SMGroup

SM Journal of Reproductive Health and Infertility

Research Article

Maternal Plasma Levels of Antithrombin-III versus Inhibin-A in Prediction of Second Trimester Miscarriage

El-Gharib MN, El Sabae TM and Morad MA*

Departments of Obstetrics and Gynecology, Tanta University, Egypt

Article Information

Received date: Nov 05, 2015
Accepted date: Oct 25, 2016
Published date: Oct 28, 2016

***Corresponding author**

Morad MA, Clinical Pathology, Faculty of Medicine, Tanta University, Egypt, Email: mohgharib@hotmail.com

Keywords Inhibin-A; Antithrombin-III; Second trimester miscarriage

Abstract

Objective: To study whether changes in maternal plasma thrombin inhibitor complex level and Inhibin-A are associated with second trimester miscarriages or not.

Design: Prospective study.

Setting: Tanta University Hospital.

Methods: The study included 200 asymptomatic pregnant women with singleton pregnancy of 15-20 weeks divided into two groups: 154 pregnant women with no history of abortion and 46 expectant women with a prior history of abortion. Each case was subjected to detailed obstetric and gynecological history, clinical examination, determination of gestational age, fetal viability, routine antenatal laboratory investigations, ultrasound examination and measurement of human Antithrombin-III and Inhibin-A.

Results: The mean values of maternal plasma levels of Inhibin-A and Antithrombin-III were significantly lower in patients with a history of abortion than those without a history of abortion. The area under Receiving Operating Characteristic (ROC) curve denoting sensitivity and specificity of maternal plasma levels of Inhibin-A compared to Antithrombin-III as a prognostic factor to miscarriage among pregnant women during the second trimester.

Conclusions: We concluded that Inhibin-A could be useful in monitoring of miscarriage in patients during the second-trimester pregnancy.

Introduction

Abortion is the ending of pregnancy by removing a fetus or embryo before it can survive outside the uterus. An abortion, which occurs spontaneously, is also known as a miscarriage [1]. Second-trimester pregnancy termination comprises 10 to 15 percent of the approximately 42 million abortions performed annually worldwide. [2] The United States Centers for Disease Control and Prevention (CDC) reported that 7.0 percent of abortions were performed between 14 to 20 weeks and 1.3 percent at or after 21 weeks [3]. Second-trimester abortion is associated with more morbidity and mortality and, for some women, more social or emotional challenges than first-trimester terminations. [4]. Human Antithrombin-III is a plasma α2 glycoprotein and belongs to the serpent (serine protease-inhibitor) superfamily. It is a single polypeptide chain of 432 amino acids with three disulfide bridges and four glycosylation sites. Antithrombin-III is synthesized within the liver. It is one of the most important natural inhibitors of blood coagulation. During pregnancy, women have a four to five-fold increased risk of thromboembolism compared with women who are not pregnant [5].

Conditions associated with second trimester pregnancy loss overlap those of the first trimester, to a certain extent, but some are characteristic of second trimester losses [6].

Compelling evidence suggests that women having a history of recurrent miscarriage are in a pro-coagulant state even when they are not pregnant [7]. Evidence also suggests that, just before a miscarriage, deficiencies occur in the naturally-occurring anticoagulants are well recognized conditions predisposing to recurrent venous thromboembolism. Since thrombotic phenomena have been implied as a cause of abortion and stillbirth, we hypothesized that these deficiencies increase the risk of fetal demise [8].

Aharon et al; revealed that only three types of thrombophilia may be related to recurrent pregnancy loss: elevated homocystine level, factor V Leiden or activated protein C resistance and antiphospholipid antibodies associated with second trimester loss [9].

High concentrations of serum Inhibin-A is found in the circulation of women with normal

pregnancies. Inhibin-A is from the feto–placental unit and the corpus luteum [10]. There is evidence for the secretion of these proteins by placental explants and trophoblasts in culture. Both placental cytotrophoblast and the syncytiotrophoblast synthesize Inhibin-A. Inhibin is a heterodimeric glycoprotein hormone with α and β1 subunit. There is limited information about the biological role of this protein throughout pregnancy [11].

Muttukrishna et al. have also shown that Inhibition-A is lower in missed abortions compared with ongoing IVF pregnancies. They suggested that these proteins may play a role in placentation [12].

The aim of this work is to study whether changes in maternal plasma thrombin inhibitor complex level and Inhibin-A are associated with second trimester miscarriages or not.

Patients and Methods

This prospective observational study consisted of 200 asymptomatic pregnant women with singleton pregnancy of 15-20 weeks (calculated from the first day of LMP and by ultrasound) who were selected from Obstetrics and Gynecology Clinic in Tanta University during the period between January 2015 and October 2015. They were divided into two groups: 154 pregnant women with no history of abortion and 46 expectant women with a previous history of abortion. All patients were thoroughly counseled about the procedure, stating the values, the hazards and the aim of the study. A written consent was obtained and signed by each participant.

Inclusion criteria:

- Singleton pregnancy
- Healthy pregnant women from the 15-20 weeks and follow-up of cases until delivery

Exclusion criteria:

- Major fetal anomalies
- Current cervical cerclage
- Placenta praevia
- Thrombophilia
- Pre-eclampsia
- History of previous preterm birth

Methods:

Each case in the study was subjected to the following:

- Detailed obstetric and gynecologic history was fulfilled
- Clinical examination was done
- Gestational age was determined according to the last menstrual period and ultrasound findings
- Fetal viability was confirmed by ultrasonography
- Routine antenatal laboratory investigations, including blood group and Rh typing, full blood count and urine analysis

Ultrasound examination: Trans-abdominal ultrasound was done with the assessment of fetal viability, fetal Biometry, EGA, placental (site and maturity), liquor (amount and turbidity) and congenital fetal malformation.

Table 1: The age and BMI of the two studied groups.

	Age		BMI	
	PCO	Non PCO	PCO	Non PCO
Mean±SD	28.83±3.47	27.65±3.67	28.98±3.74	25.88±4.86
p-value	0.254		0.014	
Significance	NS		S	

*t-value was significant at 0.05significance level.
SD: Standard Deviation; **p-value:** Probability value; **S:** Significant; **NS:** NOT Significant.

Sample collection, maternal blood sample (10ml) was withdrawn from each participant at 15-20 weeks of gestation and collected into tubes containing ethylene Di-Amine-Tetra-Acetic acid. The plasma immediately separated from the whole blood by centrifugation at 2500/rm for 10 minutes and store at -70°C for assay.

- Measurement of human Anti-Thrombin-III. [13]
- Measurement of human Inhibin-A. [13]
- Statistical analysis: The collected data were organized, tabulated and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 16, SPSS Inc. Chicago, IL, USA) [14].

Results

The results of the current study are depicted in [5] tables and one figure.

The mean age of the control group was 23.57±4.46 compared with 24.09±4.80 years among cases with mid-trimester abortion. No significant difference between the groups was found. The mean duration of gestation was 17.13±2.13 weeks compared with 17.61±1.97 weeks among cases with mid-trimester abortion. There was a non-significant difference between patients with no history of abortion and patients with a history of abortion regarding gestational age ($p>0.05$).

The mean value of Inhibin-A was lower in patients with a history of abortion (408.91±256.24) than in those without a history of abortion (565.91±423.23). The difference was significant ($P<0.05$). Antithrombin-III-III was 208.73±107.79 in patients without a history of abortion and 177.96±33.66 in patients with a history of abortion ($P\geq0.05$).

Table 1 shows that there were substantial differences between patients with no history of abortion and patients with a history of abortion regarding gynecological data ($p<0.001$), except for a history of vagina bleeding, which exhibited an insignificant difference

Table 2: Mean values of Homocysteine scores of PCO and Non-PCO groups.

	PCO group	Non PCO group
Mean±SD	26.66±18.70	12.97±5.17
p-value	0.0025	
Significance	S	

*t-value was significant at 0.05significance level.
SD: Standard Deviation; **p-value:** Probability value; S: Significant.

Table 3: Mean values of HOMA2-IR scores of PCO and Non-PCO groups.

	PCO group	Non PCO group
Mean±SD	3.51±1.91	1.77±0.76
p-value	0.0004	
Significance	S	

*t-value was significant at 0.05significance level.
SD: Standard Deviation; **p-value:** Probability value; **S:** Significant.

(p>0.05). There was statistically a significant difference between patients with no history of abortion and patients with a history of abortion regarding miscarriage of the current pregnancy (p<0.05).

Table 2 displays the mean values of maternal plasma levels of Inhibin-A and Antithrombin-III was significantly lower in patients with a history of abortion than those without a history of abortion.

Table 3 and (Figure1) illustrated the area under ROC curve denoting sensitivity and specificity of maternal plasma levels of Inhibin-A compared to Antithrombin-III as diagnostic to prognosis to miscarriage among pregnant women in second-trimester.

The sensitivity, specificity, positive and negative predictive values and accuracy of maternal plasma levels of Inhibin-A compared to Antithrombin-III as a prognostic value for miscarriage among pregnant women in second-trimester are expressed.

Multiple regression analysis of inhibition-A level compared to Antithrombin-III levels as predictors of the occurrence of miscarriage among the studied pregnant women is established.

Discussion

Inhibins are hetero-dimeric proteins that suppress the secretion of FSH (follicle stimulating hormone) from the pituitary. Inhibin consists of two distinct chains, or subunits (alpha and beta), linked together. Inhibin-A consists of the alpha subunit and ß-subunit. Only the dimeric forms of the molecule, containing both the alpha and beta subunits, are bio-active. The free subunit forms exist in circulation as well. Inhibin-A is secreted by ovarian granulosa cells. At the onset of menstruation during the early follicular phase, very low levels of Inhibin A are found. Levels increase dramatically in the late follicular phase and maximize in the mid-luteal phase. During the menstrual cycle and very early pregnancy, Inhibin-A is produced by the corpus luteum [15].

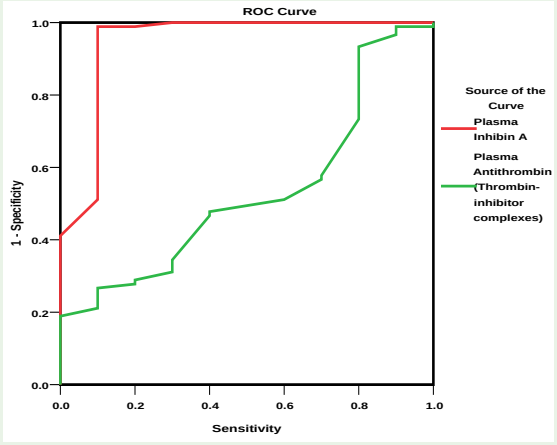

Figure 1: It illustrates the area under Receiving Operating Characteristic (ROC) curve representing the sensitivity and specificity of maternal plasma levels of Inhibin-A compared to Antithrombin-III as prognostic factors to miscarriage among pregnant women in the second trimester.

The fetoplacental unit appears to be the major source of increased circulating concentrations of Inhibin-A in early pregnancy. Production occurs at a number of sites, including the fetus and placental and fetal membranes. Maternal serum levels of Inhibin-A increase during the first trimester and decline after about 10 weeks. Levels remain stable at 15 to 25 weeks and then increase, reaching a peak [16].

Antithrombin (AT) is a natural anticoagulant that inactivates thrombin by covalently binding to the active serine of thrombin and Activated Factor X (FXa). AT can also inactivate other coagulation factors, including factors IXa, XIa, and XIIa. AT has a binding site for heparin and, in the absence of heparin, has low inhibitory activity against thrombin. In contrast, when heparin is present, inhibitory activity can be induced at least 1000-fold [17].

Miscarriage is the most common complication of pregnancy. Early pregnancy loss occurs in 10-20% of clinically recognized gestations [18]. Recurrent miscarriage is defined as three or more consecutive pregnancy losses and affects 1% of couples trying to conceive [19].

The present study was designed to study whether changes in maternal plasma thrombin inhibitor complex level and Inhibit-A are associated with second trimester miscarriage or not.

Our results showed that there were non-significant differences between patients with no history of abortion and patients with a history of abortion regarding demographic data and gestational age. There were statistically high significant differences between patients with no history of abortion and patients with a history of abortion regarding obstetric history, except for a history of vagina bleeding, which exhibited a non-significant difference. There was statistically a significant difference between patients with no history of abortion and patients with a history of abortion regarding miscarriage of the current pregnancy.

The present study evaluated the possible role of plasma thrombin-inhibitor complex and Inhibin-A measurement in the prediction of early second trimester miscarriage. There were significant differences between patients with no history of abortion and patients with a history of abortion regarding maternal plasma levels of Inhibin-A and Antithrombin-III. Also, there was statistically a high significant difference between patients with no miscarriage and patients with miscarriage regarding the maternal plasma level of Inhibin-A and a non-significant difference regarding the maternal plasma level of Antithrombin-III.

Phipps et al; have shown that Inhibin-A level is low in those patients with clinical symptoms of spontaneous miscarriage [20].

Our study reported 90% sensitivity and 98.88% specificity of Inhibin-A and 60% sensitivity and 94.44% specificity of Antithrombin-III for prediction of the occurrence of miscarriage. Cut off value of Inhibin-A for prognosis to miscarriage was 929 and cut off value of Antithrombin-III for prognosis to miscarriage was 285.

In our study, there was statistically a high significant positive correlation between Inhibin-A and Antithrombin-III and non-significant correlations between Inhibin-A and age of mother and gestation age and between Antithrombin-III and age of mother and gestational age.

France et al; have reported ir-Inhibin in recurrent miscarriage patients and found there was no correlation between hCG and ir-inhibin in these patients. A radio-immunoassay was used for inhibition, which cross reacted with dimeric Inhibins (A and B) and monomeric α subunit [21].

Muttukrishna et al; investigated the clearance of Inhibin-A after pregnancy termination and suggested that Inhibin-A concentrations clear from circulation within hours, unlike hCG, which has a longer half-life. This indicated that any variation in Inhibin-A production will quickly be reflected in the circulation, rendering it a sensitive marker of early placental dysfunction [22].

Phipps et al; reported that lower levels of Inhibin-A was found in the maternal circulation in IVF missed abortions and spontaneous non-viable pregnancies with clinical symptoms of miscarriage [20].

Muttukrishna et al; have evaluated whether circulating level of Inhibin-A is an endocrine marker of early pregnancy loss in patients with a history of recurrent miscarriage and is linked with other serum markers such as estradiol, progesterone and hCG. They showed that Inhibin-A in combination with hCG could be useful in monitoring unexplained recurrent miscarriage patients to predict a subsequent miscarriage as early as six-week gestation [22].

Prakash et al; looked at the role of Inhibin-A and activin in predicting pregnancy outcome in patients with a history of recurrent miscarriage. They concluded that Inhibin-A and Activin-A may be used as markers to predict pregnancies that are likely to miscarry [10].

Phupong and Hanprasertpong examined the value of combined maternal serum Inhibin-A and embryonic/fetal heart rate to predict the pregnancy outcome in a first-trimester threatened abortion. They concluded that combined motherly serum Inhibin-A and embryonic/fetal heart rate is not better than embryonic/fetal heart rate for predicting the pregnancy outcome in a first-trimester threatened abortion [23].

Lastly, we concluded that Inhibin-A could be useful in monitoring unexplained recurrent miscarriage patients to predict a subsequent miscarriage.

References

1. Grimes DA, Stuart G. “Abortion Jabberwocky: the need for better terminology”. Contraception. 2010; 81: 93-96.
2. ACOG Practice Bulletin No. 135: Second-trimester abortion. Obstet Gynecol. 2013; 121:1394-1406.
3. Pazol K, Creanga AA, Zane SB, Burley KD, Jamieson DJ; Centers for Disease Control and Prevention (CDC). Abortion surveillance-United States, 2009. MMWR Surveill Summ. 2012; 61:1-44.
4. Henshaw SK, Finer LB. The accessibility of abortion services in the United States, 2001. Perspect Sex Reprod Health. 2003; 35:16-24.
5. Flint S and Gibb DM. Recurrent second trimester miscarriage. Curr Opin Obstet Gynecol. 1996; 8: 449-453.
6. ACOG: American College of Obstetricians and Gynecologists. Management of recurrent pregnancy loss. ACOG Practice Bulletin No.24. 2001.
7. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ 3rd. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med. 2005; 143: 697-706.
8. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. Lancet. 2003; 361: 901-908.
9. Aharon A, Lanir N, Drugan A, Brenner B. Placental TFPILs decreased in gestational vascular complication and can be restored by maternal enoxaparin treatment. J Thromb Hemost. 2005; 3: 2355-2357.
10. Tulppala M, Viinikka L, Ylikorkala O. Thromboxane dominance and prostacyclin deficiency in habitual abortion. Lancet.1991; 337: 879.
11. Prakash A, Laird S, Tuckerman E, Li TC, Ledger WL. Inhibin A and activin A may be used to predict pregnancy outcome in women with recurrent miscarriage. Fertil Steril. 2005; 83: 1758-1763.
12. Muttukrishna S, Jauniaux E, Greenwold N, McGarrigle H, Jivraj S, Carter S, et al. Circulating levels of inhibin A, activin A and follistatin in missed and recurrent miscarriages. Human Reproduction. 2002; 17: 3072-3078.
13. Mohan A, Asselin J, Sargent IL, Groome NP, Muttukrishna S. Effect of cytokines and growth factors on the secretion of inhibin A, activin A and follistatin by term placental villous trophoblasts in culture. Eur. J. Endocrinology. 2001; 145: 505-511.
14. Sehat Z, Goshetasbi A, Taheri-Amin M. Investigating association between second trimester maternal serum biomarkers and preterm delivery. Iran J Reprod Med. 2013; 11: 127-132.
15. Dawson BD, Trapp RG. Reading the medical literature: Basic & Clinical Biostatistics. Lange Medical Book/ McGraw-Hill. Medical Publication Division, New York. 2001; 7-9: 161-218.
16. Mohan A, Asselin J, Sargent IL, Groome NP, Muttukrishna S. Effect of cytokines and growth factors on the secretion of inhibin A, activin A and follistatin by term placental villous trophoblasts in culture. Eur. J. Endocrinology. 2001; 145: 505-511.
17. Muttukrishna S, George L, Fowler PA, Groome NP, Knight PG. Measurement of serum concentrations of inhibin A (αβA dimer) during human pregnancy. Clin. Endocrinology. 1995; 42: 391-397.
18. Patnaik MM, Moll S. Inherited antithrombin deficiency: a review, Haemophilia. 2008; 14: 1229-1239.
19. Alberman E. Spontaneous abortion: epidemiology. In Stabile, S., Grudzinskas, G. and Chard, T. (eds) Spontaneous abortion: diagnosis and treatment. Springer-Verlag, London, UK. 1992: 9-30.
20. Phipps MG, Hogan JW, Peipert JF, Lambert-Messerlian GM, Canick JA, Seifer DB. Progesterone, inhibin and hCG multiple marker strategy to differentiate viable from non viable pregnancies. Obs. Gyn. 2000; 95: 227-231.
21. France JT, Keelan J, Song L, Liddell H, Zanderigo A, Knox B. Serum concentrations of human chorionic gonadotrophin and immunoreactive inhibin in early pregnancy and recurrent miscarriage: a longitudinal study. Aust. New Zea. J. Obs. Gyn.1996; 36: 325-330.
22. Muttukrishna S, Child TJ, Groome NP, Ledger WL. The source of circulating levels of inhibin A, pro-alpha C containing inhibins and activin A in early pregnancy. Hum. Reprod. 1997; 12: 1089-1093.
23. Phupong V, Hanprasertpong T. Combined maternal serum inhibin A and embryonic/fetal heart rate in the prediction of pregnancy outcome in a first-trimester threatened abortion. J Med Assoc Thai. 2011; 94: 529-534.