Fetal Lung Maturity in Complicated Pregnancy, as Predicted from Microviscosity of Amniotic Fluid

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We measured the microviscosity of amniotic fluid between 28 and 40 weeks of gestation in 252 normal pregnancies and in 172 pregnancies complicated by factors known to influence fetal lung maturation, including chronic high blood pressure, pregnancy-induced hypertension, diabetes mellitus, and therapy with betamethasone. Comparison of the microviscosity value distributions and regression analysis indicated significantly lower microviscosity values in hypertensive disorders, in Class D and Classes F or R diabetes, and after 48 h of treatment with betamethasone. Few changes were observed in Classes A, B, or C diabetes. These observations are consistent with the accelerated maturation of surfactant observed in chronic intrauterine stress and the lower incidence of hyaline membrane disease reported after glucocorticoids.

Additional Keyphrases: fetal status · diabetes · therapy with betamethasone · hypertension

Microviscosity (MV) of amniotic fluid as measured by the technique of fluorescence polarization apparently is an excellent predictor of fetal lung maturity (1). In this method, the fluorescent hydrocarbon probe, 1,6-diphenylhexatriene, embedded in the amniotic fluid liposomes is excited by plane-polarized light. The fluorescence polarization response depends on the rotational freedom of the probe in the hydrocarbon region of the phospholipids (2). Both the MV of the lipid dispersions and the fluorescence polarization decrease with gestation, and these changes parallel quantitative and qualitative changes in the composition of surfactant (2–5).

Some complications of pregnancy, such as hypertensive disorders and diabetes mellitus, may induce modifications in fetal lung surfactant, reflected in the amniotic fluid phospholipids (6–9). Glucocorticoids, given for 48 h in premature labor, decrease the incidence of hyaline membrane disease (9). Although the same complications may also influence MV, there are few such data. This study was intended to determine whether complications that accelerate or delay the maturation of surfactant also affect the MV of amniotic fluid.

Materials and Methods

We studied two groups of pregnant women. The first group consisted of 252 women with singleton pregnancies and no complications other than premature labor. None of these women received tocolytics or glucocorticoids. The second group consisted of 172 pregnant women with conditions capable of altering the surfactant maturation, that is: diabetes mellitus (n = 78) grouped according to White’s (10) classification: Class A (n = 16), Class B (n = 23), Class C (n = 18), Class D (n = 11), Class F or R (n = 10); chronic high blood pressure (n = 27); pregnancy-induced hypertension (n = 54); and premature labor between 28 and 34 weeks of gestation, treated with a glucocorticoid (betamethasone) for 48 h (n = 13). All women given glucocorticoids were concurrently treated with beta-agonists (terbutaline or ritodrine). Insulin-dependent gestational diabetics were included in Class B diabetes.

Gestational age was calculated from clinical data such as the dates of the last menstrual period, the first auscultation of unamplified fetal heart sounds, and the first perception of quickening; and uterine size in the first half of gestation, as substantiated by the ultrasound technique between 18 and 26 weeks of gestation and (or) by Dubowitz score (11). In all cases, amniotic fluid was collected either by indicated trans-abdominal amniocentesis or by puncture of the amniotic sac during cesarean section or at the time of amniotomy during labor. Specimens visibly contaminated with meconium or specimens containing erythrocytes (hematocrit >0.05%) were excluded.

After collection, specimens of amniotic fluid were centrifuged at 1500 × g for 10 min at room temperature. The supernate was either analyzed immediately or stored at −20 °C until tested. Duplicate suspensions of a mixture of 0.5-mL portions of the supernatant fluid and 2 mL of a 2 mmol/L solution of 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co., Milwaukee, WI 53223) in tetrahydrofuran were incubated for 30 min at 37 °C. After cooling to room temperature, the samples were placed in the cuvette of a microviscosimeter (FELMA; Elscint Inc., Hackensack, NJ 07602) for measurement of fluorescence polarization.

The standard deviation of the method, as determined by repeated measurements of pooled samples of amniotic fluid, was ±0.003 fluorescence polarization units. Reported values are the mean of two determinations. For this analysis, fluorescence polarization (FP) values (nonlinear scale) were converted into MV (poise) units (linear scale) according to the formula (12): MV = (FP × 2)/(0.46 – FP).

The regression line for data from normal pregnancies, with 68% and 95% confidence intervals, was used as a baseline for statistical comparison with the data from complicated pregnancies. Significance of the slope was evaluated by Student’s t-test. We also calculated regression lines for pregnancies with diabetes and hypertensive disorders, and compared the slopes for each of the complicated conditions with the slope for normal pregnancy, again with Student’s t-test.

To assess goodness of fit of the distributions of values for complicated pregnancies with the value distribution for normal pregnancy, we used the chi-square test. At corresponding gestational ages, we made single-point comparisons (inde-
Fig. 1. Microviscosity as a function of gestational age for amniotic fluid from 81 pregnant women with hypertensive disorders

- Chronic hypertension; O pregnancy-induced hypertension. Regression lines: mean ± 1 and 2 standard errors of MV values from 252 women with normal pregnancies

Results

We determined fluorescence polarization values on all amniotic fluids and converted those values into MV units. The MV value we found to correspond to fetal lung maturity was 4.8 or less (equivalent to a fluorescence polarization value of 0.325 or less) (13).

Normal group: MV values decreased as gestation neared term. Nine (14%) of the 65 MV values for fluids sampled between 30 and 34 weeks were <4.8. The regression equation relating MV (y) to gestational age (x) between 28 and 40 weeks was: y = 19.61 - 0.41x. The correlation coefficient was -0.77. The standard error of the microviscosity estimate was 1.82. The slope proved highly significant (p <0.001). The regression line and its confidence intervals are shown in Figures 1, 2, and 3.

Hypertensive disorders (Figure 1). We evaluated amniotic fluid in 81 women with hypertensive disorders who were between 32 and 40 weeks of gestation. Of these, 27 (35%) had chronic high blood pressure and 54 (67%) had pregnancy-induced hypertension. Thirty-eight (70%) of the 54 women with pregnancy-induced hypertension and 22 (81%) of the 27 women with chronic high blood pressure had MV values below the mean MV values for corresponding gestational age in normal pregnancy. Five women, four with chronic high blood pressure and one with pregnancy-induced hypertension, had MV values below the 95% confidence interval for normal pregnancy condition. The differences from the normal pregnancy distribution proved statistically significant for both pregnancy-induced hypertension ($\chi^2 = 7.40$, $p <0.05$) and chronic high blood pressure ($\chi^2 = 10.70$, $p <0.005$).

The slope of the regression line for the pregnancy-induced hypertension group (Figure 4) was statistically significant, indicating a relationship with gestational age ($t(52) = -5.81$, $p <0.001$). Comparison of the regression lines for normal pregnancy and pregnancy-induced hypertension also indicated a statistically significant difference: $t(52) = 2.87$, $p$

Fig. 2. Microviscosity units as a function of gestational age for amniotic fluid from 78 pregnant diabetics

- Class A; O Class B; ▲ Class C; Δ Class D; ■ Class F or R diabetes. Regression lines as in Fig. 1

Fig. 3. Microviscosity as a function of gestational age for amniotic fluid from 13 women with premature labor being treated with betamethasone for 48 h

Regression lines of MV values from 252 women with normal pregnancies shown for comparison. Regression lines as in Fig. 1

Fig. 4. Comparison of regression lines of microviscosity values as a function of gestational age in amniotic fluid from normal and complicated pregnancies

N, normal pregnancy ($y = 19.60 - 0.41x$; r = -0.77; n = 252); HBP, chronic high blood pressure ($y = 10.54 - 0.19x$; r = -0.38; n = 27); PIH, pregnancy-induced hypertension ($y = 14.10 - 0.27x$; r = -0.62; n = 54); A, Class A diabetes ($y = 15.70 - 0.30x$; r = -0.53; n = 16); B, Class B diabetes ($y = 24.50 - 0.55x$; r = -0.50; n = 23); C, Class C diabetes ($y = 15.94 - 0.32x$; r = -0.81; n = 18); DFR, Class D, F, or R diabetes ($y = 14.31 - 0.29x$; r = -0.40; n = 21)
<0.01. In contrast, the regression analysis of the data from patients with chronic high blood pressure showed no correlation with gestational age. The slope of the regression line (r(25) = −0.20, p > 0.05) was not statistically significant, and the correlation coefficient (r = −0.37) failed to show significance at the 0.05 level (critical values for r for the Pearson’s correlation coefficient).

Diabetes (Figure 2). We obtained amniotic fluid from 78 women with diabetes mellitus, at 31 through 40 weeks of gestation. All MV values fell within the 95% confidence interval for normal pregnancy.

We evaluated the distribution of MV values we obtained for each group of diabetes with the distribution of values for normal pregnancies. For diabetes Class A, nine (56%) of the 16 MV values were above the normal pregnancy regression line; for Class B, 14 (61%) of the 23 MV values were above the line; for Class C, 13 (72%) of the 18 MV values were below the line; for Class D, all 11 (100%) MV values were below the line; for Class F or R, 9 (90%) of the 10 MV values were on or below the line. The MV value distributions of Classes A and B were each insignificantly different from the normal pregnancy distribution (Class A: χ² = 0.25, p > 0.05, Class B: χ² = 1.09, p > 0.05). The MV value distribution of Class C barely proved significant at the 0.05 level (χ² = 4.263, p < 0.05). The MV values of Class F or R diabetes were combined with those of Class D diabetics to form a group suitable for statistical analysis. The combined distribution for Class D and Classes F or R was significantly different from the normal pregnancy distribution (χ² = 13.76, p < 0.01), a difference that was only significant until 36 weeks of gestation (t(100) = 3.96, p < 0.01).

Figure 4 shows the regression lines for each class of diabetes. The slopes of the regression lines do not differ from normal in Class A (t(14) = 0.88, p > 0.05), Class B (t(21) = 0.66, p > 0.05), or Class C diabetes (t(16) = 0.84, p > 0.05). The critical values for the Pearson’s correlation coefficient indicated a significant correlation with gestational age in Class A, B, and C diabetes. No significant correlation with gestational age could be demonstrated in Class D or in F or R diabetes.

Betamethasone treatment (Figure 3). Amniotic fluid was obtained at 30 through 34 weeks of gestation from 13 women after 48 h of betamethasone therapy. Two 12-mg injections of betamethasone were given intramuscularly at 24-h intervals, together with ritodrine or terbutaline. Of these 13 women, seven (54%) vs nine (14%) of 65 women in the normal group between 30 through 34 weeks of gestation had MV values <4.8 (χ² = 25, p < 0.005). Eleven (85%) women had MV values below the regression line for normal pregnancy; of those, two had values below the 95% confidence interval for normal pregnancy.

The distribution of values for pregnancies associated with betamethasone therapy differed significantly from the distribution of values from normal pregnancies (χ² = 6.23, p < 0.05). Regression line comparisons were not performed because of the truncated range of gestational ages for the betamethasone-treated group. From two women, amniotic fluid samples were obtained before and after 48 h of treatment with betamethasone. In one case, the MV value decreased from 5.7 to 2.5; in the second case, the MV value decreased from 5.7 to 4.9.

Discussion

This study produced evidence that hypertensive disorders, diabetes of long-standing or with vascular complications, or glucocorticoids administered with beta-agonists can all affect amniotic fluid MV.

Hypertensive disorders. We observed an accelerated decrease of MV in both pregnancy-induced hypertension and chronic high blood pressure. In some women, most of them with chronic high blood pressure, MV values were well below the 95% confidence interval for normal pregnancy, confirming an observation previously made by Elrad et al. (14).

Regression analysis indicated a greater effect of chronic high blood pressure on fetal lung maturation as compared with pregnancy-induced hypertension, presumably representing the effect of fetal stress of longer duration. For this reason, the significant correlation between MV and gestational age observed in normal pregnancy was decreased in pregnancy-induced hypertension and could not be demonstrated in women with chronic high blood pressure.

The accelerated decrease of MV in hypertensive disorders is consistent with the alterations of the lung profile described in the literature, that is, higher amounts of disaturated lecithin, early increase in the L/S ratios, and (or) early appearance of phosphatidylglycerol (6, 8, 15–18). The accelerating effect on surfactant maturation is probably mediated through increased cortisol concentrations in response to fetal stress (19).

Diabetes. MV values for all diabetic women were within normal limits for uncomplicated pregnancies. When the diabetic pregnancies were classified according to White, there was no significant difference between values for the normal group and Class A and B diabetes. There was a tendency toward more “mature” values in Class C diabetics. In Class D, F, and R diabetics, an accelerated decrease of MV could be demonstrated until 37 weeks of gestation. No significant correlation between MV and gestational age was observed in these patients.

Whereas low L/S ratios have been found in diabetes of Classes A through R (16), several investigators have reported an acceleration of fetal lung maturity in Class D or Class F or R diabetes, reflected in an early increase in L/S ratio and (or) early appearance of phosphatidylglycerol (7, 8, 15–20). Very little change in the lung profile is demonstrable in Class C diabetes (8). Classes A and B diabetes have been associated with low (16), normal (8, 21), or even increased (8) L/S ratios as well as persistence of phosphatidylinositol and delay in appearance of phosphatidylglycerol (8, 22). These varied findings reflect the complexity of the influence of diabetes on fetal lung maturity, that is, on the degree of control of carbohydrate metabolism (21, 22), of associated hypertensive disorders (17, 18), and of vascular complications responsible for chronic fetal stress (19).

In this study, the effect of Class D or Classes F or R diabetes on MV was consistent with the effect of those complications on the results of other tests of fetal lung maturity. No significant change in MV was found in the other classes of diabetes.

Betamethasone treatment. Betamethasone given for 48 h in conjunction with beta-agonists seems to decrease MV in some women. Most of the MV values were lower for a given gestational age as compared with those for normal pregnancy. Before 34 weeks of gestation, more MV values compatible with fetal maturity were observed than we expected. When we studied paired amniotic fluid samples, we saw an obvious difference between pre- and post-treatment MV values.

Liggins and Howie have shown that glucocorticoids, administered for at least 24 h, decrease the incidence of hyaline membrane disease (9), a beneficial effect at least partly explained by accelerated synthesis or release of surfactant (23). Whereas increase in the L/S ratio has been reported after the administration of dexamethasone (24, 25) and hydrocortisone (26), no change or only a modest increase has been observed with betamethasone (27–29), although betamethasone reportedly increases the amount of disaturated lecithin in the amniotic fluid (27, 30). Beck et al. (31) reported a slight de-
crease in sphingomyelin and in total phospholipids together with a relative increase in the proportion of lecithin in response to betamethasone administered to the pregnant rhesus monkey. Moreover, glucocorticoids influence enzymes controlling the production of phospholipids other than lecithin such as phosphatidylglycerol and phosphatidylinositol (23). The effect of betamethasone on MV may reflect subtle changes in surfactant composition induced by treatment. Indeed, changes in the relative concentrations in amniotic fluid of lecithin, sphingomyelin, phosphatidylglyerol, and phosphatidylinositol as well as the degree of saturation of the phospholipids' hydrocarbons are factors known to influence MV (2-4). Although the matter is still controversial (32), there are reports that beta-agonists may cause release or synthesis of surfactant (33-36). Therefore, the possibility that both beta-agonists and glucocorticoids affect MV cannot be excluded in this study.

In summary, we have demonstrated an accelerated decrease in MV in amniotic fluid from pregnancies complicated by hypertensive disorders; Classes D, F, or R diabetics; and in premature labor after 48 h of therapy with betamethasone given in conjunction with beta-agonists. These same conditions are also known to diminish the incidence of hyaline membrane disease and to accelerate several other indexes that reflect fetal pulmonary surfactant production. Microviscosity determinations should identify those fetuses that have achieved fetal lung maturity, regardless of gestational age, when premature delivery is contemplated in a high-risk pregnancy.

References