Acta Genet Med Gemellol 28:319–325 (1979)
The Mendel Institute/Alan R. Liss, Inc.

The Value of Measurement of Pregnancy-Specific Proteins in Twin Pregnancies

V. Jandial, C. H. W. Horne, R. G. Glover, A. D. Nisbet, D. M. Campbell, I. MacGillivray

Department of Obstetrics and Gynaecology and Department of Pathology, University of Aberdeen, UK

In this study of 53 twin pregnancies, the plasma concentrations of the placental proteins, placental lactogen (hPL), and pregnancy-specific glycoprotein (SP1) were measured. Placental lactogen was found to be of more value than SP1 both in the detection of twins and in assessment of fetal growth. Serial measurement of either protein would seem to be more useful in assessment of fetal growth than single measurements. Preliminary studies of the less well known placental protein, PP5, in a small series of twin pregnancies indicate that it may also prove to be of clinical value in the detection and monitoring of twin pregnancies.

Key words: Placental lactogen, Pregnancy-specific glycoprotein, Placental protein five, Plasma concentrations, Twin pregnancy

INTRODUCTION

The well-known trophoblast products placental lactogen (hPL) and chorionic gonadotropin (hCG) have been shown to be useful adjuncts to ultrasonography in the detection of twins [5, 10]. The clinical usefulness of the more recently identified trophoblast products, pregnancy-specific glycoprotein (SP1), and placental protein 5 (PP5) in this condition, however, has not been reported previously.

SP1 is a product of the syncitiotrophoblast [8, 12, 15, 16] and has been detected as early as seven days postovulation [6], the maternal concentrations rising steadily until the 36th week of pregnancy, when they tend to plateau. Measurement of plasma SP1 levels has been found to be useful in the assessment of fetal growth, and especially in the detection of intrauterine growth retardation [4, 9, 17, 21]. In an earlier study of nine cases of twin pregnancy, maternal SP1 levels were around or above the upper limit of the normal range [20]. Accordingly, it was thought worthwhile to extend these observations in a series of 53 twin pregnancies. For comparison, SP1 measurements have been compared with hPL levels. In addition, we have made a preliminary assessment of the value of the relatively unknown trophoblast product PP5 [1, 2]. Like SP1, PP5 can be localised in the trophoblast [7, 15], but it is necessary to use a radioimmunoassay to quantitate maternal serum levels [14].

SUBJECTS AND METHODS

Heparinized venous blood samples were obtained from 53 patients attending the antenatal clinic at Aberdeen Maternity Hospital or from inpatients of the antenatal ward or clinical research unit. The diagnosis of twin pregnancy was made on clinical examination and was confirmed by ultrasonography. In the majority of patients only one blood sample was obtained, but in 12 patients serial samples were taken. The separated plasma was stored at −20°C until required for assay.

The plasma levels of SP1 were measured by single radial immunodiffusion [13] or electroimmunoassay [11], using rabbit anti-SP1 (Dakopatts). Placental lactogen concentrations were determined by single radial immunodiffusion using commercially available partigen plates (Behringwerke AG). Serum PP5 levels were measured in a further nine patients; a sensitive radioimmunoassay employing the double antibody solid phase method of Chesworth [3] was adapted to allow measurement of PP5. Purified PP5 (Batch 49/48) and specific antiserum prepared in rabbits (Batch 606A) were gifts of Dr. Hans Bohn, Behringwerke. Late pregnancy samples were diluted tenfold before assay.

From the clinical data the centile baby weight in each case was calculated using the tables published by Thomson et al [19]. Intrauterine growth retardation was defined as birth weight at or below the 10th centile for gestational age after correction for sex, parity, maternal height, and natural weight.

RESULTS

Fetal growth was considered "normal" (N) when the birth weight of each baby was greater than the 10th centile for the period of gestation and corrected for sex and parity. When the birth weight was below the 10th centile it was presumed that the infant growth was retarded (GR). The distribution of cases is shown in Table 1.

SP1 levels in the N/N group and the N/GR group were significantly higher than for a singleton fetus (Table 2). Although mean SP1 levels for the GR/GR group were reduced, the levels were still significantly higher than that for singleton fetuses.

Placental lactogen (hPL) values for the N/N and N/GR groups were again significantly higher than for singleton fetuses. However, in the GR/GR group hPL levels were considerably reduced, and there was no significant difference between the level for singleton fetus and twins (Table 3).

Table 1. Distribution of Cases

	Number	%
Normal growth (N/N)	23	43.4
Growth retarded (GR/GR)	13	24.5
One normal, one growth retarded (N/GR)	17	32.1

TABLE 2. Mean Maternal SP1 Levels in Twin Pregnancies

	Outcome	N/N	N/GR	GR/GR
Mean maternal plasma concentration (μg/ml)	Twins	184.3 ± 69.9	189.9 ± 80.5	177.6 ± 51.6
	Singletons	140.5 ± 35.4	144.9 ± 30.8	148.2 ± 32.0
		($t = 2.68$) $P < 0.01$	($t = 2.16$) $P < 0.025$	($t = 1.74$) $P < 0.05$

https://doi.org/10.1017/S0001566000008850 Published online by Cambridge University Press

TABLE 3. Mean Maternal hPL Levels in Twin Pregnancies

	Outcome	N/N	N/GR	GR/GR
Mean maternal plasma concentration (μg/ml)	Twins	9.8 ± 3.2	9.7 ± 3.0	7.7 ± 2.2
	Singletons	6.0 ± 1.5	6.5 ± 1.3	6.6 ± 1.3
		(t = 5.13) P < 0.0005	(t = 4.17) P < 0.0005	ns

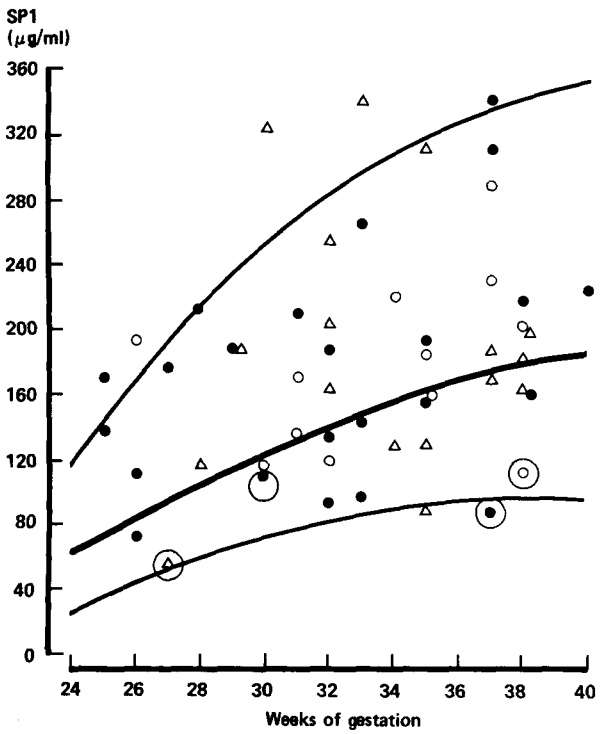

Fig. 1. SP1 levels in randomly selected single samples from twin pregnancies. Mean ± 2 SD for normal singleton pregnancy shown. • Normal growth (N/N); ○ Growth retarded (GR/GR); △ One normal, one growth retarded (N/GR). The four patients who produced predominantly the α-form of SP1 are circled.

Single sample measurement of SP1 was not helpful in the assessment of fetal growth (Fig. 1). There were four patients in this group who predominantly produced the α-form of the protein, which is difficult to measure both by radioimmunoassay and electro-immunoassay. Similarly, single sample measurement of hPL was unhelpful in assessing fetal growth (Fig. 2).

The serial sample measurements of SP1 appear to be more useful. There was only one patient in this group who produced growth-retarded babies, and this was the only case in which SP1 levels failed to rise as the pregnancy advanced (Fig. 3).

https://doi.org/10.1017/S0001566000008850 Published online by Cambridge University Press

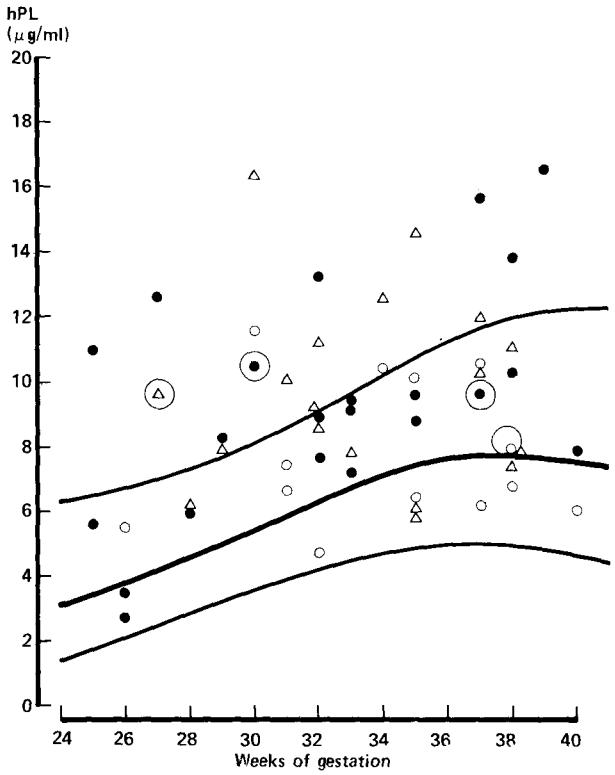

Fig. 2. hPL levels in randomly selected single samples from twin pregnancies. Mean ± 2 SD for normal singleton pregnancy shown. ● Normal growth (N/N) ○ Growth retarded (GR/GR) Δ One normal, one growth retarded (N/GR). The four patients who produced predominantly the α-form of SP1 are circled.

Serial hPL measurements provided slightly better information (Fig. 4). In the one patient with growth retarded babies, the levels decreased to below the mean for singleton fetuses as the pregnancy progressed. In patients with normal growth there was continued rise in hPL levels.

PP5 levels, although measured in a preliminary set of patients (nine), appeared to reflect the results for hPL. Levels were high, particularly for the N/N group, whereas the GR/GR group showed no significant difference from singletons. Serial samples were available for four patients, and only the one from the GR/GR group showed no increase with weeks of gestation.

DISCUSSION

The results indicate that hPL is better than SP1 both in the diagnosis of twins (although there are many false negatives, particularly in the GR/GR group) and in the assessment of fetal growth in twins. Levels of both proteins were significantly higher than in singletons except for the hPL levels of the GR/GR group, which were comparable with singletons. Even in this group, however, there were patients with levels more than 2 SD above the mean for singletons. Serial samples were available for only one patient in the GR/GR

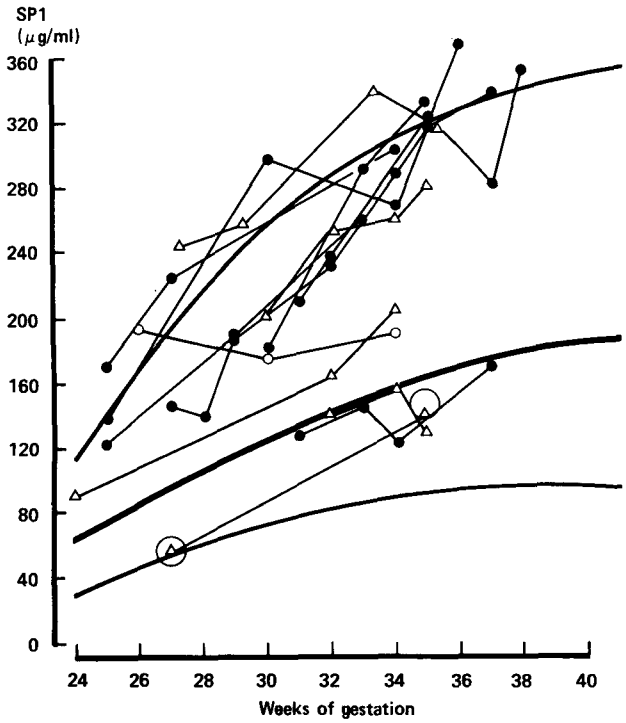

Fig. 3. SP1 levels in serial samples from twin pregnancies. Mean ± 2 SD for normal singleton pregnancy shown. • Normal growth (N/N); ○ Growth retarded (GR/GR); △ One normal, one growth retarded (N/GR). One patient who produced predominantly the α-form of SP1 is included (circled). The one patient in the GR/GR group showed constant SP1 levels.

group and seemed more promising, since a decrease in hPL levels was observed, and all other patients showed an increase. In addition, a patient in the GR/GR group of the PP5 series had constant PP5 levels on consecutive weeks, whereas all others showed an increase. Serial samples from many patients in the GR/GR group will be necessary to indicate whether this is a universal phenomenon and to allow calculation of the detection rate for this group.

The distinction between growth-retarded and normal has been made using data for singleton birth weight. It is accepted that this may not be adequate, but sufficient information for calculation from twin birth weights was not available.

The four patients who produced predominantly the α-form of SP1 were detected by the presence of "fuzzy" rockets in electroimmunoassay [22]. Subsequently, it has been suggested that all patients have a proportion of the α-form that is constant throughout pregnancy but varies from patient to patient [18]. It is not known whether the presence of SP1α has had an effect on the SP1 levels measured.

PP5 levels measured in plasma were reported as being approximately half those measured in serum [14]. For this reason, PP5 was not measured for the 53 patients, but sera from a separate nine patients were used to obtain preliminary results. It has since been discovered that the assay used in this laboratory does not distinguish between serum

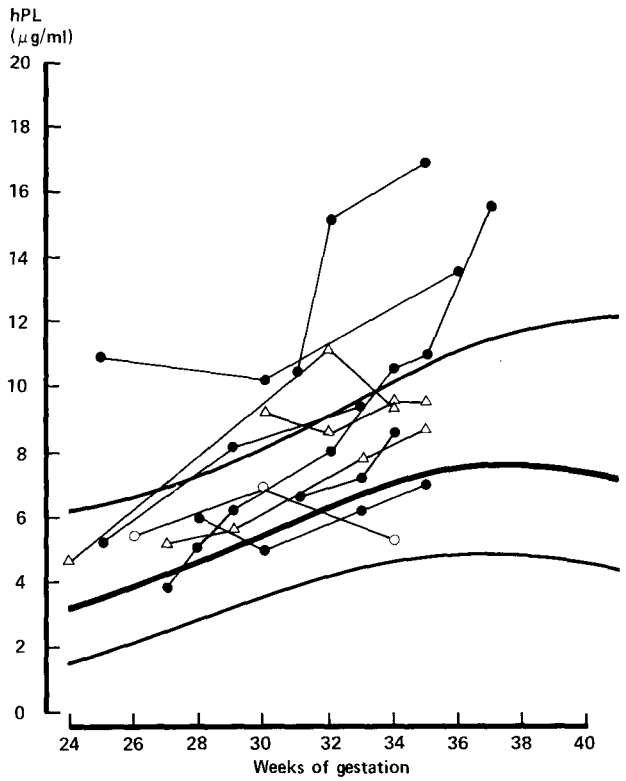

Fig. 4. hPL levels in serial samples from twin pregnancies. Mean ± 2 SD for normal singleton pregnancy shown. ● Normal growth (N/N); ○ Growth retarded (GR/GR); △ One normal, one growth retarded (N/GR). The one patient in the GR/GR group showed a decrease in hPL levels.

and plasma. Measurement of the 53 patients will give a better assessment of the value of PP5 compared to SP1 and hPL. The results of PP5 look similar to hPL rather than SP1 and are therefore promising.

Acknowledgments. A.D. Nisbet is in receipt of a Wellcome research fellowship. Antiserum to SP1 was generously donated by Dakopatts A/s Copenhagen, Denmark.

REFERENCES

1. Bohn H (1972): Detection and characterization of soluble antigens in the human placenta. Arch Gynäkol 212:165–175.
2. Bohn H, Winckler W (1977): Isolation and characterization of the placental protein PP5. Arch Gynäkol 223:179–186.
3. Chesworth JM (1977): Radioimmunoassays of ovine LH and ovine prolactin using polymerized second antisera. Anal Biochem 80:31–40.
4. Gordon YB, Grudzinskas JG, Jeffrey D, Chard T, Letchworth AT (1977): Concentrations of pregnancy-specific β_1-glycoprotein in maternal blood in normal pregnancy and in intrauterine growth retardation. Lancet 1:331–333.
5. Grennert L, Persson P-H, Grennser G, Kullander S, Thorell J (1977): Ultrasound and human-placental-lactogen screening for early detection of twin pregnancies. Lancet 1:4–6.

6. Grudzinskas JG, Gordon YB, Jeffrey D, Chard T (1977): Specific and sensitive determination of pregnancy-specific β_1-glycoprotein by radioimmunoassay. A new pregnancy test. Lancet 1:333–335.
7. Horne CHW (1978): Unpublished data.
8. Horne CHW, Towler CM, Pugh-Humphreys RGP, Thomson AW, Bohn H (1976): Pregnancy-specific β_1-glycoprotein – A product of the syncitiotrophoblast. Experientia 32:1197–1199.
9. Jandial V, Towler CM, Horne CHW, Abramovich DR (1978): Plasma pregnancy-specific β_1-glycoprotein in complications of early pregnancy. Br J Obstet Gynaecol 85:832–836.
10. Jovanovic L, Landesman R, Saxenna BB (1977): Screening for twin pregnancy. Science 198:738.
11. Laurell CB (1966): Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15:45–52.
12. Lin T-M, Halbert SP (1976): Placental localisation of human pregnancy-associated plasma proteins. Science 193:1249–1252.
13. Mancini G, Carbonara AO, Heremans JF (1965): Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235–254.
14. Obiekwe BC, Grudzinskas JG, Gordon YB, Bohn H, Chard T (1979): Circulating levels of placental protein 5 (PP5) in the third trimester of pregnancy. In Lehmann F-G (ed): "Carcino-Embryonic Proteins," Vol II. Amsterdam: Elsevier, pp 629–632.
15. Sedlacek HH, Rehkopf R, Bohn H (1976): Immunofluorescence histological localisation of human pregnancy and placenta proteins in the placenta of man and monkeys. Behring Inst Mitt 59:81–91.
16. Tatarinov YS, Falaleeva DM, Kalashnikov VV, Toloknov BO (1976): Immunofluorescent localisation of human pregnancy-specific β_1-globulin in placenta and chorioepithelioma. Nature 260:263.
17. Tatra G, Placheta P, Breitenecker G (1975): Pregnancy-specific β_1-glycoprotein (SP1). Clinical aspects. Wiener Klin Wochenschr 87:279–281.
18. Teisner B, Westergaard JG, Folkersen J, Husby S, Svehag SE (1978): Two pregnancy-associated serum proteins with pregnancy-specific-glycoprotein determinants. Am J Obstet Gynecol 131: 262–266.
19. Thomson AM, Billewicz WZ, Hytten FE (1968): The assessment of fetal growth. J Obstet Gynaecol Br Commonw 75:903–916.
20. Towler CM, Horne CHW, Jandial V, Campbell DM, Macgillivray I (1976): Plasma levels of pregnancy-specific β_1-glycoprotein in normal pregnancy. Br J Obstet Gynaecol 83:775–779.
21. Towler CM, Horne CHW, Jandial V, Campbell DM, Macgillivray I (1977): Plasma levels of pregnancy-specific β_1-glycoprotein in complicated pregnancies. Br J Obstet Gynaecol 84:258–263.
22. Towler CM, Glover RG, Horne CHW (1978): Problems encountered in the measurement of pregnancy-specific β_1-glycoprotein. Clin Chim Acta 87:289–296.