PAPERS AND ORIGINALS

Assay of a placental protein to determine fetal risk

GRAEME HUGHES, PAUL BISCHOF, GEORGE WILSON, ARNOLD KLOPPER

Summary and conclusions

Plasma concentrations and total amounts of pregnancy associated plasma protein A were determined in 272 patients at 34 weeks' gestation by immunoelectrophoretic assay. The mean plasma concentration and mean total amount of this protein were closely related ($r=0.9643$) and were significantly raised in patients who subsequently developed pre-eclampsia (28 patients), went into premature labour (12), or suffered from antepartum haemorrhage (10). Mean values in all patients delivering growth-retarded babies were also raised, but when the results for such patients who also had other complications were excluded there were no differences between the sets of means.

The assays were easily performed, and they may be a useful technique for screening pregnant women to detect those at risk of developing pre-eclamptic toxæmia, although the full potential of these assays cannot be realised until the protein's function is known.

Introduction

In 1973 Lin *et al.*¹ isolated a new protein from the plasma of pregnant women. This protein, which they named pregnancy associated plasma protein A (PAPP-A) was, like the protein hormones human placental lactogen and human chorionic gonadotropin, peculiar to pregnancy and produced in the placenta.² The concentration increased with the growth of the placenta as pregnancy advanced.³ The Miami group have advocated the measurement of PAPP-A concentrations to assess placental function.⁴ We have developed a simple immunoelectrophoretic technique for determining concentrations of this

protein⁵ and have investigated the possibility of using this assay to screen large numbers of patients by a single measurement in late pregnancy.

Patients and methods

Patients in Aberdeen Maternity Hospital receiving antenatal care under a combined scheme with their general practitioners are reviewed in hospital when they are 34 weeks pregnant. We took venous blood samples at random from 272 such patients. The results of the assays did not influence management of these patients. After delivery data about the pregnancy were taken from the case records.

Venous blood samples (10 ml) were taken and put into heparinised containers. The plasma was removed the same day and stored at -20°C until the analysis was performed. PAPP-A concentrations were measured by the Laurell "rocket" method of immunoelectrophoresis,⁵ with a monospecific antiserum to PAPP-A raised by injecting the purified protein into rabbits.⁶ The standard was a pool of late-pregnancy plasma calibrated against the pure protein. We have aliquots of a similar standard available for researchers wishing to calibrate their own standard.

Results

The 272 patients studied were divided into two groups: 176 who had normal pregnancies and 96 who developed some clinical obstetric abnormality. Some of the 96 already presented evidence of obstetric disease at the time of the 34-week examination or earlier. We excluded from the analysis those who presented with pre-eclamptic toxæmia or a history of antepartum haemorrhage before 34 weeks, six patients with essential hypertension, five who delivered sets of twins, and three diabetics. We included in the analysis 28 patients who developed pre-eclampsia (blood pressure above 140/90 mm Hg) and 10 patients who suffered an antepartum haemorrhage, however slight, after 34 weeks. Only two of the patients with pre-eclampsia were considered to be suffering from severe pre-eclampsia—that is, had albuminuria as well as hypertension. We included a further 12 patients who went into spontaneous labour before 37 weeks (preterm labour) and 38 who produced growth-retarded babies according to the criteria laid down for the Aberdeen population by Thomson *et al.*⁷ There was some overlap between the growth-retarded category and other categories: three of these patients also had antepartum haemorrhage, two developed pre-eclampsia, and one went into labour prematurely. Three of the growth-retarded babies were malformed.

Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen AB9 2ZB

GRAEME HUGHES, CHB, MRCOG, senior registrar

PAUL BISCHOF, PHD, research biochemist (now at Department of Obstetrics and Gynaecology, University of Geneva, Switzerland)

GEORGE WILSON, PHD, biochemist

ARNOLD KLOPPER, MD, FRCOG, professor of reproductive endocrinology

Table I shows the mean and standard deviation of PAPP-A values in the various categories. On inspection of the values in the normal group we found that the distribution of data was skewed, with values tending to cluster below the mean, which was increased by a few exceptionally high results. A similar finding has been recorded with other placental proteins such as human placental lactogen.⁸ Log transformations were therefore performed in determining the statistics of this material. An attempt was also made to get a more reliable result for the placental production of PAPP-A by allowing for the different maternal plasma volumes in which the protein was distributed. This was achieved by multiplying the plasma PAPP-A concentration by the woman's body weight, giving a result representing the total amount of PAPP-A. Values for the total PAPP-A and the plasma concentration were closely related ($r=0.9643$), and information derived from total PAPP-A values was similar to that derived from PAPP-A concentrations.

TABLE I—Plasma concentrations and total amounts of PAPP-A in 272 patients at 34 weeks' gestation. Results are means, with 1 SD above mean then 1 SD below mean given in parentheses

	PAPP-A	
	Plasma concentration (μg/ml)	Total (μg)*
Normal patients (n = 176)	97.0 (167.5, 56.2)	6530 (11 374, 3749)
Abnormal patients (n = 96)	131.5† (255.8, 67.6)	8549† (16 440, 4415)
Patients with:		
Pre-eclampsia (n = 28)	149.2‡ (277.9, 80.2)	10 493‡ (19 720, 5584)
Antepartum haemorrhage (n = 10)	126.7 (239.8, 67.0)	8220 (16 327, 4139)
Preterm labour (n = 12)	147.9§ (206.5, 70.3)	8951§ (20 413, 6682)
Growth-retarded baby (n = 38)	116.3† (196.2, 69.0)	6981 (11 828, 4120)

*Total PAPP-A = plasma concentration (μg/ml) × body weight (kg).

Significance of differences from normal: †2p < 0.05, ‡2p < 0.001, §2p < 0.03.

TABLE II—Percentages of patients at 34 weeks' gestation having PAPP-A values above 80th percentile of log transforms

	Percentile of log transforms							
	80th-		85th-		90th-		95th-	
	Concentration (μg/ml)	Total (μg)	Concentration (μg/ml)	Total (μg)	Concentration (μg/ml)	Total (μg)	Concentration (μg/ml)	Total (μg)
Normal patients	19.9	19.8	14.8	15.0	9.7	9.6	5.1	4.8
Patients with:								
Pre-eclampsia	60.7	59.3	46.4	48.2	28.6	33.3	25.0	18.5
Antepartum haemorrhage	30.0	25.0	20.0	25.0	10.0	12.5	10.0	12.5
Growth-retarded baby	34.3	32.0	20.0	21.4	11.4	10.7	5.7	7.1
Preterm labour	50.0	50.0	33.3	50.0	33.3	50.0	33.3	12.5

PAPP-A values were raised above normal in patients who subsequently developed pre-eclampsia, went into preterm labour, or suffered an antepartum haemorrhage. Values in the abnormal group as a whole were also raised, but this group was so heterogeneous that this finding largely reflected the numerical distribution of the subgroups composing it. The patients with growth-retarded babies were also a mixed group, and when patients with complications such as pre-eclampsia or antepartum haemorrhage were excluded the mean PAPP-A values for patients in this group were not raised.

Table II shows the percentage of patients in each category who had values above the 80th, 85th, 90th, and 95th percentiles of the log transforms. Sixty per cent of patients who would later develop pre-eclampsia already had values above the 80th percentile by 34 weeks, and 25% had values greater than the 95th percentile. Half the women who went into preterm labour had values greater than the 80th percentile, as did 30% of patients who subsequently had some antepartum bleeding. Thirty-four per cent of all patients who delivered a growth-retarded baby had values above the 80th percentile, but when patients who also had pre-eclampsia and antepartum haemorrhage were excluded from this group the figure fell to 26%, which was barely above the normal incidence.

Discussion

Our findings suggest that PAPP-A assays may be a useful screening technique for detecting a group at risk requiring special surveillance in antenatal care. Pre-eclamptic toxæmia

is a major cause of perinatal fetal loss, and it is of particular interest that the plasma PAPP-A concentration has some predictive value for the development of this disease. The fact that the test does not require advanced technology, expensive instruments, or radioactive materials is an attractive feature. The protein is stable at room temperatures, and hospital laboratory centres could provide a rural service by post.

PAPP-A is a placental protein, and its concentration in maternal plasma probably reflects placental activity. Not surprisingly, therefore, the assays are of no value in diagnosing fetal growth retardation, a disease that often has a primary fetal element. Indeed, the coexistence of growth retardation and pre-eclampsia may impair the value of the test in predicting pre-eclampsia.

The potential of PAPP-A assays cannot be realised until more information on the physiological function of the protein is available. Klopper *et al*⁹ have suggested that it is a locally active compound performing a function, presumably immunosuppressive, at the trophoblast-decidual interface. It probably does not enter the maternal circulation by active secretion from the chorionic villi into the retroplacental blood but by dissolution of migrating or embolic trophoblast within the maternal tissues.⁹ Perhaps the association of raised PAPP-A values with preterm labour is not causal but a consequence of increased uterine activity preceding the onset of labour.

Raised values of PAPP-A in established pre-eclampsia have been noted,¹⁰ but we have shown for the first time that the plasma concentration of this protein is raised before clinical signs become evident. The protein may contribute towards causing pre-eclampsia. Untoward immune responses have

often been invoked in pre-eclampsia, and there is increasing evidence that PAPP-A participates in the immunological interaction between trophoblast and maternal tissues.¹¹ If PAPP-A is indeed a locally active and non-toxic biological immunosuppressive agent it raises many possibilities in subjects such as transplant surgery. The function of this protein should be thoroughly investigated.

We thank Miss Nan Scott for computer analysis of our data, Mrs Pamela Cunningham for technical help, and Miss Gina Cowie for secretarial help.

References

- Lin TM, Halbert SP, Kiefer D, Spellacy W, Gall S. Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol* 1974;118:223-36.
- Lin TM, Halbert SP. Placental localization of human pregnancy-associated plasma proteins. *Science* 1976;193:1249-52.
- Lin TM, Halbert SP, Spellacy WN. Measurement of pregnancy-associated plasma proteins during human gestation. *J Clin Invest* 1974;54:576-81.
- Halbert SP, Lin TM. Pregnancy-associated plasma proteins: PAPP-A and PAPP-B. In: Klopper A, Chard T, eds. *Placental proteins*. Heidelberg: Springer-Verlag, 1979:89-104.
- Bischof P, Bruce D, Cunningham P, Klopper A. Measurement of pregnancy associated plasma protein A (PAPP-A). *Clin Chim Acta* 1979;95:243-7.

- ⁶ Bischof P. Observations on the isolation of pregnancy-associated plasma protein A. In: Klopper A, Chard T, eds. *Placental proteins*. Heidelberg: Springer-Verlag, 1979:105-18.
- ⁷ Thomson AM, Billewicz WZ, Hytten FE. The assessment of fetal growth. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1968;**75**:903-12.
- ⁸ Chard T. Normality and abnormality. In: Klopper A, ed. *Plasma hormone assays in evaluation of fetal wellbeing*. Edinburgh: Churchill Livingstone, 1976:1-19.
- ⁹ Klopper A, Smith R, Davidson I. The measurement of trophoblastic proteins as a test of placental function. In: Klopper A, Chard T, eds. *Placental proteins*. Heidelberg: Springer-Verlag, 1979:23-42.
- ¹⁰ Lin TM, Halbert SP, Spellacy WN, Berne BH. Plasma concentrations of four pregnancy proteins in complications of pregnancy. *Am J Obstet Gynecol* 1977;**128**:808-10.
- ¹¹ Klopper A. The new placental proteins. *Placenta* 1980;**1**:77-89.

(Accepted 17 December 1979)

Relation between extent of coronary artery disease and blood viscosity

G D O LOWE, MAUREEN M DRUMMOND, A R LORIMER, I HUTTON, C D FORBES, C R M PRENTICE, J C BARBENEL

Summary and conclusions

Blood viscosity (shear rate 100/s) and its major determinants (packed cell volume, plasma fibrinogen concentration, and plasma viscosity) were measured before coronary angiography in 50 men aged 30-55 and related to the extent of coronary artery disease. Twenty-six men had extensive disease (stenosis of two or three major coronary vessels), and 24 had either stenosis of one vessel or no stenosis. The 26 men with extensive disease had significantly higher mean blood viscosity than those with mild or no disease and 25 healthy controls ($p < 0.001$). The increased viscosity was due partly to a higher packed cell volume and partly to a higher fibrinogen concentration; plasma viscosity was not significantly increased. These differences could not be explained by smoking history.

These results suggest an association between increased blood viscosity and extensive coronary artery disease in men, which merits further investigation.

Introduction

Increased blood viscosity has been described in patients with clinical manifestations of occlusive arterial disease (previous myocardial infarction, angina, claudication) compared with control subjects.¹⁻³ The relation of viscosity to the extent of coronary artery occlusion—the most important determinant of mortality in ischaemic heart disease⁴—is, however, not known. We therefore studied blood viscosity and its major determinants (packed cell volume, plasma fibrinogen concentration, and plasma viscosity) in relation to the extent of coronary occlusion in men undergoing coronary arteriography.

University Department of Medicine, Royal Infirmary, Glasgow G4 0SF

G D O LOWE, MRCP, lecturer
C D FORBES, MD, FRCP, senior lecturer
C R M PRENTICE, MD, FRCP, reader

Bioengineering Unit, University of Strathclyde, Glasgow

MAUREEN M DRUMMOND, biochemist
J C BARBENEL, PHD, senior lecturer

University Department of Medical Cardiology, Royal Infirmary, Glasgow G4 0SF

A R LORIMER, MD, FRCP, consultant
I HUTTON, MD, FRCP, senior lecturer

Patients and methods

We studied 75 men aged 30-55 years after they had been admitted to Glasgow Royal Infirmary. Fifty were studied before they underwent selective coronary arteriography for assessment of chest pain: we excluded those with a history of myocardial infarction in the previous three months, those receiving treatment with diuretics (which cause haemoconcentration) or clofibrate (which lowers fibrinogen concentration and viscosity⁵), and those with overt cardiac failure. The extent of angiographic coronary occlusion was graded according to the number of major vessels (right, left anterior descending, and left circumflex coronary arteries) in which the lumen was occluded by 50% or more: patients were thus classified as having no disease or disease of one, two, or three vessels. Twenty-five men admitted for elective minor surgery served as controls: none had clinical evidence of vascular disease and in all a resting electrocardiogram was normal. Cigarette smokers were defined as those who had regularly smoked cigarettes within the previous three months.

Venous blood samples were taken from resting subjects between 11 am and 1 pm and anticoagulated with EDTA (1 mg/ml) for measurement of blood viscosity at 37°C (rhombospheroid viscometer,⁶ shear rate 100/s); packed cell volume (Hawksley microhaematocrit, 13 000 g for five minutes); and plasma viscosity at 37°C (BS M3 capillary viscometer). Fibrinogen was determined by a thrombin-time method⁷ in citrated plasma. The coefficient of variation for all these variables as measured by us is under 2%. Differences in mean values were analysed by Student's *t* test, and correlations by covariance analysis.

Results

Twenty-six men had extensive coronary occlusion: 16 had stenosis of two and 10 stenosis of three vessels. This group was compared with the 24 men who had relatively normal coronary arteries (eight with no stenosis and 16 with stenosis of a single vessel) and the 25 control subjects who had no clinical vascular disease. Table I shows that the three groups were comparable with regard to age and prevalence of smoking; history of smoking, weekly consumption of tobacco, and prevalence of inhaling were also comparable. The two groups of patients undergoing arteriography did not differ significantly in current use of beta-adrenergic blocking drugs or history of myocardial infarction.

TABLE I—Clinical data on patients studied

	Controls	Extent of stenosis	
		None, or one vessel	Two or three vessels
No of patients	25	24	26
Mean age ($\pm$ SD) (years)	46.1 $\pm$ 5.9	45.6 $\pm$ 5.5	48.2 $\pm$ 5.3
No of smokers	15	14	16
No receiving beta-blockers		14	19
No with previous infarct		12	16