ORIGINAL ARTICLE

SERUM ANGIOPOIETIN-1 AS A BIOMARKER OF MISSED ABORTION

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Background: Waiting in cue for vaginal ultrasound is a stressful situation faced by patients with missed abortion. In Pakistan, it is not a usual practice to go for screening of Angiopoietin-1 for confirmation of missed abortion. This study was conducted to assess the serum Angiopoietin-1 levels in patients with missed abortion. **Methods:** A total of 60 women aged 20–40 years were investigated after written informed consent on the basis of convenience sampling. Out of 60, 30 women having normal viable intrauterine pregnancy with the gestational age of 6–8 weeks were enrolled as control group, and the study group included other 30 women who presented to the hospital with missed abortion at same gestational age. **Results:** The serum Angiopoietin-1 levels were decreased significantly in women with missed abortion compared to women having normal viable intrauterine pregnancy (780.50±134.30 *vs* 1102.50±112.40, p=0.001). **Conclusion:** The level of serum Angiopoietin-1 in missed abortion decreases and it can be used as an early and effective biomarker for diagnosis of missed abortion.

Keywords: Angiopoietin-1, missed abortion, intrauterine fetal demise, Biomarker

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INTRODUCTION

Spontaneous abortion is a condition in which foetal demise occurs at a time before which foetus is unable to survive outside the uterus.[1] Spontaneous abortion can be divided into various subtypes: threatened, inevitable, incomplete, complete, and missed abortion.[2] Missed abortion is a condition with retained products of conception, with no cardiac activity but the uterus is still silent making no attempt to expel the fetus.[3] The aetiological factors for missed abortion include chromosomal abnormalities, maternal, foetal and embryonic malformations, placental and uterine anomalies, history of recurrent abortions, sexually transmitted diseases, thyroid disease and maternal diabetes.[4–6] No symptoms for missed abortion appear for several weeks and if appear, these vary from spotting to heavy vaginal bleed and loss of pregnancy symptoms. Signs of missed abortions include loss of foetal heart sounds and closure of cervical os.[7,8] Complications of missed abortions may include pain, fever, haemorrhage, retained products of conception, septic shock, bladder, bowel and uterine injuries and perforation.[9–11] Patients with missed abortion report to the hospital with mild to severe vaginal bleeding with no pain initially. Diagnosis of missed abortion is confirmed by serial measurement of beta human chorionic gonadotrophin and pelvic ultrasound.[12–13]

Angiopoietin-1 is an angiogenic protein which belongs to a family of growth factors.[14] It is released from the mesenchymal cells and the placental syncytiotrophoblasts.[15] Angiopoietin-1 is responsible for growth and proliferation of all vessels including placental and brain and also for the foetal cardiovascular system development.[15–17] The diagnosis of missed abortion can be made by trans-vaginal ultrasound and in most of the cases ultrasound is to be done serially to confirm the diagnosis. The availability of Angiopoietin-1 screening can prove to be the first test to confirm the diagnosis of nonviable pregnancy instantly, as early as 6–8 weeks of gestation.[15] The estimation of serum Angiopoietin-1 can minimize the psychological trauma that the patients with missed abortion face when they have to undergo serial ultrasounds. The aim of this study was to determine the usefulness of serum Angiopoietin-1 as a biomarker of missed abortion as early as 6–8 weeks of gestation by comparing its levels with viable intrauterine pregnancies of same gestational age.

MATERIAL AND METHODS

This cross-sectional comparative study was conducted at Sheikh Zayed Postgraduate Medical Institute, Lahore, in collaboration with Gynaecology Department of Sheikh Zayed and Jinnah Hospitals, Lahore from June to November 2015 after obtaining institutional approval. Power and precision software was used to calculate the sample size. Considering the input of alpha as 5%, power as 90% and Angiopoietin-1 levels of 963.5±200 pg/ml for normal and 810±200 pg/ml for missed abortion group, a sample size of 30 per group was calculated making a total sample size of 60. Pregnant women aging 20–40 years with gestational age 6–8 weeks were included through convenience sampling after obtaining written informed consent. Thirty women with evidence of viable intrauterine pregnancy by pelvic scan (group A) and 30 women with evidence of missed abortion on pelvic scan (group B) were included. Patients who conceived after *in vitro* fertilization, had multi-foetal pregnancy, medical abortion, or any tumour were

excluded from this study. Presenting complaints, and past medical and gynaecological history were noted. Height and weight of each subject was measured by height and weight scale. Body mass index (BMI) was calculated by standard formula.[18]

After using aseptic measures, 3 ml blood was collected from a visible vein and centrifuged at 10,000 rpm for 10 minutes. The separated serum was transferred into another tube and stored at -20 °C. Estimation of Angiopoietin-1 was done using ELISA. Immobilized capturing antibody for Angiopoietin-1, in wells of microtitre plate were reacted with concentration of Angiopoietin-1 present in standards and samples. Buffer was used to remove the unbound Angiopoietin-1 from wells of microtitre plate. Specific monoclonal antibody Angiopoietin-1, conjugated with horse reddish peroxidase was added as detecting antibody. After removing the unbound fraction, substrate solution tetra methyl benzidine (TMB) was added to develop the colour depending upon the amount of Angiopoietin-1 bound in the initial step. Reaction was stopped by addition of stop solution and absorbance was measured at 450–650 ηm.

Data was analysed using SPSS-24. Mean and standard deviation were calculated for normally distributed variables and median and inter quartile range for non-normally distributed variables were calculated. Frequency and percentage were calculated for categorical variables. Comparison between the two groups was performed by independent *t*-test for normally distributed variables. For non-normally distributed variables Mann Whitney U-test was used. Maximum alpha error was kept at 5%.

RESULTS

Both the groups were similar for age and duration of pregnancy; however, BMI of group B was significantly lower as compared to group A as shown in Table-1. Comparison of mean levels of Angiopoietin-1 between group A and B is shown in Table-2.

Table-1: Comparison of age, BMI and duration of pregnancy between group A and B

Parameters	Group A n=30	Group B n=30	*p*
Age (years)	29.7±4.8	31.9±5.1	0.082
Duration of pregnancy by scan (IQR)	6.5 (6–8)	6.0 (6–8)	0.770
Body mass index (Kg/m²)	24.2±2.7	22.3±2.5	0.007*

*significant

Table-2: Comparison of Angiopoietin-1 between group A and B

Parameters	Group A n=30	Group B n=30	*p*
Angiopoiein-1 Levels (pg/ml)	1102.50±112.40 (1028.5–1196.3)	780.50±134.30 (712.3–881.8)	0.001*

*significant

DISCUSSION

The present study was planned to determine the usefulness of serum Angiopoietin-1 as a biomarker of missed abortion as early as 6–8 weeks of gestation by comparing its levels with viable intrauterine pregnancies of same gestational age. The serum Angiopoietin-1 levels were found to be significantly lower in women with missed abortion compared to women with viable intrauterine pregnancy.

Daponate *et al*[19], in their study showed that optimal levels of serum Angiopoietin-1 were around 963.5 pg/ml (793.9–1277.6) in normal pregnant women with gestational age 6–8 weeks, and 810 pg/ml (595–767.9) in women with missed abortion. The current study showed that these values were relatively higher in South Asian population but, overall, there was a significant fall in the levels of Angiopoietin-1 in cases of failed pregnancies compared to normal viable intrauterine pregnancies. These findings are consistent with those of Deponate *et al*[19] and Schneuer *et al*[20] who also tried to find out the role of Angiopoietin-1 in failed pregnancies. Increasing level of Angiopoietin-1 is a marker of progression of pregnancy as it is an angiogenic factor; it induces the sprouting of new vessels which is the first step in new vessel formation. This fact was studied by Kim *et al*[21]. High angiogenesis is a prerequisite for the maintenance of normal pregnancy as shown by a study conducted by Sugino N *et al*[22].

Angiopoietin-1 is responsible for vascular growth and maturation of placenta, so decreased levels of Angiopoietin-1 lead to increased chances of failure of pregnancy because of the defective vascular formation. When every step of vessel formation is impaired starting from sprouting of vessels to maturation, then the chances of survival of the foetus decrease due to lack of exchange of nutrients and waste products. Impaired placental vascular development is related to imbalances in angiogenic factors, as implicated in pathological pregnancies.[15]

In this study, the physical and gestational age matched women showed a statistically significant positive relationship between missed abortion and low body mass index. The women who reported with missed abortions were found to be having low body mass index. Low body weight and low body mass index can prove to be a cause of missed abortion as chances of survival of the foetus decline with low maternal BMI. Although, the limitation of this study is that the BMI of patients of both groups could not be kept the same because of multiple factors, but it can be said that data should be collected on a larger scale, including the patients having same BMI to prove the correlation of low BMI and missed abortion in our population. Studies conducted by Allison *et al*[8] proved the same fact.

In various studies, multiple biomarkers have been identified, the serial measurement of which can predict the adverse pregnancy outcomes but, Angiopoietin-1 is a marker that gives instant diagnosis that either a missed abortion or viable pregnancy is there. This study in South Asia shows that there is a definite relationship between decreasing levels of Angiopoietin-1 and poor pregnancy outcome. No serial measurement of Angiopoietin-1 needs to be done. Availability of this biomarker would strengthen the diagnosis made through ultrasonography without the need of further evaluation and follow-up visits.

CONCLUSION

Serum Angiopoietin-1 levels decrease during pregnancy failure. The measurement of serum Angiopoietin-1 can be used as a first tool, to support the confirmation of diagnosis of missed abortion made by ultrasound.

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