EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Changes in the Microheterogeneity of Serum α_1-Acid Glycoprotein in Pregnancy

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Immunodiffusion studies show that serum concentration of α_1-acid glycoprotein remains virtually the same during all trimesters of pregnancy. A positive correlation (r=0.87) between the term of pregnancy and the concentration of the α_1-acid glycoprotein fraction with bi-, tri- and tetra-antenna side chains is established using crossed affine immunoelectrophoresis in the presence of concanavalin A.

Key Words: *glycoproteins; α_1-acid glycoprotein; glycosylation; concanavalin A; pregnancy*

Variations of the degree of glycosylation are a manifestation of microheterogeneity of glycoproteins. Changes in the composition and structure of oligosaccharide chains result in the formation of glycoproteins (GP) with bi-, tri-, or tetra-antenna organization. So far, it remains unclear whether the microheterogeneity of GP is associated with various pathological and physiological states and what is the functional significance of such an association [10]. In this study we attempted to find out whether the microheterogeneity of human blood GP is related to the term of pregnancy.

All serum proteins, except albumin, are glycoproteins. α_1-Acid GP (α_1-aGP) has the highest (up to 41% of total weight) carbohydrate content [1]. This GP has five bi-, tri- and tetra-antenna oligosaccharide chains [5], is a positive marker of the acute phase, and exhibits immunosuppressive activity [2,6]. Its functions are unclear. The data on the blood concentrations of α_1-aGP during pregnancy are contradictory [11-13]. The microheterogeneity of this GP was studied only at early [13] and late [11,13] periods of pregnancy.

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In this study we measured the total blood content of α_1-aGP and assessed changes in its microheterogeneity during pregnancy. Crossed affine immunoelectrophoresis (CAIEP) in the presence of concanavalin A (ConA) was used [3]. This method is based on different electrophoretic mobility of GP that depends on different affinity of their carbohydrate chains for the lectin. The prerequisite for the binding of α_1-aGP to ConA is the presence of unsubstituted hydroxyl groups at the third, fourth, and sixth carbon atoms in mannose residues, which has been observed in N-GP with biantenna oligosaccharide chains. Glycoproteins with tri- and tetra-antenna chains display a lower affinity for ConA [8].

MATERIALS AND METHODS

Fifty-three pregnant women aged 17-40 years (mean age 28.6±0.4 years) were enrolled in the study. They were divided into three groups according to the trimester of pregnancy: group 1 (weeks 1-14), group 2 (weeks 15-28), and group 3 (weeks 29-40). Control group consisted of 11 healthy women aged 18-42 years (mean age 26±2.4 years). Blood was collected from the ulnar vein. Serum was frozen and stored at -18°C.

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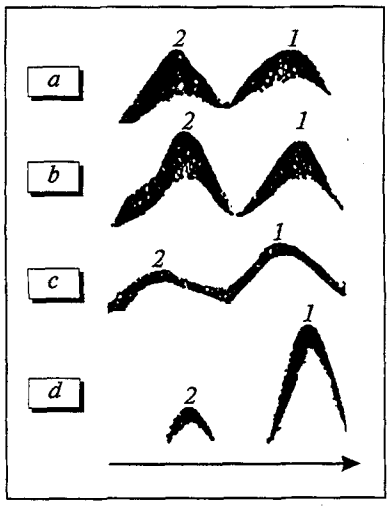

Fig. 1. Immunoaffine electrophoregrams of serum α_1-aGP at I, II, and III trimester of pregnancy. Crossed affine immunoelectrophoresis in the presence of ConA. Control (*a*), first (*b*), second (*c*), and third (*d*) trimesters. *1*) peak corresponds to a_1-aGP fractions unreactive with ConA; *2*) peak correspond to α_1-aGP fraction reactive with ConA. The arrow shows the direction of current in the first-dimension gel.

Crossed affine immunoelectrophoresis was carried out in as described previously [4] with some modifications. Agarose-M gel (1%, 1.5-mm thick, LKB) was used. Electrophoresis buffer (pH 8.6) contained 100 mM medinal, 11 mM veronal, and 3 mM sodium azide (Serva). Concanavalin A (Serva) was dissolved in this buffer and added to the first-dimension gel to a final concentration of 1.5 mg/ml (163 ml/cm^2). Rabbit antiserum to human α_1-aGP (Mikroflora, G. N. Gabrichevskii Institute of Epidemiology and Microbiology) was added to the second-dimension gel to a final concentration of 21.3 μl/cm^2. The volume of serum samples (5 μg α_1-aGP) varied from 3 to 10 μl depending on the individual blood content of α_1-aGP. Electrophoresis in the first dimension was carried out for 1.5-2 h (21 V/cm) and for 18-20 h (6 V/cm) in the second dimension.

The gels were stained and dried, and Xerox copies of the electrophoregrams were made. The areas under each peak were cut and weighed. The contents of α_1-aGP fractions with different affinity for ConA were expressed as a percentage of the total area of the electrophoregram.

Serum concentration of α_1-aGP was measured by radial immunodiffusion [9] using standard human serum with known concentration of α_1-aGP (Mikroflora).

Results were analyzed using the Student—Fisher *t* test.

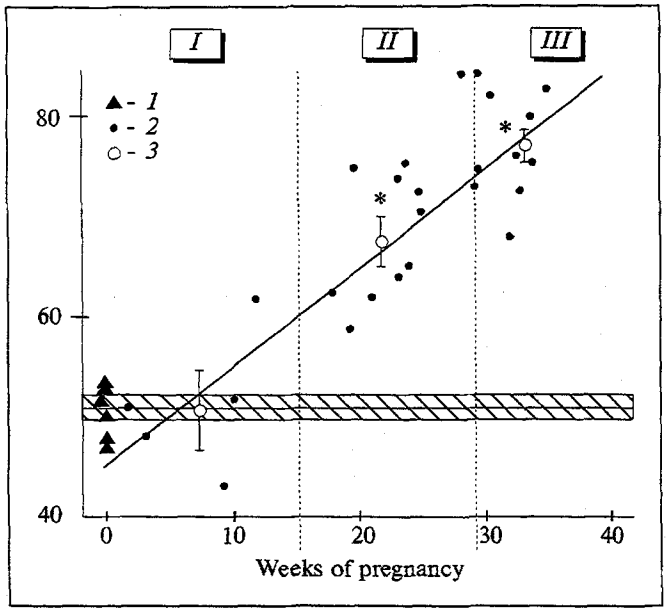

Fig. 2. Relative blood content of the α_1-aGP unreactive with ConA in the blood at different trimesters of pregnancy. Ordinate: content of α_1-aGP unreactive with ConA (percent of the total area of protein precipitation). *1*) control; *2*) pregnancy; *3*) mean values for the I, II, and III trimesters. The *M*±*m* zone for the control is shaded. *Significantly different from the control (p<0.001).

RESULTS

The mean content of α_1-aGP in the control sera was 796±63 mg/liter, which agrees with the literature data [1,6]. Blood content of α_1-aGP remained virtually the same during pregnancy. This finding argues with the reported decrease in the α_1-aGP content at the third trimester [12].

Figure 1 shows a typical electrophoregram obtained by CAIEP with ConA. Control sera are characterized by two peaks of α_1-aGP (Fig. 1, *a*). Peak *1* corresponds to the α_1-aGP fraction unreactive with ConA and peak *2* to the fraction reactive with this lectin [5]. It was reported that α_1-aGP produces 3-4 peaks in CAIEP with ConA. The sensitivity of CAIEP can be increased by adding a specific monosaccharide, for example methyl-α-D-glucopyranoside, to the second-dimension gel [11]. This monosaccharide competes with GP for the binding sites on ConA molecules, thus preventing the formation of complexes between ConA and the high affinity α_1-aGP fractions. These complexes are poorly soluble and have an extremely low electrophoretic mobility. However, the contribution of these fractions to the microheterogeneity of blood α_1-aGP is very small in nonpregnant women. This holds true for α_1-aGP during pregnancy [11,13]. Therefore, we did not add any specific monosaccharide to the second-direction gel and did not measure minor fractions of α_1-aGP.

As seen in Fig. 1, *b-d*, the height and shape, but not the number of α_1-aGP peaks, vary as a function of the trimester of pregnancy. The ratio between the fractions reactive and unreactive with ConA changes considerably during pregnancy (Table 1). At the first trimester, the contents of these fractions were similar to those in the control. At the beginning of the second trimester, the content of

the reactive fraction (peak *2*) decreased to 66% of the control ($p<0.01$), while that of the unreactive fractions increases by 33% ($p<0.001$) compared with the control. At the third trimester, the differences in the contents of these fractions were more pronounced (Fig. 1, *d*, Table 1).

Figure 2 shows the relationship between the term of pregnancy and the relative content of α_1-aGP fraction unreactive with ConA. A strong correlation between these parameters ($r=0.87$, $n=25$) points a cause-and-effect relationship between the period of pregnancy and changes in the oligosaccharide chains of this GP.

The carbohydrate moiety of α_1-aGP unreactive with ConA (peak *1*) is represented only by tri- and tetra-antenna glycans [5]. Therefore, an increase in the content of this α_1-aGP indicates that the structure of circulating α_1-aGP becomes more complex. Similar changes in oligosaccharide chains occur in other serum GP during pregnancy and estrogen therapy [7,11] irrespective of variations of the blood concentrations of these GP and their positive or negative reactivity in the acute phase. Estrogen levels are increased in pregnancy, which may affect glycosylation of serum proteins. Although no direct relationship between blood estrogen content and microheterogeneity of α_1-aGP has been detected [13], it can be hypothesized that female sex hormones act on glycosylation indirectly.

The physiologic significance of the increase in the content of serum GP with a more complex structure during pregnancy is unclear. Two possibilities can be suggested. First, this increase is associated with transferrin. Changes in the transferrin glycans during pregnancy correlate with increased transport of iron through the placenta [7] that provides sufficient resources of iron in the fetus and prevents anemia in infants during the first year of life. Second, since there is a relationship between the structure of the carbohydrate chains of α_1-aGP and its immunosuppressive activity [2,6], one can expect that structural modifications of α_1-aGP may contribute to the modulations of the immune response in pregnancy.

TABLE 1. Shares of α_1-aGP Fractions with Different Affinity for ConA as a Function of the Trimester of Pregnancy (Percent of the Total Area of Protein Precipitation on Immunoelectrophoregrams, $M\pm m$)

Group	Number of samples	Peak *2*	Peak *1*
Control	6	49.60±1.0	50.4±1.0
First trimester	7	47.80±4.9	52.2±4.8
Second trimester	10	32.80±2.3*	67.1±1.7**
Third trimester	10	23.65±1.5**	75.4±1.5**

Note. *$p<0.01$, **$p<0.001$ *vs.* the control.

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