International Journal of A J Institute of Medical Sciences 2 (2013) 13-16

Original Research Article

Immunoreactive α2-macroglobulin in tubal pregnancy: A possible tool for early diagnosis

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Abstract

About 2% of all pregnancies are ectopic, where the implantation of the developing blastocyst occurs outside the uterus. More than 98% of ectopic pregnancies occur in the fallopian tubes. Haemorrhage from ectopic pregnancy is still the leading cause of maternal death in the first trimester, presenting a diagnostic challenge to the clinician. Besides signs and symptoms, the clinician depends only on 2 tools - the ultrasound and the quantitative estimation of β-hCG (human chorionic gonadotropin) for diagnosing the condition. In cases where both these do not give a clear picture as to the location of the implantation, precious time is lost waiting for a repeat β-hCG reading which might still prove to be inconclusive. Currently, there is no single serum marker available to detect tubal pregnancy. This study found a significant rise in immunoreactive serum α2-macroglobulin determined by radial immunodiffusion in ectopic pregnancy ($p<0.001$) when compared to normal nonpregnant and normal pregnant women, and discusses the relevance of this finding in the clinical setting.

Keywords: α2-macroglobulin; Ectopic pregnancy; Immunoreactivity; Tubal pregnancy

Received 4th September, 2012; Revised 5th November, 2012; Accepted 1st December, 2012.

1. Introduction

An ectopic pregnancy (EP) occurs when implantation of the embryo occurs outside the uterus. Currently, the incidence of EP is about 2 % of all pregnancies. Though the common site of ectopic implantation is the fallopian tube (98%), implantation can also be abdominal, cervical, interstitial or ovarian.[1] If tubal pregnancy (TP) is left untreated, the developing fetus will continue to grow, and may lead to life-threatening consequences for the mother.Tubal pregnancy has been the leading cause of maternal deaths in the first trimester of pregnancy.[1,2,3]

A major difficulty with the diagnosis of EP is that, methods of detection are limited. The clinician relies on a combination of clinical findings, ultrasound and repeated quantitative determination of serum beta-human chorionic gonadotropin (β-hCG) levels to arrive at a diagnosis.[4] Detection of β-hCG in the serum or urine, 8 to 10 days post-conception or later, confirms pregnancy.[5] However, the qualitative or

quantitative estimation of β-hCG does not give any clue regarding the location of the implantation. Transabdominal ultrasound (TAU) cannot diagnose pregnancy at less than 6 weeks gestation and transvaginal ultrasound (TVU) can detect pregnancies of 4.5 to 5 weeks gestation.[2,3,6,7] Currently, no single effective serum test exists to distinguish an EP from an intrauterine pregnancy (IUP).[8] When TVU does not reveal IUP in a patient with serum β-hCG level of >1500 mIU/ml, EP is suspected. An empty uterus at this stage may indicate an early pregnancy or an ectopic one. If the patient is haemodynamically stable, the clinician would wait for a doubling of β-hCG in 48 hours. If this occurs, the diagnosis of EP becomes unlikely, though it cannot be ruled out. Non-visualization of the gestational sac in the uterus, along with inadequate rise in β-hCG level (after 48 hours following the first estimation of β-hCG), strongly suggests ectopic pregnancy.[1,8] Since non-doubling or plateauing of β-hCG is known to occur also in normal intrauterine pregnancies, the clinician has no option but to wait in such cases hoping to visualize the gestational sac in the coming days. This delays diagnosis, and when the implantation is ectopic, it increases the risk of precipitating an obstetric emergency like a tubal rupture, necessitating surgical intervention.

At present, easy and early diagnosis of TP is not always possible. This study was an attempt to assess the usefulness of estimation of serum alpha2-macroglobulin (α2M) level as a tool in the early diagnosis of TP.

2. Materials and methods

This prospective case control study was conducted at the Department of Biochemistry, Kasturba Medical College, Manipal, India. Normal non-pregnant women (NNP, n = 20) in the age group of 25 to 35 years formed the normal control group. Women with normal intrauterine pregnancy (NIUP, n=20) and women with tubal pregnancy (TP, n =20) in the first 4 to 7 weeks of gestation calculated from the last menstrual period (LMP), attending the Obstetrics and Gynecology Department of the Kasturba Medical College Hospital, Manipal, India, were included in the study. This study was approved by the Institutional Ethics Committee and informed consent was taken from the subjects before starting the study. Sample selection in these cases was based on serum β-hCG levels of more than 1000 mIU/ml and ultrasound findings of NIUP or TP. Samples, where diagnosis of ectopic pregnancy was initially inconclusive due to unclear ultrasound findings, but later were confirmed to be tubal, (based on inadequate rise in β-hCG levels in 48 hours after first estimation of β-hCG) and ultrasound findings were also included in the study. Among cases of ectopic implantation, only cases of tubal pregnancy were selected, as availability of other types of ectopic implantation were rare. None of the women suffered from any other type of disease.

Purified human α2M and antibodies to human α2M were obtained from Sigma Chemicals, USA. All other reagents were of analytical grade. Estimation of immunoreactive serum α2M levels was performed by single radial immuno-diffusion.[9] Serum β-hCG levels were measured using the enzyme chemiluminescence immunoassay kit in Elecsys 2010 immunoassay analyzer, using a hCG+β kit from Roche Diagnostics, Germany. Statistical analysis was done using SPSS version 16 using one way ANOVA and values were expressed as mean ± SD and p values were tabulated. Statistical significance was defined at $p<0.05$.

3. Results

As shown in Table 1, α2M in normal non-pregnant women (NNP) was in the range 398 ± 28.98mg/dl and in normal intrauterine pregnancy (NIUP) it was decreased, i.e., 190.0 ± 16.99mg/dl. Though the decrease was considerable, it was not found to be significant. On the contrary, in TP, the level of

immunoreactive α2M was highly elevated, i.e., 1530± 577mg/dl, with p<0.001 when compared to both, i.e., normal non-pregnant women and normal women with intrauterine pregnancy. When compared with the mean α2M in normal pregnant women, α2M was significantly elevated, in all the 20 samples of TP.

4. Discussion

An earlier study estimated plasma proteinase inhibitory activity, i.e., antitryptic, antichymotryptic and α2M activity per se by estimating the chymotrypsin-bound esterase activity of the protein.[10] It was found that biologic activity of α2M was significantly increased in tubal pregnancy, and decreased in normal intrauterine pregnancy.[10] The assay used in the earlier study, though specific for α2M, was time consuming. The present study based on measuring the immunoreactive concentration of α2M confirms the significant elevation of α2M. It is also less tedious and much faster, which is significant if it is to be done in the emergency setting.

It has been reported earlier that trophoblastic invasion of maternal tissues differs in EP compared to IUP.[11] Studies by other workers have suggested serum vascular endothelial growth factor (VEGF) with a threshold value of 174 pg /ml as diagnostic for EP.[12] Triple marker analysis, based on VEGF, pregnancy associated plasma protein-A (PAPP-A) and progesterone is also suggested as a marker for EP.[13] Multiple marker tests have been reported recently.[14] Placental growth factor and cysteine rich secretory protein have been suggested as promising markers in TP. [15,16] Rausch et. al. have evaluated 12 different proteins as markers for ectopic pregnancy and inhibin A, progesterone, activin A, VEGF, pregnancy specific β-1 glycoprotein and PAPP-A to have fair diagnostic properties.[14] In this study, we have selected patients in the range of 4-7 weeks from last menstrual period, during which time diagnosis of EP using USG and β-hCG is difficult in some cases. α2M affords easy detection due to its very high concentration in serum. Need to estimate only a single protein is cost effective and time saving. It is proposed that, an immunoreactive α2M above 500 mg/dl would discriminate TP from NIUP. Together with USG and β-hCG, α2M looks very promising as a tool for the early diagnosis of TP, thus helping to manage TP medically rather than surgically, thereby helping to preserve the fertility of the patient. The study would have to be extended to a larger number of cases. Also, women with other sites of implantation such as abdominal, ovarian, interstitial and cervical should be investigated to determine the specificity and sensitivity of this potential diagnostic marker.

Table 1: Serum levels of immunoreactive α2M

Groups	Immunoreactive α2Min mg/dl
Normal non-pregnant (NNP, n=20)	398 ± 28.98
Normal intrauterine pregnancy (NIUP, n=20)	190.0 ± 16.99
Ectopic pregnancy (EP, n=20)	1530 ± 577 (p < 0.001)

5. Conclusion

This preliminary study suggests that as early as 4 weeks from the LMP when ultrasound findings are inconclusive, estimation of α2M in serum can be of possible use to differentiate a normal intrauterine pregnancy from a tubal pregnancy. Estimation of α2M by immunologic method affords an accurate and quicker method, which is crucial in the emergency setting. However, the

study has to be conducted with a larger sample size and should include other sites of ectopic implantation.

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