C₃ and C₄ complement components and acute phase proteins in late pregnancy and parturition

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SUMMARY To determine whether changes in complement or acute phase proteins can predict onset of labour, complement components C₃ and C₄, α -1-antitrypsin, and α -1-acid glycoprotein were measured serially in the third trimester of pregnancy and throughout labour in 11 women. C-reactive protein (CRP) concentration, C₃ conversion products, and factor B activation was studied in five pregnancies one week before delivery, during labour, and six weeks after delivery. C₃, C₄, and CRP concentrations were unchanged and there was no evidence of complement conversion, either immediately before or during labour, by the classical or alternative pathways. A wide variation among subjects but a small variation within subjects for all proteins assayed was noted.

It is important to use serial rather than cross sectional biochemical data when determining normal physiological patterns in pregnancy. The proteins measured have no predictive value in the timing of the onset of normal labour.

Preterm labour remains an important cause of fetal morbidity and mortality. There is presently no practical predictor of its occurrence to aid the clinician. The concentration of many serum proteins changes with increasing gestation to term.¹ C-reactive protein (CRP) increases in the third trimester of pregnancy and labour.² The data on complement activity in pregnancy are contradictory. Total haemolytic complement activity (CH₅₀) and C₃ have been reported both to be increased in the third trimester³ and to be normal⁴ while fetal concentrations increased towards term.⁵

Material and methods

Eleven women were studied at two weekly intervals from about 28 weeks' gestation to delivery, and five women six weeks after having given birth. Gestation was timed by date of the last menstrual period and by routine early ultrasound scan. The onset of labour was spontaneous in all cases and the pregnancies uncomplicated. The clinical factors which may influence protein concentrations are shown in table 1.

Blood was collected into edetic acid at routine antenatal visits and in labour on presentation to the labour ward, at 4 cm cervical dilatation and

immediately after delivery. Plasma was separated within two hours of collection and assayed within 24 hours. Serum samples were stored at -20°C until assay.

Complement components C₃ and C₄ were assayed in fresh edetic acid plasma by radial immunodiffusion⁶ with 0.1 M edetic acid added to the agarose plates. Alpha-1-antitrypsin and α -1-acid glycoprotein were measured in serum by rocket immunoelectrophoresis.⁷ All antisera were obtained from Atlantic Antibodies (Maine, USA). An "in-house" standard with assigned g/l values was used for C₃ and C₄; the standard for α -1-antitrypsin was calibrated against purified protein

Table 1 Clinical factors likely to affect acute phase proteins (mean (range))

Maternal age (years)	29 (23-41)
Parity	0.82 (0-3)
Infection (prenatal/intrapartum)	0/11 patients
Allergy	0/11 patients
Drugs in pregnancy	Iron supplements 11/11
Gestation at delivery (wks)	38 (33-42)
Birthweight (g)	3226 (2560-4175)
Anaesthetic	7 epidural/4 nil
Vaginal examinations in labour	4 (2-7)
Delivery type	8 spontaneous/3 forceps
Intrauterine monitoring	10/11
Fetal scalp electrode	10/11
Pressure catheter	9/11

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Fig 1 Third trimester protein profiles in two typical patients. C₃, ▲—▲, C₄ ○—○, α₁-antitrypsin □—□, and α₁-acid glycoprotein ■—■. Delivery is indicated by arrow.

kindly provided by Dr C B Laurell. A standardised serum (Behringwerke, Marburg, West Germany) was used as the standard for α₁-acid glycoprotein.

C₃ and factor B conversion products were investigated in fresh edetic acid plasma by immunofixation.⁸ C-reactive protein was measured in serum using rate nephelometry (ICS, Beckman Instruments Inc, California, USA). The interbatch coefficient of variations for the quantitative measurements were 6.5% for C₃, 8.4% for C₄, 4% for α₁-antitrypsin, 5.3% for α₁-acid glycoprotein, and 3.3% for CRP, respectively. The samples before and after delivery were compared by the Wilcoxon matched pairs signed rank test.

The study was approved by the hospital ethics committee.

Table 2 C₃, α₁-antitrypsin, and α₁-acid glycoprotein in third trimester of pregnancy, labour, and after delivery (mean (range))

Gestation (wks)	C ₃ (g/l)	C ₄ (g/l)	α ₁ -Antitrypsin (g/l)	α ₁ -Acid glycoprotein (g/l)
26–28	1.61 (1.10–2.38)	0.34 (0.15–0.69)	2.06 (1.53–2.43)	
28–30	1.60 (1.02–2.03)	0.31 (0.17–0.71)	2.59 (1.76–3.31)	
30–32	1.66 (1.02–2.10)	0.31 (0.18–0.76)	2.75 (1.83–3.31)	
32–34	1.54 (1.00–1.95)	0.32 (0.16–0.76)	3.12 (2.49–3.86)	
34–36	1.49 (1.20–2.03)	0.27 (0.12–0.69)	2.91 (2.49–3.59)	
36–38	1.45 (1.05–1.87)	0.29 (0.12–0.71)	3.27 (2.65–3.53)	0.50 (0.31–0.77)
38–40	1.38 (1.07–1.60)	0.35 (0.21–0.50)	2.99 (2.43–3.31)	
40+	1.42 (1.07–1.75)	0.27 (0.20–0.39)	3.00 (2.31–3.32)	0.44 (0.33–0.61)
Labour				
Presentation	1.56 (1.15–1.91)	0.30 (0.21–0.44)	2.91 (2.65–3.31)	0.52 (0.46–0.65)
4 cm dilatation	1.55 (1.10–1.95)	0.30 (0.10–0.76)	3.01 (2.76–3.31)	0.57 (0.38–0.92)
Delivery	1.52 (1.10–1.91)	0.33 (0.09–0.76)	3.03 (2.49–3.32)	0.58 (0.38–0.91)
6 wks after delivery	1.52 (0.90–1.86)	0.37 (0.13–0.72)	1.39 (1.16–1.69)	0.77 (0.46–1.02)

Fig 2 CRP concentration one week before delivery, during labour (A, at presentation; B, at 4 cm dilatation; C, at delivery), and six weeks after delivery.

Results

The third trimester concentrations of α₁-antitrypsin, α₁-acid glycoprotein, and complement components C₃ and C₄ in all patients showed little variation within a subject but considerable variation among patients. The mean data are shown in table 2. Two typical profiles are shown in fig 1.

There was no significant difference between the concentrations of C₃ and C₄, before and after delivery, while there was a significant ($p < 0.05$) increase in α₁-acid glycoprotein and a significant decrease ($p < 0.05$) in α₁-antitrypsin after birth. There were no conversion products of C₃ and factor B either in the initial sample (at about 28 weeks' gestation) or during labour. There was no significant difference in CRP

between values before and after delivery. The concentrations remained within the normal range one week before delivery, during labour, and six weeks after delivery (fig 2).

Discussion

Complement and other acute phase proteins were not shown to predict the onset of labour. We measured the complement components C_3 and C_4 as indicators of total complement concentrations; C_3 and factor B conversion products were estimated to exclude the possibility of complement activation during pregnancy and labour in the presence of normal concentrations of these complement components. Conversion products were not found, and C_3 and C_4 remained stable through the last trimester and labour.

Alpha-1-antitrypsin and α -1-acid glycoprotein were selected as reference markers of protein changes as their pattern is well documented in pregnancy,⁹ and again no changes were found through labour. Surprisingly, there was no change in CRP. The finding of a normal concentration in uncomplicated pregnancy suggests that baseline values in pregnancy may be similar to those of non-pregnant women. In a previous study¹⁰ CRP was found to be increased in only 22% of pregnancies, and in only some could this be explained by infection, suggesting that CRP has little value as a predictor of clinically important disease in pregnancy.

Our study interestingly shows a wide range of individual protein values but remarkable stability within a subject. This suggests that a woman's own baseline value may be preferable to assess change rather than using a broad reference range (< 10 mg/l C-reactive protein).¹¹ The use of reference ranges within subjects would permit a more sensitive estimation of change in disease states. A quantitative estimation of CRP and the detection of products of complement activation may, however, be the exception; the role of measurement of these plasma acute phase proteins in the management of complicated pregnancy is, however, uncertain. C-reactive protein and reduced serum complement activity may provide a useful marker for infection where there is prolonged rupture of membranes, suggesting that delivery or antibiotics would be advisable.

In pre-eclampsia, zymosan induces active complement components (C_{56}),¹² and low C_4 and C_1 esterase inhibitor values have been reported¹³; the

importance of these changes, however, is unknown.

The proteins we studied would seem to be of little predictive value in the timing of onset of normal labour, but investigation in pregnancies with known pathology would seem appropriate. The study underlines the importance of serial data to determine normal physiological patterns in pregnancy.

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References

- 1 Von Studnitz W. Studies on serum proteins in pregnancy. *Scand J Clin Lab Invest* 1955;7:324-8.
- 2 Nesbitt REL, Hays RC, Mauro J. Behaviour of C-reactive protein in pregnant and puerperal women, fetal blood and in newborn infants under normal and abnormal conditions. *Obstet Gynaecol* 1960;16:659-66.
- 3 Baines MG, Millar KG, Millis P. Studies of complement levels in normal human pregnancy. *Obstet Gynaecol* 1974;43:806-10.
- 4 Soliman MDE, Fadel HE, El-Mehairy MM. Serum complement activity during normal pregnancy. *Int J Gynecol Obstet* 1971;9:181-4.
- 5 Kovar I, Ajina NS, Hurley R. Serum complement and gestational age. *J Obstet Gynaecol* 1983;3:182-6.
- 6 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J Immunochem* 1965;2:235-54.
- 7 Laurell CB. Quantitative estimate of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966;15:45-52.
- 8 Whicher J, Higginson J, Riches PG, Radford S. Clinical applications of immunofixation: detection and quantitation of complement activation. *J Clin Pathol* 1980;33:781-5.
- 9 Laurell CB. Orosomucoid and alpha-1-antitrypsin in maternal and fetal sera at parturition. *Scand J Clin Lab Invest* 1968;21:136-8.
- 10 Fuchs F, Cederqvist L, Land Spiegel HE. Maternal plasma proteins. In: Klopfer A, Genazzani A, Crosignani P, eds. *The human placenta, proteins and hormones*. London: Academic Press, 1980:389-99.
- 11 Statland BE, Winkel P, Killingworth LM. Factors contributing to intra-individual variation of serum constituents. *Clin Chem* 1976;22:1635-8.
- 12 Stark JM, Johansen KA, Rees JA, Williams JH. Acute-phase sera in pre-eclampsia and latent hypertension of pregnancy. *Lancet* 1977;ii:299.
- 13 Hsieh C, Cauchi MN. Platelet and complement changes in pre-eclampsia. *J Obstet Gynaecol* 1983;3:165-9.

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