Classical complement activation induced by pregnancy: Implications for management of connective tissue diseases

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Abstract

Aims: To determine the effect of pregnancy on C4d concentrations and to assess whether C4d remains a useful disease activity marker in the management of connective tissue diseases during pregnancy.

Methods: Plasma C3, C4, and C4d concentrations were measured in 83 women at various stages of normal pregnancy and compared with those in 80 non-pregnant controls.

Results: C3 concentrations in the pregnant women were significantly raised (p = 0.0001) and the C4 concentrations were reduced (p = 0.0007), and accompanied by a significant increase in C4d (p = 0.0001). The C4d:C4 ratio was higher in the pregnant women (p = 0.0001).

Conclusions: Pregnancy induces activation of the classical complement pathway. C4d concentrations cannot be used to monitor disease activity in patients with connective tissue diseases during pregnancy.

Activation of the complement system is considered to be central to the inflammatory mechanisms in systemic lupus erythematosus (SLE). The measurement of complement breakdown products gives a quantitative assessment of such activation and has become established in some centres as a marker of disease activity. C3dg is a product of C3 activation by both the classical and the alternative pathway; C4d is a product of C4 activation by the classical pathway alone. Both have been shown to correlate with disease activity in SLE.

The management of pregnant lupus patients entails careful monitoring. This can be even more problematical because certain laboratory variables are affected by normal pregnancy. A sustained increase in the erythrocyte sedimentation rate occurs during pregnancy, and increased concentrations of C3 and C4 have been shown, although this was significant only for C3 in serum samples assayed by rocket immunoelectrophoresis. Plasma C3 degradation products are not affected during the course of normal pregnancy and hence this variable remains a useful activity marker in pregnant patients with SLE.

The effect of pregnancy on C4d concentrations is unknown and therefore concentrations were measured in a group of pregnant women and normal controls to evaluate possible physiological changes.

Methods

Eighty-three women (age range 16–42 years) at various stages of pregnancy were randomly selected to be studied. Samples were collected during the second and third trimesters and compared with those from 80 non-pregnant controls. EDTA plasma was separated within two hours of venepuncture and stored at −18°C in a liquid nitrogen. A control group comprised 80 subjects (47 men and 33 non-pregnant women, aged 16–84 years) attending for routine venepuncture but who were otherwise well. The study was approved by the hospital ethical committee.

C3 and C4 concentrations were measured using a turbidometric assay on a centrifugal fast analyser. C4d concentration was quantified using rocket immunoelectrophoresis, based on the method of Nitsche et al, using an anti-human C4 whole (Behring) where the faster rocket is C4d. After drying and staining, the rocket heights were measured and C4d values calculated from a standard curve. The C4d concentration of the standard (normal serum plus heat aggregated IgG, incubated at 37°C for one hour) was assigned an arbitrary value of 100 Units/ml. C4d:C4 ratios were calculated for both groups to give indices of complement activation independence of the initial concentration of C4.

Results

C3 concentrations were significantly higher in the pregnant women compared with controls (1.10 (0.16) g/l vs 0.91 (0.15) g/l; p = 0.0001); C4 concentrations were significantly lower in the pregnant women (0.24 (0.08) g/l vs 0.28 (0.08) g/l; p = 0.007). Both C3 and C4 concentrations, however, remained within the normal range for the laboratory.

The distribution of C4d values in the two groups is shown in figure 1. The mean (SD) value in the normal group was 12.4 (4.3) Units/ml giving a reference range of 4–21
the pregnant women were compared with a control group of men and women of a wider range, no effect of either gender or age on C4d concentrations was found in our laboratory (data not shown). Our findings are no different if women of childbearing age only are compared with the pregnant women. The rise in plasma C3 concentrations in normal pregnancy concurs with previous findings.45

Complement components have been identified in both mature and immature human placental tissue in association with stromal cells in areas of fibrinoid necrosis within the trophoblastic mantle and in the walls of fetal blood vessels.44,49 Thomson et al.,10 however failed to show clinically important complement activation in plasma collected from normal pregnancies as judged by normal concentrations of Clq, C4, C3 and factor B, and both C3 and factor B conversion products. Likewise, Jenkins et al.46 failed to show changes in the C3 conversion product, C3dg, in normal pregnancies widely distributed in the second and third trimesters. Although trophoblastic basement membrane-bound C3d, accompanied by C9 but not by Clq or C4, might suggest alternative pathway activation, C4 has been noted in areas of intervillous fibrin deposition.11 This deposition of C4 suggests classical pathway activation and our findings of increased plasma C4d concentrations would further support classical pathway activation in addition to alternative pathway activation.

From a clinical standpoint, plasma C4d concentrations cannot be used as a marker of disease activity during pregnancy in patients with connective tissue diseases as pregnancy itself leads to C4 activation, and measurements of C4 must therefore be treated cautiously.

We thank Lupus UK for financial support.

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doi: 10.1136/jcp.45.1.66

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