Original Article

Serum angiopoietin -1 as a biomarker of missed abortion

Heba M. Abozeid Hegazy[1], Boshra N. Ali[1], Hala W. Abdel Halim[1], Nashwa M. Abd El-baky[2]

[1] Obstetrics and Gynaecology Department, Faculty of Medicine for Girls, Cairo, Al-Azhar University, Egypt.
[2] Clinical Pathology Department, Faculty of Medicine for Girls, Cairo, Al-Azhar University, Egypt.

ABSTRACT

Background: Missed abortion (MA) is a pregnancy with an embryo without cardiac pulsation or gestational sac without embryo without attempt for expulsion of the product of conception outside the uterus.

Objective: to detect the efficacy of serum Angiopoietin-1(Ang-1) as a biomarker of missed abortion at 6 to 8 weeks of pregnancy

Methodology: This case-control study comprised eighty pregnant women aged 20–40 years; they were divided into; A) control group which composed of 40 women with normal viable pregnancy at the 6-8 weeks of pregnancy , B) study group that included 40 women with missed abortion diagnosed by TVUS at same gestational age.

Results: This study revealed that, there was highly statistically significant decrease in serum levels of Ang-1 in missed abortion .The mean ±SD of serum Ang-1 were 926.16 ± 211.48 (700 – 1620.3) pg/ml in normal pregnant women, and 700.04 ± 87.87 (435.9-797.8) pg/ml in women with missed abortion. The cutoff point was ≤ 780.99 pg/ml with high sensitivity and specificity (87.5.% and 90.00% respectively). There was no correlation between the Ang-1 levels and (age, parity, and body mass index).

Conclusion: Serum levels of Ang-1 were highly significantly decreased in a status of missed abortion as early as 6 to 8 weeks of conception when compared with Ang-1 levels in normal viable pregnancies of similar gestational age which indicate its role in pathogenesis of missed abortion. Angiopoietin 1 is not a reliable biomarker for instant diagnosis of missed abortion as some patients need further transvaginal ultrasound (TVUS) and regular follow up.

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INTRODUCTION

Missed abortion is a disorder with retained embryo without cardiac pulsation or gestational sac without an embryo; however, the uterus is dormant yet without any trying to expel this product [1]. No symptoms for miscarriage emerge for some weeks and if exist, these may be spotting or moderate to severe bleeding per vagina with disappearance of symptoms of conception.

Signs of missed abortions appear in picture of foetal demise with closed cervix [2]. Missed abortion can be diagnosed by two successive measurements of beta human chorionic gonadotrophin (BHCG) with transvaginal ultrasound (TVUS). Some patients may need to repeat this TVUS to confirm the diagnosis. [3]

Angiopoietin-1 (Ang-1) is a member of growth factors group that is responsible for angiogenesis. It comes from the mesenchymal cells and syncytiotrophoblasts of the placenta. Ang-1 has a role in growing and sprouting of vessels in placenta, brains, and development of cardiovascular system of the fetus. The

ease to screen Ang-1 could make it a unique test to diagnose missed abortion instantly at 6–8 weeks of gestation [4]. Therefore, this study was carried out to detect the efficacy of serum Angiopoietin-1(Ang-1) as a biomarker of missed abortion at 6 to 8 weeks of pregnancy.

PATIENTS AND METHODS

This is a case-control study was carried out at the obstetrics and gynaecology outpatient clinic at Alzahraa University Hospital in the period from August 2019 to April 2020.

Inclusion criteria: Eighty pregnant women aged 20–40 years and at 6–8 weeks of gestation were incorporated in this study. They were divided to two groups:

- **Control group (A)** with normal viable intrauterine pregnancy coming to confirm their normal intrauterine pregnancy at the same early gestational age (6-8 weeks). The criteria for diagnosis of normal

intrauterine pregnancy include at 6th week, the embryonic pole must be seen. This pole is about 2 - 10 mm and has cardiac pulsation. Also, this embryonic pole must be seen once the mean sac diameter (MSD) becomes 20 mm. The maximal diameter of embryonic pole can be used for estimation of gestational age up to 9th week of gestation [5].

• **Study group (B)** with missed abortion diagnosed by TVUS. Some of them had an early pregnancy of (6-8 weeks) with vaginal bleeding and on TVUS they revealed the criteria of missed abortion. Others came with BHCG +ve with no symptoms of early pregnancy. Criteria of missed abortion includes : The crown–rump length must be ≥ 7 mm with no pulsation ,the mean sac diameter about ≥ 25 mm without embryo , no embryo ≥ two weeks after TVUS that revealed a gestational sac devoid of a yolk sac ,no embryo with pulsation ≥ eleven days after TVUS that showed gestational sac together with yolk sac [6].

Exclusion Criteria: women with following disorders; multi-fetal pregnancy, medical abortion or any tumor noted, complicated 1st trimesteric pregnancy, and women who get pregnant with in vitro fertilization were excluded from the study.

Detailed history was including presenting complaints, past, medical and gynaecological histories were noted. Body mass index (BMI) was calculated. General and local vaginal examinations were performed. Women included in the study were subjected to transvaginal ultrasound to confirm intrauterine gestational sac to exclude ectopic pregnancy and to confirm the normal viable intrauterine pregnancies and differentiate it from missed abortion.

After aseptic circumstances, samples from venous blood were collected in a serum separator tubes (SST), clotted for 10-20 minutes at room temperature after that centrifugated for 20 minutes at 2000-3000 RPM. Serum was aspirated and stored at -80◦ c till the test.
Estimation of Angiopoietin-1 was done in the serum of all women in this study using enzyme linked immunosorbent assay (ELIZA) kit. The plate pre-coated on the wells. Next biotinylated human Angiopoietin-1 antibody was inserted and was united with Angiopoietin -1 in the sample. Then Streptavidin-horse radish peroxidase (HRP) enzyme was inserted and was united to the biotinylated Ang-1 antibody. The unbound Streptavidin-HRP was washed away during a washing step after incubation. Substrate solution was added, and color obtains in proportion to the quantity of serum Ang-1. The reaction was completed by adding acidic stop solution then the absorbance was measured at 450 nm.

Statistical analysis
The data were analysed by using IBM computer SPSS (statistical program for social science) version 20. The quantitative variables were prescribed as range, mean and standard deviation. The qualitative variables were presented as number and percentage. Using Chi-square d (x^2) to compare qualitative variables between the two groups. Fisher exact test was applied if an expected cell were less than 5. Independent t-test was used to assess the quantitative variables in parametric data, (SD < 50% of the mean). Mann Whitney Wilcoxon test was applied to assess quantitative variable in non-parametric data, (SD >50% of the mean). P value >0.05 was considered non-significant, and p-value <0.05 was considered significant Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC) of the Angiopoietin-1

RESULTS
In this study, there was statistically significant difference between the study group and control group regarding parity and body mass index, with higher BMI and lower parity in missed abortion group (p=0.013 and 0.044, respectively). There was no statistically significant difference between both groups as regard ages and modes of delivery (Table 1). Moreover, Ang-1, levels were significantly lower in missed abortion group compared to control group (Table 2 and figure 1).

At cutoff point of Angiopoietin-1 level (≤ 780.99 pg/ml), the Angiopoietin-1 level have good sensitivity and specificity (87.5% and 90.0% respectively) for diagnosis of missed abortion at 6-8 weeks of gestation (Table 3 and figure 2).

There was overlap of the Ang-1 levels (700 – 1620.3) in control group and (435.9 – 797.8) in missed abortion group as shown in (Table 2). There was no correlation between the (Ang-1) levels and the age, parity, and BMI (Table 4).

Table (1): Comparison between control group and missed abortion group as regard demographic data and obstetric history

		Control group (No. = 40)	Missed abortion group (No. = 40)	Test of significance	P-value
Age (year)	Mean ± SD Range	29.53 ± 5.22 20 – 39	28.88 ± 5.45 20 – 40	0.545[a]	0.587
Parity	Median (IQR) Range	3 (2 – 4) 0 – 7	2 (0 – 4) 0 – 7	-2.481[b]	0.013*
BMI (kg/m²)	Mean ± SD Range	29.03 ± 3.56 24.00 – 39.56	30.69 ± 3.7121 24.91 – 39.56	-2.046[a]	0.044*
Mode of delivery	NVD CS	21 (52.5%) 19 (47.5%)	21 (52.5%) 19 (47.5%)	0.001[c]	1.00

[a] : student t test, [b] : Chi-square : Chi-square, Mann Whitney Wilcoxon test , *: significant p-value, IQR: inter quadrant range, SD: standard deviation, BMI: Body mass index, NVD=normal vaginal delivery CS=cesarean section

Table (2): Comparison between control group and missed abortion group as regard the value of Angiopoietin -1

Ang-1 pg/ml	Control group (No. = 40)	Missed abortion group (No. = 40)	Test of significance	P-value
Mean ±SD Range	926.16 ± 211.48 700 – 1620.3	700.04 ± 87.87 435.9 – 797.8	6.245[a]	0.001*

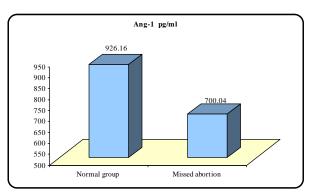[a] : student t test, *: significant p-value

Figure (1): Bar chart between control group and missed abortion group as regard the value of Angiopoietin -1 (Ang-1) Pg/ml
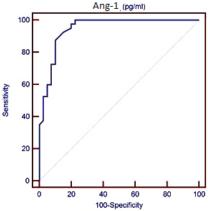

Figure (2): Receiver operating characteristic curve forAng-1 in Missed abortion

Table (3): Diagnostic performance of Ang-1 pg/ml in prediction of missed abortion

Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
Ang-1 pg/ml	0.947	≤780.99	87.5	90.0	89.7	87.8

AUC: area under a curve, PPV: Positive predictive value, NPV: Negative predictive value

Table (4): Correlation between levels of (Ang-1) and some parameters in both groups of the study

Item	Ang-1 pg/ml	
	r	P-value
Age (year)	0.003	0.987
Parity	0.039	0.811
BMI (kg/m²)	-0.125	0.442

r = correlation

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DISCUSSION

Obstetricians and gynecologists should appreciate the tools that used to differentiate between normal and failed pregnancies. Although the accurate cause and pathophysiology of abortion still unclear, it is theorized that the process of embryonic attachment to the uterus is worsened, that leads to deficient nutritional support of the embryo, inhibition of "natural selection" of viable featuses which leads to fetal demise [7]. So, this study was designed to evaluate the effectiveness of Ang-1 in serum as a biomarker for missed abortion at 6 to 8 weeks of gestation.

Regarding parity, in this study parity was statistically significantly higher in normal pregnancy group than missed abortion group, in contrast Cohain et al. [8] found that the number of abortions increased with higher parity. Additionally, they reported that the women with high parity are oldest than those of low parity usually, which support this suggestion.

In this study, the BMI was statistically significantly higher in missed abortion group compared to control group. It is possible that extreme fat amount in obese women, produce adipokines which are adipose tissue derived proteins. The adipokine leptin has a role in placental formation and maternal-fetal exchange processes that regulate the growth of the embryo and a minor rise in leptin concentrations lead to a mild rise in BMI which may be the reason of this obvious positive effect. But, with additional rise in BMI and as the woman becomes overweight to obese, a state of leptin resistance and relative insufficiency may occur that may describe the poor reproductive act like abortion in those women [9]. This study agreed with Metwally et al. [10] who concluded that women with a BMI ≥ 25 kg/m^2 may be more susceptible to abortions. In disagreement with us Rao et al. [11] found that BMI in normal pregnancy group was significantly higher than missed abortion group (24.2 ± 2.7 vs. 22.3± 2.5) kg/m^2 respectively.

In this study, there was no significant difference between the two groups, regarding the mode of delivery. This result was in contrast with Magnus et al. [12] who noticed a small increase in abortion risk after cesarean section. It is possible that the motivating dilemma that caused delivery by cesarean section raises the risk of abortion in the following pregnancy. The mechanism which explain that could be uterine scarring [13]. Intrauterine scarring may cause abortions due to lack of sufficient endometrium or lining for implantation, poor blood supply or mechanical restriction of the uterine cavity expansion during pregnancy [14] but, small sample size in this study may be the cause of such contrast.

This study demonstrated that the mean of serum Angiopoietin-1 were 926.16 pg/ml (700-1620.30) in normal pregnant women, and 700.04 pg/ml (435.9-797.8) in women with missed abortion with a cutoff point (≤780.99 pg/ml) with high sensitivity and specificity (87.5% and 90.0% respectively). Like Daponate et al. [15] who demonstrated that the levels of Ang-1 in serum were 963.5 pg/ml (793.9–1277.6) in normal pregnant women and 810.5 pg/ml (595.4–917.4) in missed abortion with cutoff point of Ang-1 (≤ 918.33 pg/ml) .Also, Rao et al. [11] showed that Angiopoietin 1 values were around 1102.50pg/ml (1028.20 – 1196.30) in normal pregnant women and 780.50pg/ml (712.3 – 881.8)in women with missed abortion.

In this study, there was not statistically significant correlation between Ang-1 and (Age, parity and BMI) of the patients in disagreement with Schneuer et al. [16] who found that there was a positive correlation between Ang-1 and maternal weight. Also, Gaebler et al. [17] identified a significant positive correlation of Ang-1 and body mass index. Rao et al. [11] reported that Angiopoietin-1 is a protein that gives prompt diagnosis of missed abortion without serial measurement of Ang-1 and could be used as a marker for abortion. The ease of use this biomarker would reinforce the diagnosis conducted by ultrasonography without additional assessment and further follow-up [11]

But due to the noted overlap of the Ang-1 levels (700 – 1620.3) pg/ml in normal pregnancy vs (435.9 – 797.8) pg/ml in missed abortion in this study, Angiopoietin -1 is not a reliable biomarker for instant diagnosis of missed abortion as some patients need further TVUS and regular follow up visits.

Collectively, our findings suggest that Ang-1 levels in serum are highly significantly decreased in a condition of missed abortion compared to its levels with viable intrauterine pregnancies similar gestational age which indicate its role in pathogenesis in missed abortion. But until now Angiopoietin -1 is not used in the practice. So further studies may be needed for more confirmation of its accuracy using larger sample sizes.

CONCLUSION

Levels of Ang-1 in serum are highly significantly decreased in a condition of missed abortion as early as 6–8 weeks of gestation compared to its levels in viable intrauterine pregnancies of similar gestational age which indicate its role in pathogenesis in missed abortion. Therefore, we recommended to measure serum Ang-1 between 6 to 8 weeks of conception because at similar age the differentiation between normal viable pregnancy and abortion is doubtful in case of irregular cycles or if the patient not sure of her last menstrual period. Angiopoietin -1 is not a reliable biomarker for instant diagnosis of missed abortion as some patients need further TVUS and regular follow up visits.

Future directions

- Another study to assess measurement of Angiopoietin-2 and calculate Ang 1/Ang 2 ratio for diagnosis of pregnancy failure and compare them with measurement of Angiopoietin -1 alone to

determine the most accurate one to be used is recommended.

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الملخص العربي

الانجيوبيوتين-1 كمؤشر حيوي للإجهاض المنسي

هبة محمد أبوزيد حجازي¹، بشرى نصر علي¹، هالة وحيد عبد الحليم¹، نشوى محمد عبد الباقي²

¹قسم التوليد وامراض النساء، كلية طب البنات، القاهرة، جامعة الأزهر، جمهورية مصر العربية.

²قسم الباثولوجيا الإكلينيكية، كلية طب البنات، القاهرة، جامعة الأزهر، جمهورية مصر العربية.

ملخص البحث

الخلفية: الإجهاض المنسي هو حمل بجنين بدون نبض أو كيس حمل بدون جنين بدون محاولة من لرحم لطرد نتائج الحمل خرجه

الهدف: الكشف عن فعالية الانجيوبيوتين-1 في دم الإنسان ليكون مؤشراً بيولوجياً للإجهاض المنسي في عمر 6 إلى 8 أسابيع من الحمل مقارنة بمستوياته في حالات الحمل الطبيعية القابلة للحياة في ذلك العمر من الحمل..

الطرق: شملت دراستنا ثمانين سيدة حامل تتراوح أعمارهن بين 20 و 40 عامًا. تتكون المجموعة أ من 40 امرأة مع حمل طبيعي قابل للحياة في عمر 6 إلى 8 أسابيع ، بالإضافة إلى مجموعة الدراسة التي تضمنت 40 امرأة أخريات كان لديهن أعراض الإجهاض المنسي في نفس عمر الحمل.

النتائج: خلصت هذه الدراسة الى وجود انخفاض معتد به إحصائيًا في مستويات الانجيوبيوتين-1 في الإجهاض المنسي. كان المتوسط والانحراف المعياري لمستويات الانجيوبيوتين- 1 حوالي 926.16 ± 211.48 (700 - 1620.3) بيكوغرام /مل في السيدات الحوامل الطبيعيات ، و 700.04 ± 87.87 (435.9-797.8) بيكوغرام / مل في النساء اللواتي يعانين من الإجهاض المنسي . كانت نقطة الفصل بين الاجهاض المنسي والحمل الطبيعي ≥ 780.99 بيكوغرام / مل مع حساسية وخصوصية عاليتين(87.5٪ و 90.00٪) على التوالي . لم يكن هناك ارتباط بين مستويات الانجيوبيوتين- 1 و (عمرالمرأه ،عدد مرات الولادة ، أو مؤشر كتلة الجسم)

الاستنتاجات: اثناء الاجهاض المنسي في عمر الحمل المبكر من 6- 8 اسابيع تنخفض مستويات الانجيوبيوتين 1 في الدم وهذا يدل على الدور الفعال للانجيوبيوتين 1فى الاجهاض المنسي.لا نستطيع الاعتماد علي الانجيوبين 1 اعتمادا كليا ففي التشخيص النهائي للإجهاض المنسي حيث تحتاج بعض السيدات الى اعادة الاشعة الموجات فوق الصوتية المهبلية ومتابعة متتالية للحمل لتأكيد التشخيص.

الكلمات المفتاحية: أنجيوبويتين 1، الإجهاض المنسي، مؤشر بيولوجي.

الباحث الرئيسي

الاسم: هبة محمد أبوزيد حجازي، قسم التوليد وامراض النساء، كلية طب البنات، القاهرة، جامعة الأزهر، جمهورية مصر العربية.

الهاتف: 01015988135

البريد الإلكتروني: abozeidheba07@gmail.com