ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Serum levels of Pregnancy Associated Plasma Protein -A , Activin-A and Inhibin-A in patients with ectopic pregnancy

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Manuscript Info

Manuscript History:

Received: 15 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

PAPP-A ,Activin-A, Inhibin-A, Ectopic pregnancy, Normal intrauterine pregnancy

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Abstract

Background: Serum Pregnancy Associated Plasma Protein -A (PAPP-A), Activin-A and Inhibin-A are markers related to embryo . The purpose of this study was to evaluate the serum level of PAPP-A ,Activin-A and Inhibin-A in early diagnosis of ectopic pregnancy. **Materials and Methods**: Serum levels of PAPP-A ,Activin-A and Inhibin-A were measured by Enzyme linked Immuno Sorbent Assay (ELISA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy in the wards of Rajkiya mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer, after taking approval from ethical committee. These values were compared by T test. **Results**: The mean serum levels of PAPP-A, Activin-A and Inhibin-A in patients with ectopic pregnancy (0.49±0.10 µg/ml, 0.13 ± 0.04 ng/ml and 13.08±5.50 pg/ml) were significantly lower than in women with normal intrauterine pregnancy (1.33±0.12 µg/ml, 0.65±0.22 ng/ml and 1.99±50.60 pg/ml, respectively) ($p<0.001$). **Conclusions:** In this study single measurement of serum Activin- A may identify patients at risk of Ectopic pregnancy (EP) with a high sensitivity(100%) and specificity (99%).

INTRODUCTION

Ectopic pregnancy (EP) is a major cause of maternal morbidity and is responsible for pregnancy-related deaths in the first trimester (1). Diagnosing an EP is a challenge to the clinician because there is no definitive non-surgical diagnostic test when the diagnosis is unclear by routine blood tests and ultrasound, and diagnosis often requires following patients over multiple visits. A rapid and accurate serum test to detect the presence of an EP would permit early treatments to prevent mortality and morbidity of this condition with preservation of fallopian tube function and fertility.1 Currently, research is underway to both identify novel biomarkers and combine new and existing markers into a multiple marker test with the goal of accurately identifying ectopic pregnancies. The following discussion will describe the current use of biomarkers in clinical practice and the present state of serum biomarker research, including the markers being investigated and the methods which are being used to discover novel candidates. PAPP-A is a glycoprotein produced by trophoblast. Normally, PAPP-A is up regulated by progesterone, which promotes the adhesion and proliferation potential of trophoblastic cells (25). It has been extensively studied and used as a marker of screening for first trimester aneuploidy (15). Mueller et al found decreased levels of PAPP-A in patients with ectopic pregnancy when compared to normal viable intrauterine pregnancy (14). Dumps et al found a cut-off of <14.3 ng/ml to have a sensitivity of 64.5% with a 99% specificity for pregnancy failure (8) But the levels were found to be lower in miscarriages making the discrimination between EP and other abnormal intrauterine pregnancy dissatisfactory. On the other hand Ugurlu et al and Daponte et al observed that PAPP-A can differentiate abnormal

pregnancy from the viable normal IUP (6,24). Inconsistency in these results prompts us to study this marker in greater details. Activin A is a dimeric glycoprotein of tumor growth factor- β (TGF-β) superfamily, with a role in cytotrophoblast invasion (2). A study by Florio et al found Activin A level at a cut-off of ≤0.37ng/mL to have 100% sensitivity and 99.6% specificity for the diagnosis of EP (11). Rausch et al found Activin A to have 80% sensitivity and 72% specificity as a single marker in a cohort of patients with EP (18). Daponte et al also observed activin A concentrations to be significantly lower in women with EP and women with missed abortion, compared to IUP, and reported that at a threshold value of 505 pg/mL, activin A had 87.9% sensitivity and 100% specificity for discriminating an ectopic pregnancy from viable pregnancies (7). On the contrary, Activin-A was not found useful in diagnosing EP (13, 26). Similarly, Elito et al demonstrated that serum activin A levels could not discriminate between an EP from a normal intrauterine pregnancy when an adnexal mass was found by transvaginal scan (9). Therefore, A validation of this biomarker in a larger cohort would be encouraged before makes it a tool for diagnostics. Inhibin A is another dimeric protein produced from corpus luteum (23). Seifer et al observed lower levels of inhibin-A in EP compared to normal viable IUP (21). Rausch et al found Inhibin A to have a sensitivity of 83% and a sensitivity of 79% at a lower cut-off of 23.67pg/ml in discriminating EP from viable IUP (18). In contrast to the above findings, Chetty et al found Inhibin-A to be unable to discriminate EP from viable IUP in their cohort of 109 patients (5). It can be concluded that although Inhibin-A is a promising marker for the early pregnancy viability, further studies need to be carried out to use it as a biomarker in the detection of EP.

Materials and Methods:

The subjects included in the study were 100 of ectopic pregnancy and 100 of intrauterine pregnancy (IUP) of different age groups (20-40 years) attending the out patient clinics or admitted in the wards of Rajkiya mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer, after taking approval from ethical committee. Serum levels of PAPP-A, Activin-A and Inhibin-A were measured by Enzyme linked Immuno Sorbent Assay (ELISA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy. SPSS.13/win statistical software was used for analyzing the data. Data were presented as mean±standard deviation. A parametric independent sample t-test was used to compare differences between two groups. Level of statistical significance was set at $p<0.05$. By determining cut-off levels of these parameters the specificity and sensitivity of each in prediction of ectopic pregnancy were estimated.

Results: Demographic data of IUP and EP is shown in tables.

Table 1: Mean serum PAPP-A, Activin-A and Inhibin-A values in normal IUP and EP.

S.No.	Parameters	IUP	EP	t-value	p- value	Statistical Significance
1	Serum PAPP-A (µg/ml)	1.33±0.12	0.49±0.10	36.6	<0.001	Highly Significant
2	Serum Activin-A (ng/ml)	0.65±0.22	0.13±0.04	52.6	<0.001	Highly Significant
3	Serum Inhibin-A (pg/ml)	1.99±50.60	13.08±5.50	22.4	<0.001	Highly Significant

Table 2: Sensitivity , Specificity and Cut off values of parameters.

S.No.	Parameters	Sensitivity	Specificity	Cut off value
1	Serum PAPP-A (µg/ml)	81%	54%	< 0.53 µg/ml
2	Serum Activin-A (ng/ml)	100%	99%	<0.37 ng/ml
3	Serum Inhibin-A (pg/ml)	83%	79%	<28.67 pg/ml

The mean serum PAPP-A, Activin-A and Inhibin-A in patients with ectopic pregnancy (0.49±0.10 µg/ml, 0.13 ± 0.04 ng/ml and 13.08±5.50 pg/ml) were significantly lower than in women with normal intrauterine pregnancy (1.33±0.12 µg/ml, 0.65±0.22 ng/ml and 1.99±50.60 pg/ml, respectively) ($p<0.001$) (Table 1). In this study, by determining the cut-off levels of < 0.53 µg/ml for PAPP-A, <0.37 ng/ml for Activin-A and <28.67 pg/ml for

Inhibin-A, the sensitivity of single measurement of serum PAPP-A, Activin-A and Inhibin-A levels were 81% , 100% and 83% and specificity levels were 54% , 99% and 79% , respectively (Table 2).

Discussion:

The word 'ectopic' means displaced, and an ectopic pregnancy is when a fertilised egg implants itself outside of the womb (uterus) , usually in one of the fallopian tubes. It is an extrauterine pregnancy. Almost all ectopic pregnancies occur in the fallopian tube , but other possible sites include: cervical, interstitial (also referred to as cornual; a pregnancy located in the proximal segment of the fallopian tube that is embedded within the muscular wall of the uterus), hysterotomy scar, intramural, ovarian, or abdominal. In addition, in rare cases, a multiple gestation may be heterotopic (include both a uterine and extrauterine pregnancy). The present study demonstrated that pregnancy associated plasma protein A (PAPP-A) is an important pregnancy protein and its serum levels were determined in normal intrauterine and ectopic pregnancy. The mean ± standard deviation values were 1.33 ± 0.12 µg/ml and 0.49 ± 0.10 µg/ml in normal intrauterine and ectopic pregnancy respectively and when compared it was found that values of PAPP-A was statistically highly significant ($p < 0.001$) i.e. PAPP-A values are lower in tubal ectopic pregnancy when compared with normal intrauterine pregnancy (Table 1). In normal intrauterine pregnancy the trophoblastic tissue continues to synthesise PAPP-A so the concentration of PAPP-A increases with term but in tubal ectopic pregnancy the concentration of PAPP-A is very low or diminished due to smaller synctio trophoblastic mass implanted in the fallopian tube. In EP PAPP-A has been found to have positive correction with serum β-HCG. Similar findings has been discussed in earlier studies done by Bischof et al, (1983), Sinosich et al (1985), Muller et al (2004), Daponate et al (2005) and Cabar et al (2008).

Two important biomarkers taken in consideration for the early detection of ectopic pregnancy are Activin A and Inhibin A. These are two closely related protein complexes that have almost directly opposite biological effects. It is that Activin A enhances FSH secretion and Inhibin A inhibits Follicle Stimulating Hormone (FSH) secretion. In the present study Activin A and Inhibin A are found to be significantly lower in ectopic pregnancy when compared to normal intrauterine pregnancy. These two are closely related protein complexes that have almost directly opposite affects i.e. stimulating and inhibiting Follicle Stimulating Hormone (FSH) release from the pituitary. Florio et al (2010) reviewed that these two protein complexes are essential protein in establishment of pregnancy. These are found in high concentration in maternal serum throughout human pregnancy. These are helpful in the establishment and maintenance of pregnancy. As a consequence of ovarian progesterone stimuli, activin A is expressed and secreted by the stromal endometrial cells,which locally induces the decidualisation process, a pre requisite for implantation. Activin A is secreted from ovary and placenta but during pregnancy the main source is the trophoblast (Petraglia et al, 1987).

Serum Activin A and Inhibin A were found to be significantly elevated in normal intrauterine pregnancy than ectopic pregnancy. These are found in high concentration in maternal serum throughout pregnancy and are helpful in maintenance of pregnancy. In ectopic pregnancy low activin A lead us to suggest that an impaired secretion of Activin-A occurs in the presence of problems related to trophoblast invasion and implantation. Decidualised endometrium from ectopic pregnancies would differ from decidulised endometrium of intrauterine pregnancies due to absence of physical contact between the trophoblast and decidualised endometrium. So as the association of both trophoblast and decidualised endometrium is absent, so the production of Activin A and Inhibin-A is low in ectopic pregnancy than normal intrauterine pregnancy. In normal intrauterine pregnancy Activin-A was found to have positive correlation with serum progesterone and β-HCG only. The results of Activin-A of the present study are in accordance to the previous studies done by Florio et al (2007), Refaat B et al (2008) and Carrwright et al (2009). Serum inhibin-A values were similar to the previous studies done by Seifer DB et al (1996), Phipps et al (2000) and Segal et al (2008).

Conclusions:

In this study single measurement of serum Activin- A may identify patients at risk of Ectopic pregnancy (EP) with a high sensitivity(100%) and specificity (99%).

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