PLASMA PROTEINASE INHIBITORY ACTIVITY IN ECTOPIC AND MOLAR PREGNANCY

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ABSTRACT

Antitryptic, antichymotryptic and alpha 2- macroglobulin activities were measured in sera of normal nonpregnant and normal pregnant women and women with tubal ectopic pregnancy and molar pregnancies in the first 5 to 7 weeks of pregnancy calculated from the last menstrual period . While alpha 2- macroglobulin decreased in early normal pregnancy compared to nonpregnant state ($p < 0.001$), in ectopic and molar pregnancies there was an increase in alpha 2- macroglobulin activity ($p < 0.001$), as compared to nonpregnant and normal pregnant women . Antitryptic activity did not increase in normal and ectopic pregnancy, however was increased in molar pregnancy ($p < 0.01$). Antichymotryptic activities did not show a change either in normal pregnancy or in cases of ectopic and molar pregnancy . Drop in alpha 2- macroglobulin activity to near normal levels in ectopic, 6 weeks post surgery, correlated well with the decrease in β -hCG. However, in molar pregnancy, alpha 2- macroglobulin remained elevated even when the β -hCG levels in serum returned to zero 10 weeks after surgery. The studies suggest a major role for circulating proteinase inhibitors especially alpha 2- macroglobulin in regulating proteinase activity in normal, ectopic and molar pregnancy.

KEY WORDS

Antitryptic , Antichymotryptic , Alpha 2 – macroglobulin , Molar , Ectopic.

INTRODUCTION

The serum proteinase and proteinase inhibitory profile of pregnant women differs from that of nonpregnant women (1). This may be attributed to the production of proteinases and their inhibitors from trophoblastic tissue and also to the production of increased amounts of inhibitors from maternal tissues like the liver. The invasion of the trophoblast into the uterine endometrium involves the digestion of the matrix by matrix metalloproteinases, which facilitates implantation. Implantation involves a delicate balance between proteinases and their inhibitors (2, 3). Alpha 2-macroglobulin (A2M) has a major role in implantation by regulating the bioavailability of proteinases and cytokines involved in implantation (4).

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Implantation process in ectopic pregnancy is abnormal (5). Proteinase – proteinase inhibitor balance in trophoblastic tissue regulates the invasiveness of the mole in gestational trophoblastic disease (6). While most studies have shown that the trophoblastic tissue per se has an imbalance of proteinase against antiproteinase activity (4), the plasma proteinase inhibitor profile has not been studied in these conditions. Status of proteinase inhibitory activity in blood in normal early gestation, i.e, 5 to 7 weeks from last menstrual period (LMP) is not known. The objective of this study is to compare normal early pregnancy (NP) with abnormal gestation such as ectopic pregnancy (EP) and molar pregnancy (MP), with regard to serum proteinase inhibitory activities, which would reflect the events at implantation and in the days that follow it. The inhibitory activity before and after surgical intervention for EP and MP have also been studied. We have selected Anti tryptic activity (AT), Antichymotryptic activity (ACT) and A2M as representatives of proteinase inhibition in blood.

MATERIALS AND METHODS

Normal nonpregnant (NNP) women (n=20) in the age group of 25 to 35 years formed the normal control group. NP (n=20) and women with EP (n=10) and MP (n=6) in the first 5 to 7

weeks of gestation, attending the Obstetrics and Gynecology Department of the Kasturba Medical College Hospital, Manipal, Karnataka, were included in the study. Informed consent was taken from the subjects prior to the study. Selection of serum samples was based on β -hCG levels more than 1000 munits/ml and ultrasound findings of NP, EP or MP. In case of EP only cases of tubal pregnancy were selected. Histopathology reports were looked into to confirm the presence of MP and only cases where β -hCG levels returned to zero after evacuation of the mole were included in the statistical analysis. Cases of invasive mole identified on basis of computerized tomography (CT) scan report and choriocarcinoma identified based on increasing β -hCG levels following evacuation of the mole were excluded from the study. For follow up of the cases after surgical intervention in EP and MP, the β -hCG and proteinase inhibitory activities were estimated on subsequent visits of the patients to the hospital. None of the women suffered from any other type of disease.

Bovine trypsin and chymotrypsin were obtained from Worthington Biochemicals, Freehold, NJ, USA. Casein (purified) and N-acetyl L-tyrosine ethyl ester (ATEE) were from Sigma chemicals, USA. All other reagents were analytical grade commercial chemicals. The modified Kunitz caseinolytic method was used for estimating AT and ACT activities in the plasma samples (7). One unit of inhibitory activity was defined as the amount that caused suppression of caseinolytic activity of trypsin or chymotrypsin by one absorbance unit. Estimation of A2M was based on the esterolytic activity of A2M bound chymotrypsin, described in detail by Rao et al (8). Plasma β -hCG levels were measured using the electrochemi-luminescence immunoassay method in Elecsys 2010 immunoassay analyzer, using a HCG+ β kit from Roche Diagnostics, Germany

Statistics : Statistical analysis was done using SPSS version 10.0 using one way ANOVA and values are expressed as Mean $\pm$ SD and p values were tabulated. Statistical significance was defined as p less than 0.05.

RESULTS AND DISCUSSION

There was a significantly decrease in A2M activity in NP ($p < 0.001$), as compared to NNP in the first 5 to 7 weeks of pregnancy, while there was no significant change in AT and ACT activity during the same period (Table 1). A2M activity in serum decreases during 12 to 14 weeks of normal pregnancy (1). Our study demonstrates that the decrease in A2M activity begins as early as 5 to 7 weeks of pregnancy. A2M generally decrease when there is increased release of proteinases from

Table 1 : A2M, AT and ACT levels in Control, Normal, Ectopic & Molar Pregnancy (mean $\pm$ SD)

Parameters	A2M	AT	ACT
Group I (n=20) Normal nonpregnant	274.19 $\pm$ 43.8	108.0 $\pm$ 30.0	117.5 $\pm$ 30.0
Group II (n=20) Normal pregnancy	140.0 $\pm$ 40.2a **	150.4 $\pm$ 48.4a ns	168.0 $\pm$ 76.7a ns
Group III (n=10) Ectopic pregnancy	423.92 $\pm$ 47.0b **	173.0 $\pm$ 50.1b ns	117.5 $\pm$ 27.6b ns
Group IV (n=6) Molar pregnancy	481.5 $\pm$ 69.7c **	243.1 $\pm$ 75.8c *	190.0 $\pm$ 75.2c ns

ns – not significant a : Group I and Group II; b : Group II and Group III; c : Group II and Group IV; ** $p < 0.001$; * $p < 0.01$

tissues into the blood and the inhibitor-enzyme complex formed is taken up by the liver and degraded. Considerable amounts of plasma proteinase inhibitors are extravasated into the interstitium in the vicinity of the implanting blastocyst (9) to regulate the activities of number of factors in the early events of implantation (4). Decreased A2M activity in NP in serum is in line with these observations. AT and ACT activities are not involved significantly in regulation of implantation as observed here.

In contrast, in EP, there was an increase in A2M activity ($p < 0.001$). Change in ACT activity was not significant. AT activities showed an increasing trend, though the rise was not significant. EP differs from normal pregnancy with respect to vascular endothelial growth factor (VEGF), pregnancy associated plasma protein A (PAPP –A) etc. A triple marker analysis for differentiating between EP and NP based on

Table 2 : β -hCG * levels before and after surgery in ectopic and molar pregnancy

	Time in weeks	Ectopic tubal pregnancy	Molar pregnancy
Before surgery	0	4199 .0	421,574.0
After surgery	1	3275.0	-
	2	652.0	489.0
	3	-	-
	4	-	73.5
	5	3.0	36.1
	6	0.9	-
	7	-	12.3
	8	-	-
	9	-	6.5
	10	-	4.5

*values of β -hCG are from one case each and are in munits/ml

VEGF, PAPP-A and progesterone levels has been proposed (5). It is worthwhile investigating whether A2M activity along with low β -hCG which is seen in ectopic pregnancy, can serve as a marker in diagnosing EP. Fall in A2M activity to near normal level only 6 weeks after surgery (Graph 1), when the β -hCG levels returned to almost zero, demonstrates that A2M is involved in long term regulation of multiple factors during implantation and thereafter. ACT activity did not show any response to surgery and continued in the normal range as found in NNP. Antitryptic activity which was in the normal range for pregnant women almost doubled one week after surgery suggesting an acute phase reaction (10) and reached normal levels in 6 weeks.

In MP, A2M was highly elevated ($p < 0.001$), with values exceeding three times of that in NP. ACT activities resembled those of normal pregnant women while AT activities were elevated ($p < 0.01$). Implantation involves the digestion of the uterine endometrial matrix by different matrix metalloproteinases which belong to the class of type IV collagenases present in trophoblastic tissue (3). While the collagenases are produced in increased amounts in trophoblastic tissue, their inhibitor concentration is decreased, which is correlated with the invasive nature of the mole and the pathogenesis of gestational trophoblastic disease (6). Marked rise in serum A2M in MP seems to be a defense mechanism against lower levels of proteinase inhibitors and higher levels of proteinases in trophoblastic tissue per se. A2M being a broad spectrum proteinase inhibitor, is capable of binding to these enzymes when they are released into systemic circulation (11). Recent reports indicate that A2M is capable of binding hydrolases required for enzyme mediated tumor invasion (12). The body seems to perceive EP and MP in a similar fashion as a tumor. The ACT activity in molar pregnancy

is not significantly elevated, showing that this activity does not contribute to regulating proteinase activity in this condition as in NP. The nature of enzymes and other molecules interacting with A2M in molar pregnancy has not been elucidated as yet although it is reported to bind cytokines and growth factors required for tumor proliferation (12). An investigation into the plasma proteinase profile of these patients is required. It is tempting to suggest here, that A2M estimation in MP may be used as an indicator of trophoblastic disease in conjunction with high β -hCG. Levels of β -hCG dropped in response to surgery in MP. (Table 2), corresponding A2M, AT and ACT activities in MP are depicted in Graph 2. In MP (Graph 2, week 0), A2M activity which was highly elevated before surgery continued to be highly elevated even upto 10 weeks after surgery. AT and ACT activities followed a similar course, falling one week after surgery and attaining normal levels as in NNP women in 6 weeks.

It can be concluded that A2M undergoes significant amount of turnover in early normal pregnancy when proteinases are released during events of implantation of the blastocyst and invasion of the uterine endometrium by the trophoblast. We hypothesise that the very rapid consumption of A2M in early EP and MP, leading to the formation of proteinase-inhibitor complexes stimulates the synthesis of markedly increased levels of A2M, probably by the maternal liver. The continued elevation of A2M even after surgical removal of the trophoblastic tissue in EP or evacuation of the mole in MP suggests a long term change in the inhibitory profile of the patients. However, it is certain that A2M and not AT or ACT activities bear the major brunt of proteinase onslaught in NP, EP and MP. To understand the elevation of A2M in EP and MP, studies into the nature of the proteinase activities in plasma of these patients is required.

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