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ASSESSMENT OF SERUM LEVELS OF α-1-MICROGLOBULIN, β-2-MICROGLOBULIN, AND RETINOL BINDING PROTEIN IN THE FETAL BLOOD. A METHOD FOR PRENATAL EVALUATION OF RENAL FUNCTION

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SUMMARY

The concentrations of α-1-microglobulin, β-2-microglobulin, and retinol binding protein were determined in fetal blood sampled by cordocentesis. The blood values of 126 fetuses without ultrasonographic findings of urinary tract abnormalities as controls were found to be independent of the week of gestation. In nine fetuses affected by a severe bilateral renal dysplasia or agenesis, elevated values of α-1-microglobulin but normal values of retinol binding protein were obtained. The authors recommend the determination of α-1-microglobulin and, with some restriction, also of β-2-microglobulin in prenatal renal function diagnosis.

KEY WORDS: microproteins; fetus; renal function

INTRODUCTION

Advances in the field of sonography have improved the diagnosis of fetal malformations as well as the sampling of fetal materials for various other examinations. Blood sampling by cordocentesis under sonographic control, for instance, has become a technique with an overall risk of about 1 per cent in our department. Biochemical measurements are now available for relating morphological findings to functional abnormalities.

About 50 per cent of all sonographic abnormalities in the fetus are malformations of the kidneys and urinary tract (Lettgen *et al.*, 1993).

The embryonal or fetal kidneys respectively develop stepwise from the pronephros to the mesonephros and the metanephros. The metanephros as the final kidney begins to excrete substances into the amniotic fluid at the 13th to 15th week of gestation (Lettgen *et al.*, 1993). At the 20th week of gestation, most of the amniotic fluid is produced by the fetal kidneys (Shackelford *et al.*, 1992). The volume of the urinary bladder at the 16th week and the volume of amniotic fluid at the 20th week of gestation can therefore be used for an approximate evaluation of renal function.

By the 17th week, the fetal kidneys and urinary tract can be checked for morphological alterations by sonography. According to different authors (Grupe, 1987; Clarke *et al.*, 1989; Shackelford *et al.*, 1992), the sonographic findings are not sufficient for functional evaluation of the kidneys. At first, amniotic fluid was taken into consideration as material for biochemical examinations.

Crombleholme *et al.* (1990) recommended the quantification of sodium, chloride, and osmolarity in the fetal urine as prognostic criteria initially

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proposed for predicting outcome and selecting the management of fetuses with obstructive uropathy. Nicolini *et al.* (1992) did not completely accept the optimistic view of other authors that sampling of fetal urine and measurement of biochemical parameters are useful for the evaluation of renal failure.

With improvements in the technique of cordocentesis and the resultant reduction in risks associated with this procedure, fetal blood has come to be used for this purpose as well. Creatinine, urea, electrolytes, and other biochemical parameters are suitable for postnatal but not for prenatal evaluation, because all these substances pass through the placenta (Burghard *et al.*, 1987, 1988; Elder *et al.*, 1990; Nolte *et al.*, 1991). Only some microproteins in the fetal plasma with a molecular weight below 40 000 D permit passage across the glomeruli but not across the placenta and therefore may be useful for diagnosis (Greiling and Gressner, 1989). These include β-2-glycoproteins I (40 000) and III (35 000), β-2-anti-collagenase (30 000), α-1-microglobulin (30 000), β-2-microglobulin (11 800), and retinol binding protein (21 000). The last three proteins have been used for postnatal diagnosis (Wibell *et al.*, 1973; Shea *et al.*, 1981; Engle and Arant, 1983; Itoh *et al.*, 1983). Serum α-1-microglobulin and β-2-microglobulin have already been proposed as prenatal parameters (Nolte *et al.*, 1991), but Nolte *et al.* only obtained cord vein blood from preterm neonates with and without renal failure for their examinations.

PATIENTS AND METHODS

Cordocentesis for sampling fetal blood was performed for different indications: malformations of the urogenital system (17 per cent), abnormal pregnancy-specific proteins in maternal serum (16 per cent), age of the pregnant woman (15 per cent), suspicion of fetal infections (12 per cent), malformations of the head and central nervous system (10 per cent), malformations of the heart and great vessels (8 per cent), placental alterations (6 per cent), malformations of the digestive organs and lungs (4 per cent), unclear chromosomal aberrations in the family (3 per cent), Rh incompatibility (3 per cent), intrauterine growth retardation (2 per cent), other reasons (3 per cent).

Only serum samples remaining after the primary investigation were used for this study, with the consent of the mothers. Blood samples of 156 fetuses in the 17th–37th week of gestation were examined. They were divided into three groups.

Group A

One hundred and twenty-six fetuses did not show any sonographic abnormalities of the urinary tract. No renal abnormalities were present postnatally among these probands available to follow-up. Three cases of the 126 showed intrauterine growth retardation (IUGR) and very low values of microproteins.

Group B

In ten fetuses we found severe kidney damage. The pregnancies had been terminated because of the expected extensive renal dysfunction or for other reasons. These ten cases are listed below (corresponding to the letters in Figs 1–3):

(a) right: aplasia; left: multicystic (Potter type IIa, Zerres *et al.*, 1984);
(b) right: multicystic, enlarged (Potter type IIa); left: small kidney with two cortical cysts (Potter type IIb);
(c) bilateral: dystopic hypoplasia (right: Potter type IIb; left: Potter type IV);
(d) bilateral: multicystic, enlarged (Potter type IIa);
(e) autosomal recessive nephropathy (Potter type I); both kidneys enlarged with numerous small cortical and medullar cysts;
(f) bilateral: multiple small cysts (Potter type IV);
(g) bilateral: multicystic, enlarged (Potter type IIa);
(h) right: aplasia; left: multicystic, hypoplastic (Potter type IIb);
(i) right: normal; left: dysplasia with tubular cystic dilation and enlargement of connective tissue, only very small residues of normal parenchyma;
(k) right: aplasia; left: multicystic (Potter type IIa).

Group C

In 20 fetuses with sonographic evidence of urinary tract malformations, renal dysfunction was not confirmed. Some of these fetuses were still in gestation. No evidence for renal dysfunction was found postnatally in some other cases of this

Table I—α-1-Microglobulin, β-2-microglobulin, and retinol binding protein (RBP) in normal fetal plasma (mg/l)

	Mean value	SD	Range minimum	Range maximum	*n*	Cut-off value
α-1-Microglobulin	29·6	10·7	5·3	71·3	125	47·2
β-2-Microglobulin	2·93	0·87	0·64	7·94	123	4·13
RBP	17	11	11	110	106	32

group. In further cases, the pregnancy termination took place in another hospital. All of them were therefore excluded from preliminary evaluation.

The nephelometric method of Behringwerke AG, 35041 Marburg, Germany, with original equipment and reagents was used for quantification of α-1-microglobulin and retinol binding protein. Both proteins react with specific rabbit antibodies to form insoluble immune complexes which scatter incident light. The intensity of the scattered light measured in a nephelometer is proportional to the concentration in the sample. The methodological coefficients of variation for these two parameters in various concentrations were 2·2–6·0 per cent in series and 2·5–6·8 per cent from day to day.

The quantification of β-2-microglobulin was performed by a two-site microparticle enzyme immunoassay with reagents and equipment produced by ABBOT GmbH Diagnostica, Max-Planck-Ring 2, 65205 Wiesbaden, Germany. Microparticles coated with one murine monoclonal antibody were used as the solid phase. The second antibody was labelled with alkaline phosphatase reacting with 4-methyl-umbelliferyl phosphate as the substrate. The coefficients of variation were 4·9–6·0 per cent in series and 6·6–7·3 per cent from day to day.

About 0·4 ml of serum was necessary for measurement of the three microproteins.

The following statistical calculations were used in our evaluation:

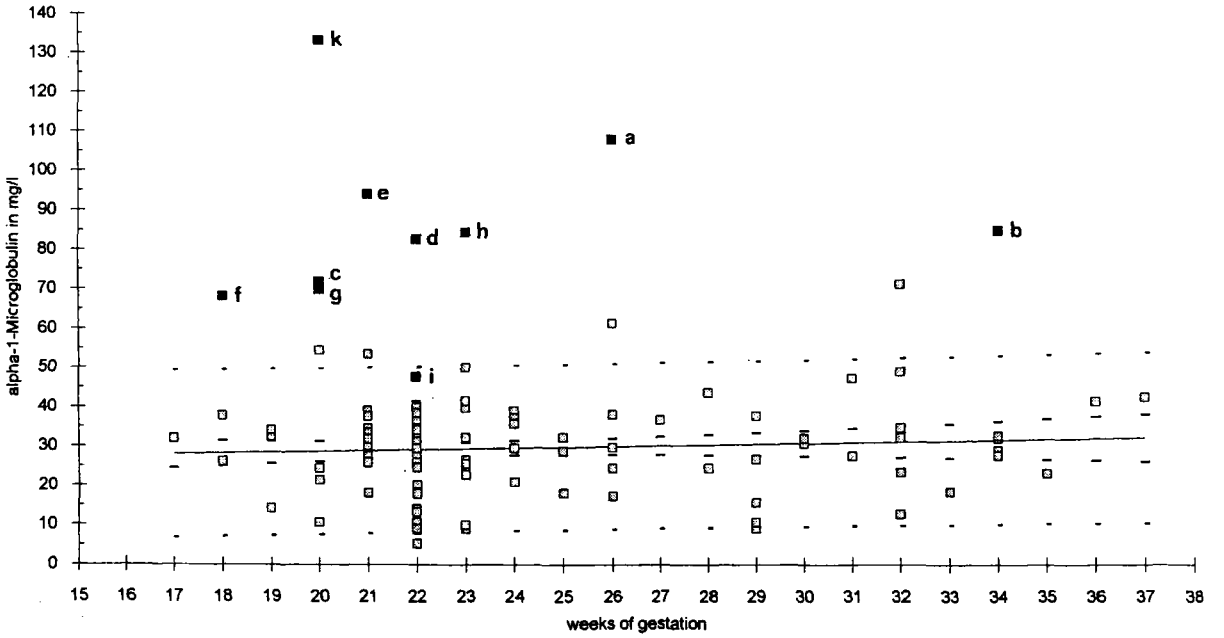

Fig. 1—Values of serum α-1-microglobulin dependent on the age of gestation in fetuses without sonographic abnormalities (group A = □) and fetuses with definite renal failure (group B = ■; the letters correspond to cases (a)–(k) in the text). Mean value ——; interval of confidence corresponding with the 95 percentile: – – –

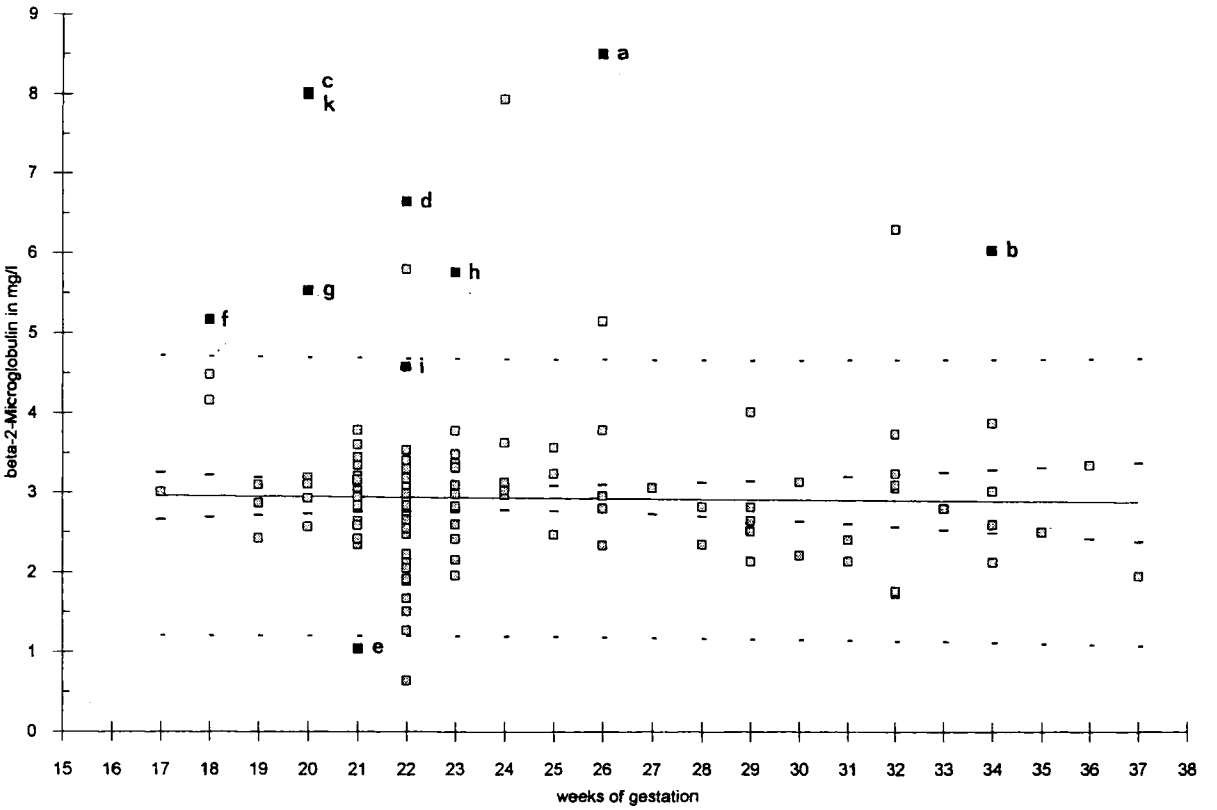

Fig. 2—Values of serum β-2-microglobulin in fetuses dependent on the age of gestation (symbols are the same as those in Fig. 1)

(1) Kolmogoroff–Smirnoff test in combination with the chi-square test to determine the normal distribution [SPSS for Windows Student Version Release 6.0.1 (21 October 1993)];
(2) Spearman's range correlation coefficient (Sachs, 1992);
(3) calculation of regression (Sachs, 1992);
(4) introduction of a cut-off value by box-whisker plots with a standard error of 5 per cent (Tukey, 1977).

RESULTS

The results are summarized in Table I.

Figures 1–3 show the values of the three microproteins in the two groups of fetuses. The normal values (group A) do not statistically correlate in their regression with the developmental age of the fetuses. Only the values of α-1-microglobulin of the controls (group A) were normally distributed. The cut-off value is therefore deduced from the mean value plus 1·64-fold standard deviation (=47·2 mg/l). The box-whisker plot shows all the values of α-1-microglobulin of the affected cases (group B) above the cut-off value.

As to the β-2-microglobulin values without a normal distribution in the controls (group A), the cut-off value is calculated according to the recommendation of Abel (1993) as the 95 percentile (=4·13 mg/l). Eight of ten affected cases (group B) are found above the proposed cut-off value. Two cases are below this limit.

Concerning retinol binding protein, the values of the controls (group A) are near or below the sensitive limit of the method. This observation excludes a normal distribution. The cut-off value is identical with the 95 percentile (=32 mg/l). Differentiation between normal and affected fetuses (groups A and B) is neither subjectively nor statistically apparent.

Our present measurements permit only the use of descriptive statistics and exploratory data analysis, because our study is not a randomized examination. The calculated cut-off value useful as the limit between normal and affected probands

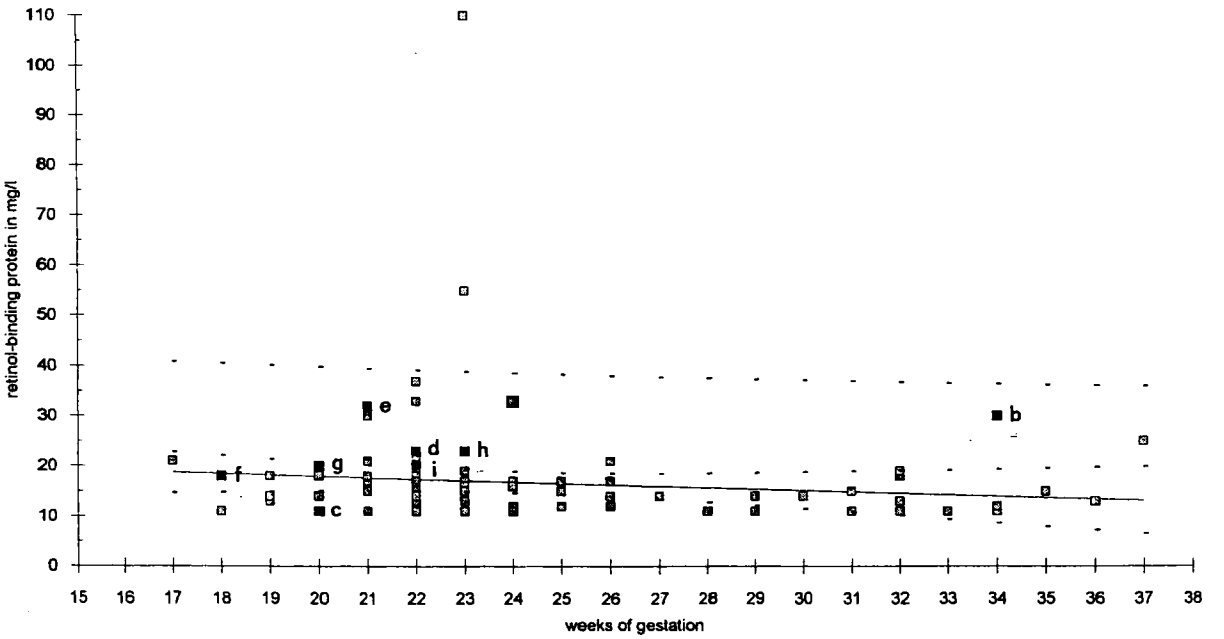

Fig. 3—Values of serum retinol binding protein in fetuses dependent on the age of gestation (symbols are the same as those in Fig. 1)

is adapted only to the group of normal fetuses. The number of fetuses with extensive damage of both kidneys (group B) is not yet sufficient for an equivalent statistical participation. A statement about the specificity and sensibility of the parameters will require more data.

DISCUSSION

The three studied microproteins are found in the fetal plasma at least from the 17th week of gestation in measurable concentrations. In contrast to the results of Nolte *et al.* (1991), we observed no decrease in the microproteins in relation to advancing gestation. Our observation permits the recommendation of a cut-off value independent of the week of gestation. Moreover, our values of α-1-microglobulin and β-2-microglobulin in normal fetuses are similar to the values of newborns published by Nolte *et al.* (1991; Nolte, 1991). The normal retinol binding protein is found in lower concentrations than those published by Sklan *et al.* (1985). But agreement of results cannot preliminarily be expected, because it is not permitted to compare studies using different methods (Itoh and Kawai, 1990).

In our results, α-1-microglobulin and β-2-microglobulin show elevated concentrations in all cases of severe renal dysplasia or agenesis except two. Therefore both parameters may be useful in the prenatal evaluation of renal dysfunction.

Elevated concentrations can obviously be expected in cases with a loss of more than 50 per cent of the renal parenchyma demonstrated by case (i). Further studies of affected cases are necessary, especially for β-2-microglobulin, for final confirmation.

Retinol binding protein is not suitable for the diagnosis of renal damage because there is no difference between controls and affected fetuses in this parameter. All values of the affected cases are below the proposed cut-off value.

The different results concerning the three proteins can be attributed to different metabolic influences. Normally all three proteins are eliminated from plasma by the glomeruli. Afterwards they are taken up by the proximal tubules and then catabolized in the lysosomes. Elimination by diffusion via the placenta is possible for substances with a molecular size below 1000 D. Active transport or carrier systems through the placenta could lead to elimination of the microproteins in spite of fetal renal dysfunction, and thus to a false-negative

result. Such an active transport system was not found for α-1-microglobulin or β-2-microglobulin (Burghard *et al.*, 1987) but could not be excluded for retinol binding protein (Sklan *et al.*, 1985). The fetal plasma level could also be influenced by variations in the rate of synthesis. Tumours can be responsible for false-positive values (Weber *et al.*, 1985; Vincent *et al.*, 1987).

We conclude that the quantification of microproteins should not be the first step in the diagnostic programme in cases where renal or urinary tract defects are suspected by ultrasonography. According to Lettgen *et al.* (1993), the following procedure should be recommended. Karyotyping and exclusion of metabolic defects (for example, Pätau or Zellweger syndrome) are necessary in cases of additional malformations besides those of the urinary tract. Obstructive renal alterations in the fetus must be excluded by volume estimation of the urinary bladder and amniotic fluid. If there is oligohydramnios, the furosemide test (Wladimiroff and Campbell, 1974) as well as imaging of the kidney arteries by colour Doppler sonography should be performed. In cases where the furosemide test does not lead to diuresis, bilateral renal dysfunction should be confirmed by microprotein quantification. α-1-Microglobulin and β-2-microglobulin may be used for this purpose.

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