.781
BMI	7 18-25	21.30*	2.23	8 17-25	20.20	2.08	2.267	0.027*
Duration of miscarriage (Day)	9 5-14	7.93	2.52	9 5-14	8.25	2.77	0.521	0.601
Gestational Age (wk)	2 6-8	6.87	0.68	7 1-8	6.79	0.98	0.399	0.691

Table (2) show 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 age between study groups.

Table (2) Estimate size of effects for parameters in patient's group comparison with control group

		B	S.E.	Wald	df	Sig.	OR	95% C.I. for OR	
								Lower	Upper
Patients vs control	SAA (ng/ml)	0.321	0.106	9.236	1	0.002	1.378*	1.121	1.695
	Age	-0.178-	0.272	0.426	1	0.514	0.837	0.491	1.427
	BMI	0.422	0.442	0.912	1	0.339	1.525	0.641	3.626
	Gastational Age (wk)	-0.294-	0.892	0.109	1	0.741	0.745	0.130	4.281
	Constant	-20.541-	12.177	2.846	1	0.092	0.000		

The mean of SAA in women with missed abortion was significantly higher than between control group ($p<0.001$) Table (3).

Table (3): The statistical difference between patients and control regarding the serum amyloid A.

Study groups / Biomarker	Patients N=30			Control N=61			t-test	Sig.
	Range Min-Max	Mean	SD.	Range Min-Max	Mean	SD.		
SAA (ng/ml)	66.2 33.0-99.2	72.800*	13.59	45.0 11.2-56.2	32.918	12.10	14.184	<0.001

Table (4) summarizes the sensitivity, precision, positive and negative probability ratio, positive and negative predictive value, and value of that cut off stages. key unknown REPL the SAA level was detected as a missed miscarriage-based indicator.

Table (4): Serum amyloid level A is used to distinguish between women with or without early missed abortion

	Estimate	95% CI
Category	91	
Patients (Positive group)	30 (32.97%)	
Control (Negative group)	61(67.03%)	
Area under the ROC curve (AUC)	0.978	0.923 - 0.997

	Estimate	95% CI
Youden index J	0.90	NA
Standard Error [a]	0.0161	
Optimum cutoff level of SAA (ng/ml)	>45.6	NA
Sensitivity	93.33	77.9 - 99.2
Specificity	85.25	73.8 - 93.0
+LR	6.33	3.4 - 11.7
-LR	0.078	0.02 - 0.3
Auc (0.05)	<0.001	

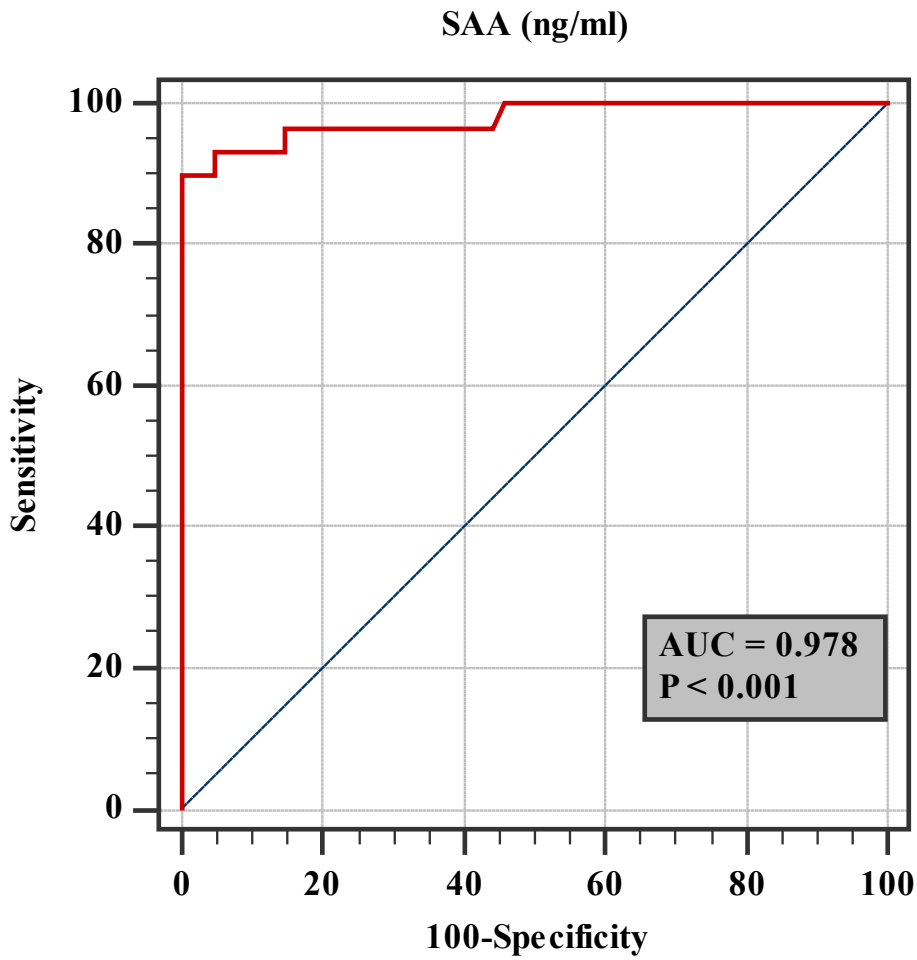

Figure (1): Receiver operating characteristic curve to assess amyloid A level capacity to distinguish among women with or without early missed abortion. The area under the curve was 0.978

The Hosmer–Leme show test (*AUC*=95% (0.923-0.997). While there was no correlation (P. value>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. There was linear correlation (P. value< 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) table (5).

Table (5): The correlation of serum amyloid A levels with age (years), BMI, and gestational age in patients and control groups.

Study Groups	Variables	Correlation coefficient	Age (year)	BMI	Gestational Age (wk)
Patients N=30	SAA (ng/ml)	r	0.651*	0.678*	0.370*
		Sig.	<0.001	<0.001	0.022
	Age (year)	r		0.729*	0.157
		Sig.		<0.001	0.204
Control N=61	SAA (ng/ml)	r	0.019	0.149	0.124
		Sig.	0.441	0.127	0.170
	Age (year)	r		0.312*	-0.044
		Sig.		0.007	0.369

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[10]. There are a lot of studies concluded that infection and inflammations whether in the gestational tissue or elsewhere in the body, are an important cause of early recurrent misscarrige.[14] 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[10].

The decidual cells and synsiotrophoplast cells and extra villous cytotrophoblasts released amyloid A.serum amyloid a have important role in placentation when present at low concentrations of amyloid A.[39] 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 miscarriage with REPL followed by group of missed miscarriage with no history of any miscarriage previously. the mean serum amyloid A 72.800mg/L,32.918mg/L respectively. *Moustafa I. Ibrahim*,*et al., (*2017),mentioned that Patient with recurrent missed abortion demonstrated SAA significantly higher than in women in control group after adjustment with maternal age and gestational age as an in-dependency predictor of this pregnancy complication. This analysis agrees with our outcome[7].

Sandri S et al., (2015), serum amyloid A facilitate Invasion trophoblastic into the decidua (a critical phase during the early stages of pregnancy) through receptors modulator activation In comparison, SAA level are increase this is associated with disturbed trophoblastic tolerance this agree with our result[11].

Knebel FH, Ruano R, et al., (2014), High levels of SAA observed in the potential represent anInverted physiological role for this protein in early recurrent missed abortion, leading todisturbedinvasiontrophoblaste and syncytialisation. SAA levels were measured in early pregnancy (6–10 weeks), indicating a significant early fetal development role[12].

Sharma S, et al ., (2010); Such SAA-induced syncytialization impairment could be facilitated by another mediator and tumor necrosis factore.[13] These results may support this study by association of high SAA levels with the incidence of consecutive miscarriage in women with recurrent early missed abortion through impairing syncytialization.[13]

Engin-Ustunet al.,(2007), recorded that increase in SAAIN patient with pre eclampsia leading to recurrent early missed miscarriges[14]. agree with our research.

Leisser C et al.,(2006), SAA stimulatedtrophoplasticinvation and effect on both metalloprotease gene and enzymetic activity therefore, SAA had no affect trophoblast syncytialisation and decreased human chorionic gonadotropin β isoform secretion. The researchers have find the biochemical syncytialisation signal (β-HCG) in a concentration-dependent manner[15], this is disagree with our result.

Urieli-Shovalet al., (2000), Trophoblast invasion is impairment when level of SAA IS HIGH, suggesting

aADVERSE effect of THAT protein that may be activated during an acute-inflammatory responsewhere serum SAA level can rise over 1000times. [16]

Trophoblastic invasion failure can also occur through rise levels of inflammatory mediators like TNF, this leads to early pregnancy loss. In equilibrium development of PROINFLAMMATORY mediator and failure of trophoblasticinvasion can show in preeclampsia and restriction of intra uterine growth[17,18]. The stressful condition that associated with these disorders courses release of SAA.[19] this study agrees with our findings.

Conclusions

Serum amyloid A theoretically a promising marker that prompts further study for primary unexplained missed miscarriage. in the Studies may be performed, for example,measurement of SAA level in women with history of early recurrent missed abortion during pregnancy and before and also compare SAA levels in women with non-pregnant state Such studies may direct the timing for initiating new therapies the future.

Conflicts of Interest: No

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Ethical Clearance: Was taken from the scientific committee of the Iraqi Ministry of health

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