

The Usefulness of Angiotensin-1 as Biomarker in Missed Abortion

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Abstract

Objectives: To detect serum angiotensin-1 level as effective biomarker for missed abortion in early pregnancy.

Methods: The study included 100 pregnant women; 50 pregnant women with missed abortion (group A) and 50 pregnant women with viable intrauterine pregnancy in first trimester (group B). Both groups were test for serum level of angiotensin-1 using Enzyme linked immunosorbent assay technique.

Results: The mean serum angiotensin-1 level is significantly decrease in missed abortion compare with control group women (771.12 ± 165 vs 1381.12 ± 340.36) respectively at *P*-value (0.001). There was correlation between missed abortion and (body mass index, reproductive history) in groups of study.

Conclusion: The current study showed that serum levels of angiotensin-1 are significantly decrease in pregnant women with missed abortion compared with its level in normal viable intrauterine pregnancy of matched gestation.

Keywords: Angiotensin-1, biomarkers, abortion, missed

Introduction

Abortion is usually defined by pregnancy duration and fetal birthweight for statistical and legal purposes. National Center for Health Statistics, the Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO) all define abortion as any pregnancy termination—spontaneous or induced—prior to 20 weeks' gestation or with a fetus born weighing <500 gram.¹ Because the incidence of conception is unknown, the incidence of spontaneous abortion (miscarriage) cannot be determined with certainty.² Fifteen percent of clinically recognized pregnancies spontaneously miscarry; more will be so early as to go unrecognized. The rate of miscarriage increases with maternal age.³ Women younger than 18 years and older than 35 years are more likely have miscarriage, which rise with both increasing parity and number of prior losses.⁴ Maternal mortality is uncommon from miscarriages, but morbidities like psychological stress, hemorrhage, sepsis, secondary infertility and recurrent miscarriages can occur.⁵ Called also (silent miscarriage) is the condition when the uterus retained the product conception with no attempt to expel the fetus despite of the fetal death.⁶ Usually defined as more than two menstrual cycles, and it can occur in first and second trimester.⁷ The body does not recognize death of the fetus or embryo and continue to release hormones with this condition.⁸ These patients have an absence of uterine growth and may have lost some of the early symptoms of pregnancy. Many women have no symptoms during this period except persistent amenorrhea.⁷ Because coagulation problems may develop, fibrinogen levels should be checked weekly until the fetus and placenta are expelled.² Retention of a dead fetus in the uterus beyond 5 weeks following the demise can be associated with consumptive coagulability and hypofibrinogenemia.⁹ If the missed abortion terminates spontaneously, and most do, the process of expulsion is the same as in any spontaneous abortion.⁷ Angiotensin-1 Is an angiogenic protein, which belongs to a family of growth factors. It is released from the mesenchymal cells and the placental syncytiotrophoblasts.¹⁰ Aim of the study: To investigate the usefulness of

serum angiotensin-1 as a biomarker of first trimester missed abortion.

Methods

This is a case-control study conducted at the Department of Obstetrics & Gynecology of Al-Yarmouk Teaching Hospital/ Baghdad in collaboration with the laboratories department of the hospital and Al-Shameem Scientific Office through a period from 1st of June to end of December 2020. The study protocol was approved by the Scientific Council of Obstetrics & Gynecology Specialization/Arab Board for Health Specializations. The study included 100 pregnant women presented in the first trimester attending the consultant clinic or inpatient ward with a gestational age confirmed by accurate last menstrual period and early abdominal ultrasonography. These women were informed about the nature of study & verbal consent were taken from them. The data were collected on questionnaire which was designed for the study. The women included in the study were divided into two groups as following:

Group A: includes 50 women with a diagnosis of first trimester missed abortion, which was confirmed by accurate last menstrual period and early abdominal ultrasonography.

Group B: includes 50 women with confirmed viable intrauterine pregnancy by abdominal ultrasonography.

Exclusion Criteria

- ▶ Patient who conceived after in vitro fertilization.
- ▶ Multi-fetal pregnancy.
- ▶ History of chronic medical diseases (including thyroid, liver, renal, diabetes, or hypertensive diseases) or blood dyscrasias (hypercoagulopathy or bleeding tendencies) or previous/current history of malignancies.

Detailed history was obtained from all patient including presenting complaint, age, last menstrual period, obstetrical history, gynecological history, past medical and surgical history.

General examination, vital signs and systemic examination. The studied groups were investigated for the following:

1. Maternal blood sample were collected at the time of admission for group A (before any intervention and before receiving any medication) and for group B when they visit the consulting clinic, then samples were send to the laboratories for the blood group/cross match – if needed-, full blood count, random blood sugar and coagulation profile.
2. Maternal blood samples were send for measurement of serum angiotensin-1 level for both groups using ELISA test.
3. Abdominal ultrasonography was done for assessment of the gestational age and viability.

After using aseptic measure, 3 ml of blood was collected from a visible vein & centrifuged at 10000 rpm for 10 minutes; the separated serum was transferred into another tube & stored at -20°C . Enzyme-linked immunosorbent assay (ELISA) kit was used for the purpose. This method is based on biotin double antibody sandwich technology to assay human angiotensin-1 to confirm the diagnosis. Statistical analysis done by SPSS 22, frequency and percentage used for categorical data, mean, median and SD for continuous data. Chi-square used for assessed association between variables, pearson correlation shows the correlation between continuous data. *T*-test used for evaluation differences between mean and median of continuous variables. ROC curve also used to show more specific and sensitive cutoff point. *P*-value less or equal to 0.05 is consider significant.

Results

This study is a case control study included 100 pregnant women. They were divided into two groups; the study group (Group A) includes 50 pregnant women presented with missed abortion, and (Group B) Includes 50 pregnant women presented with viable fetus. Shows that the mean age of (Group A) 25.78 ± 5.30 years was significantly lower than that of (Group B) 27.90 ± 5.319 years, (*P*-value = 0.049). The mean of BMI of (Group A) 19.65 ± 2.50 was significantly lower than that of (Group B) 22.31 ± 3.32 , (*P*-value = 0.001). While no significant difference was noticed between mean gestational age of (Group A) 8.76 ± 1.379 weeks and that of (Group B) 9.06 ± 1.609 weeks, (*P*-value = 0.319).

Table 2 shows that pregnant women with higher number of gravidity, parity and history of previous abortion significantly have higher rate of missed abortion in comparison with control group, (*P*-value = 0.016, 0.028, 0.019) respectively.

Table 3 shows that no significant differences in fibrinogen level, APTT, PT, INR between Group A and Group B (*P*-value 0.171, 0.084, 0.266, 0.948) respectively.

Table 1. Comparison between means of age, BMI, and gestational age according to study groups

Variable	Group A		Group B		<i>P</i> -value
	No.	Mean \pm SD	No.	Mean \pm SD	
Maternal age (year)	50	25.78 ± 5.46	50	26.52 ± 5.30	0.59
BMI (Kg/m ²)	50	19.65 ± 2.50	50	22.31 ± 3.32	0.001
Gestational age (weeks)	50	8.76 ± 1.379	50	9.06 ± 1.609	0.319

Angiotensin-1 level was significantly lower among Group A than that of Group B (*P*-value=0.001).

ROC curve in Figure 1 and Tables 4–6 show that angiotensin-1 test can differentiate correctly between viable fetus and missed abortion (total area under the curve = 0.951, *P*-value = 0.001), At cut off point of Angiotensin-1 ≤ 1004.5 pg./ml one can detect missed abortion with sensitivity = 0.94, specificity = 0.90 and accuracy = 0.92.

Table 2. Reproductive characteristic

Characteristic	Total	Group A		Group B		<i>P</i> -value	
		No.	%	No.	%		
Gravidity	<5	80	35	72%	45	88%	0.016
	≥ 5	20	15	28%	5	12%	
Parity	0	36	13	36.11%	23	63.89%	0.028
	1–3	35	17	48.57%	18	51.43%	
	≥ 3	29	20	68.97%	9	31.03%	
Abortion	Yes	24	17	66.67%	7	33.33%	0.019
	No	76	33	44.74%	43	55.26%	

Table 3. Association between coagulation factors in groups of study

Lab. investigation	Group A		Group B		<i>P</i> -value
	Mean	S. Deviation	Mean	S. Deviation	
Fibrinogen level	210.12	60.152	228.26	69.906	0.171
APTT	29.76	3.577	28.70	2.358	0.084
PT	14.22	1.529	13.88	1.507	0.266
INR	1.112	0.156	1.11	0.150	0.948

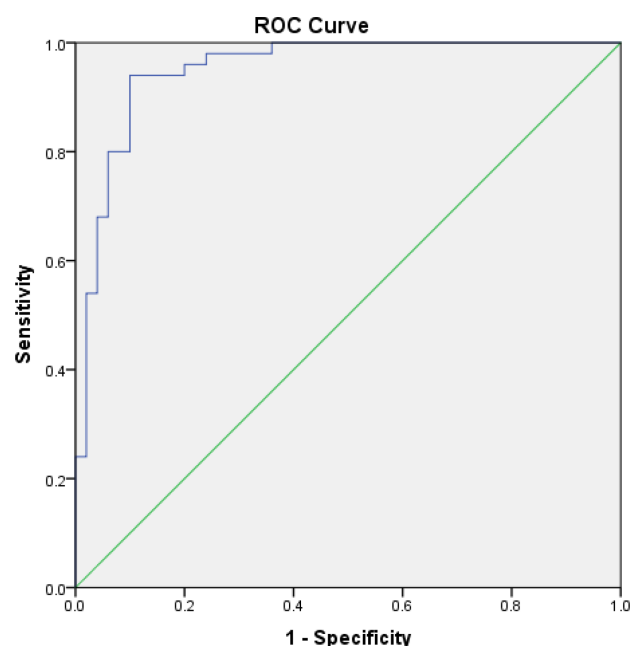


Fig. 1 ROC curve of Ang-1 in the diagnosis of missed abortion.

Table 4. Serum angiotensin-1 level of study groups

Test result variable(s)	Group A		Group B		P-value
	No.	Mean \pm SD	No.	Mean \pm SD	
Angiotensin-1 Pg./ml	50	771.12 \pm 165	50	1381.12 \pm 340.36	0.001

Table 5. The ability of Angiotensin-1 for correct diagnosis (Area Under the Curve)

Test result variable(s)	Area	Standard error	P-value	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Angiotensin-1	0.951	0.021	0.001	0.910	0.993

Table 6. Angiotensin-1 screening test

	(Ultrasound)		Total No.
	Missed	Viable	
Positive	47	5	52
Negative	3	45	48
Total No.	50	50	100

Sensitivity = 0.94, specificity = 0.90, PPV = 0.90, NPV = 0.94, accuracy = 0.92.

Discussion

Missed abortion describe dead products of conception that were retained for days or weeks in uterus with a closed cervical Os. Diagnosis is imperative prior to intervention and avoids interruption of a potentially live intrauterine pregnancy.¹¹ Obstetricians and gynecologists aim to differentiate between normal viable intrauterine pregnancy and missed abortion by using other than TVU, so this study help to prove that.¹² In this study, Table 1 shows there were no significant differences noticed between study groups regarding maternal age ($P = 0.59$), these explained by small sample size in our study. Our observations agreed with the study done by E. Destegul et al., (2020); among 80 pregnant women involve 40 pregnant women with missed abortion and 40 pregnant women with viable intrauterine pregnancy and found no significant differences regarding the association between maternal age and missed abortion ($P = 0.35$), the small sample size also play a role in this result.¹³ Regarding maternal BMI in this study, missed abortion was significantly high in pregnant women with lower BMI ($P = 0.001$), this states a low BMI has been associated with missed abortion as a risk factor because dietary quality may play a role. The observations were in accordance with a study done by Rao. M et al., (2018); among 60 pregnant women includes 30 pregnant women with missed abortion and 30 pregnant women with viable intrauterine pregnancy. They revealed that the women having low BMI noticed higher rate of missed abortions ($P = 0.007$), missed abortion may be associated with low body weight as survival rate of the fetus fall with low maternal BMI.⁶ In the current study, Table 2 shows there were significant differences noticed between study groups concerning gravidity, parity and history of previous abortion. Regarding maternal gravidity and parity, the current result shows missed abortion was higher significantly in pregnant women with high gravidity and parity

($P = 0.016, 0.028$) respectively. These may be interpreted by higher gravidity associated with maternal morbidity and the genetics may be a cause. The result of current study is compatible with a study done by Najah et al., (2020); which was found that a pregnant woman with higher gravidity had higher rate of missed abortion ($P = 0.02$).⁵ Also, the findings of a current study were similar to results of study done by Yang J et al., (2017); a retrospective analysis was performed on clinical data of 492 singleton pregnant women which found significantly higher correlation between higher gravidity and missed abortion ($P = 0.001$).¹⁴ Regarding laboratory investigations in Table 3. The current study revealed no significant differences between study groups in consideration of APTT, PT & serum fibrinogen levels (29.76 \pm 3.577 sec. and 28.70 \pm 2.358 sec., 14.22 \pm 1.529 sec. and 13.88 \pm 1.507 sec., 210.12 \pm 60.152 mg/dl and 228.26 \pm 69.906 mg/dl) respectively. This result in consistent with Shuo et al., (2021); a total 11182 pregnant women who had missed abortion and 5298 healthy pregnant women in the first trimester. The study revealed there were no differences significantly in APTT, PT and fibrinogen level between patients with missed abortion and the healthy controls. APTT were 30.76 \pm 2.86 sec. and 30.99 \pm 3.63 sec. PT were 10.92 \pm 0.64 sec. and 10.75 \pm 0.36 sec., fibrinogen were 299 \pm 39 mg/dl and 307.6 \pm 64 mg/dl in missed abortion and normal pregnant women respectively.¹⁵ Regarding maternal serum angiotensin-1 level, the current study in Table 4 showed there were a significant fall in the levels of serum Ang-1 in cases of missed abortion compared to normal viable intrauterine pregnancy. It shows that Ang-1 value were around 771.12 \pm 165 Pg./ml in women with missed abortion & 1381.12 \pm 340.36 Pg./ml in normal pregnant women, these account for Ang-1 is angiogenic factor that indicate the normal progression of pregnancy, so when decline its level is highly suggested of missed abortion. The current finding is consistent with Rao M. et al., (2018); they showed that the optimal levels of serum Angiotensin-1 were around 780.5 pg./ml in women with missed abortion, and 1102.5 pg./ml (1028.5–1196.3) in normal pregnant women with the first trimester.⁶ Similarly, H. M. Abozeid et al., (2021); the study demonstrated that the mean of serum Angiotensin-1 were around 700.04 pg./ml in women with missed abortion and around 926.16 pg./ml in women with viable intrauterine pregnancy.¹⁶ Likewise, the current study findings regarding the serum angiotensin-1 ($P = 0.001$) is in agreement with results of Alsamarai et al., (2016); a study which included 547 women with poor past obstetric history and 291 women with normal pregnancy outcome. Serological study carried out to determine IL-6, IL-17 and Angiotensin-1 using ELISA kits and observed a significantly higher correlation between low angiotensin level and pregnant women with missed abortion ($P = 0.0032$).¹⁷

Conclusion

The current study showed that serum levels of angiotensin-1 are significantly decreased in pregnant women with missed abortion compared with its level in normal viable intrauterine pregnancy of matched gestation with high Sensitivity and specificity. It may indicate its role in pathogenesis of missed abortion.

Conflict of Interest

None. ■

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