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PLASMA AND INTERNAL ERYTHROCYTE VISCOSITY IN UMBILICAL ARTERY AND VEIN OF PREMATURE INFANTS WITH AND WITHOUT ACUTE ASPHYXIA

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ABSTRACT The successful management of marked biochemical and hemorheological abnormalities in preterm neonates, especially in babies with perinatal asphyxia, requires a thorough understanding of the causes and consequences of such changes. We divided 41 preterm newborns (< 37 weeks of gestational age) into two groups -with and without perinatal asphyxia- to study biochemical, hematological and hemorheological variables (plasma viscosity and internal erythrocyte viscosity measured with a Harkness type capillary viscosimeter, osmolality, anion gap, osmol gap) in blood samples from the umbilical artery and vein. The results were analyzed with comparison of the t-test, correlation and regression studies. The most noteworthy findings were: 1.) similar values in biochemical, hematological and hemorheological variables in both vessels and in both groups, and 2.) evidence of a similar relation between plasma protein concentration and plasma viscosity in umbilical artery and vein in preterm neonates with acute fetal distress.

Key words: Perinatal asphyxia, acute fetal distress, hemorheology, preterm, viscosity.

INTRODUCTION

Perinatal asphyxia is recognized as an important cause not only of death (1), but also of neonatal hyperviscosity syndrome (2). Increased cellularity (hematocrit) due to placental transfusion (3), variations in erythrocyte filterability (4) and changes in erythrocyte aggregation potential (5) are among the causes of increased viscosity in the perinatal period. Perinatal asphyxia has been linked to modifications in plasma protein composition. Increased concentrations of fibrinogen and beta-globulin (6) may well account for increased erythrocyte aggregation potential in these patients (5). Increases in other plasma protein fractions also increase plasma viscosity and undoubtedly affect microcirculatory blood flow and tissue O_2 exchange.

The objective of this study was to investigate hemorheological properties in preterm neonates with and without acute fetal distress (AFD), as hemorheological abnormalities may have repercussions on subsequent morbidity.

MATERIAL AND METHODS

A total of a 41 preterm neonates (gestational age < 37 weeks) were divided into two groups. The control group consisted of 26 neonates (33.5 +/- 2.4 weeks) with umbilical artery blood pH > 7.20 at birth. Acute fetal distress group (AFD) contained 15 neonates (34.7 +/- 1.5 weeks) in which umbilical blood pH was < 7.20 as measured in the scalp (in partum) or umbilical artery blood (at birth). The protocol was approved by our hospital's clinical trials ethical committee. Informed consent was obtained from the parents of all neonates to obtain blood samples. Umbilical arterial and venous blood samples (maximum volume 3 ml) were collected for biochemical analysis with disposable syringes. All samples were divided into two volumes, one was transferred to a dry glass test tube (containing no anticoagulant) and centrifuged at 2500 x **g** for 10 min. The serum fraction was used for laboratory assays. The other volume was transferred to a glass test tube containing 10 mcg/ml 10 % EDTA, then centrifuged as described above to separate the supernatant. Plasma viscosity was measured at 37° C over the following 8 h, as recommended by The International Committee for Standardisation in Haematology (8), with a Harkness 8052 series capillary viscosimeter (Coulter Electronics).

The samples were processed with the technique described by Reinhart (9) for the measurement of intraerythrocytic viscosity. The blood was centrifuged at 2.000 x **g** for 10 min. The pellet was resuspended in Ringer solution. These steps were repeated, and after a third centrifugation step the red cell pellet was mixed with an equal volume of toluene by vigorous shaking, which led to complete hemolysis of the cells. The lysate was transferred to cellulose nitrate tubes and centrifuged at 30.000 x **g** for 30 min. to remove the remains of the cell membranes in the hydrophobic (upper) phase together with the toluene. The hydrophilic (lower) phase of the ultrafiltrate was collected with a thin capillary tube; this hemoglobin solution behaved as a Newtonian fluid (10) whose viscosity was measurable with a Harkness capillary viscosimeter. The protein fractions were determined by starch gel electhrophoresis (Beckman), and the resulting subfractions (albumin, alpha1-globulin, alpha2-globulin, beta-globulin, gamma-globulin and total protein) were quantified densitometrically (Beckman Appraise). The data were analyzed statistically with correlation studies (Pearson's "r"), simple and multiple regression, and comparison of the means (t-test).

RESULTS

TABLES I and II summarize the values obtained in umbilical arterial and venous blood in both groups of neonates. No significant difference between the groups was found for plasma viscosity.

TABLE I

Mean values in preterm infants. Results of umbilical artery.

	Preterm (Control)	Preterm (Asphyxia)
Plasma viscosity (mPa.s)	0.89 (+/-0.05)	0.91 (+/-0.07)
Internal viscosity (mPa.s)	2.75 (+/-0.76)	2.31 (+/-0.38)
Osmolality (mOsm/Kg)	285.7 (+/-21.18)	287.2 (+/-20.1)
Osmol GAP (mOsm/Kg)	7.84 (+/-20.01)	8.28(+/-22.53)
Anion GAP (mEq/L)	10.98 (+/-7.52)	22.03 (+/-30.84)
Hematocrit (%)	45.7 (+/-6.9)	50 (+/-9.4)
Albumin (g/dl)	3.42 (+/-0.71)	3.65 (+/-0.49)
Alpha1-globulin (g/dl)	0.18 (+/-0.04)	0.21 (+/-0.05)
Alpha2-globulin (g/dl)	0.35 (+/-0.06)	0.37 (+/-0.06)
Beta-globulin (g/dl)	0.49 (+/-0.16)	0.52 (+/-0.19)
Gamma-globulin (g/dl)	0.64 (+/-0.15)	0.7 (+/-0.23)
Total protein (g/dl)	4.86 (+/-1.03)	5.15 (+/-1.36)
pH	7.28 (+/-0.05)	7.14 (+/-0.07)

TABLE II

Mean values in preterm infants. Results of umbilical vein.

	Preterm (Control)	Preterm (Asphyxia)
Plasma viscosity (mPa.s)	0.90 (+/-0.1)	0.90 (+/-0.07)
Internal viscosity (mPa.s)	2.77 (+/-0.85)	2.4 (+/-0.49)
Osmolality (mOsm/Kg)	284.6 (+/-22.87)	287 (+/-11.41)
Osmolal GAP (mOsm/Kg)	6.34 (+/-25.75)	7.74 (+/-13.27)
Anion GAP (mEq/L)	12.01 (+/-6.64)	12.71 (+/-5.41)
Hematocrit (%)	45.5 (+/-7.01)	50.80 (+/-9.92)
Albumin (g/dl)	3.45 (+/-0.61)	3.71 (+/-0.53)
Alpha1-globulin (g/dl)	0.19 (+/-0.04)	0.23 (+/-0.08)
Alpha2-globulin (g/dl)	0.37 (+/-0.07)	0.37 (+/-0.05)
Beta-globulin (g/dl)	0.45 (+/-0.15)	0.49 (+/-0.16)
Gamma-globulin (g/dl)	0.70 (+/-0.17)	0.66 (+/-0.22)
Total protein (g/dl)	5.09 (+/-1.32)	5.46 (+/-0.74)
pH	7.36 (+/-0.05)	7.25 (+/-0.07)

Statistical comparison of the means in arterial and venous blood likewise failed to detect significant differences in any of the variables between neonates with and without acute fetal distress. Despite the apparent lack of difference, further analysis revealed some interesting relationships between variables (TABLE III). The umbilical vein shows the placental situation. In this medium we found the effect of plasma proteins on viscosity very similar in preterm neonates with and without acute fetal distress.

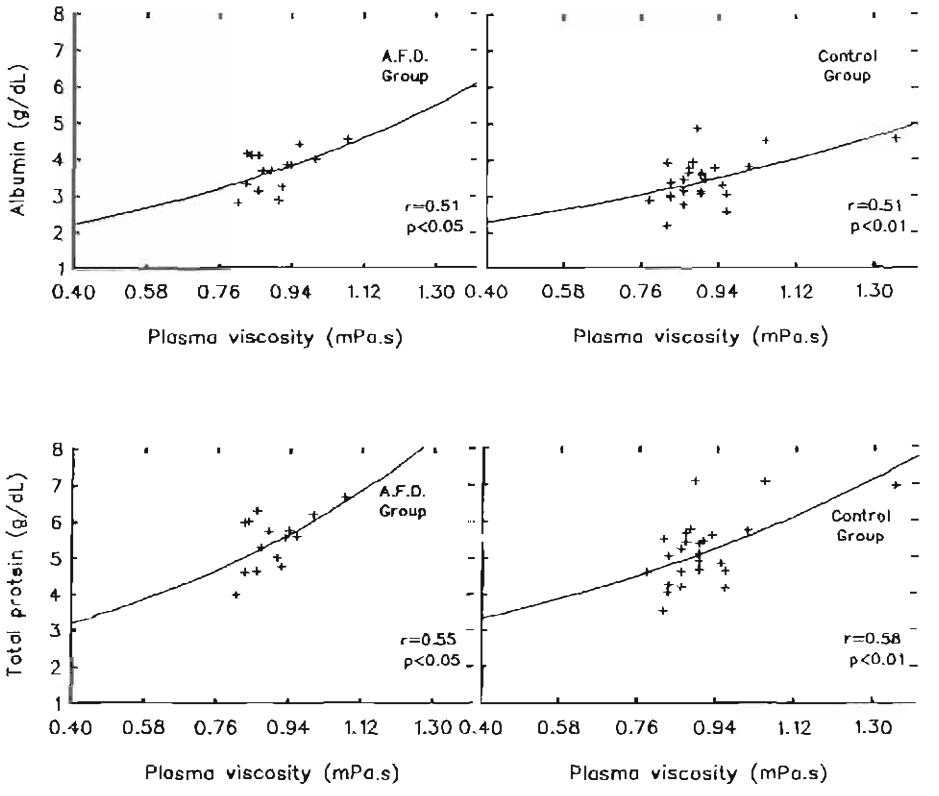

FIG. 1

Significant relationships between plasma viscosity (P.V.) and plasma proteins of umbilical vein in preterm neonates without (control) and with acute asphyxia.

TABLE III

Correlation study with comparison of "r" values for preterm infants with and without acute fetal distress.

	Umbilical artery		Umbilical vein	
	Control	Asphyxia	Control	Asphyxia
P.V./Albumin	r = -0.12	r = 0.51	r = 0.51	r = 0.51
	p = NS	p < 0.05	p < 0.01	p < 0.05
P.V./alpha1-globulin	r = 0.27	r = 0.28	r = 0.27	r = -0.05
	p = NS	p = NS	p = NS	p = NS
P.V./alpha2-globulin	r = 0.11	r = 0.49	r = 0.40	r = 0.38
	p = NS	p < 0.05	p < 0.05	p = NS
P.V./beta-globulin	r = 0.11	r = 0.24	r = 0.50	r = 0.46
	p = NS	p = NS	p < 0.01	p = NS
P.V./gamma-globulin	r = 0.28	r = 0.55	r = 0.51	r = 0.28
	p = NS	p < 0.05	p < 0.05	p = NS
P.V./Total protein	r = 0.02	r = 0.57	r = 0.58	r = 0.55
	p = NS	p < 0.05	p < 0.01	p < 0.05

In umbilical arterial blood which informs us of fetus state, we didn't find relations between the plasma viscosity and protein fractions of healthy premature neonates. Although in premature neonates with acute fetal distress (pH < 7.20) the effect of plasma proteins on viscosity was similar to that found in umbilical vein. Fig 1 illustrates some of these relationships, and shows that some protein fractions in umbilical venous blood were able to predict changes in plasma viscosity in preterm neonates with and without acute fetal distress.

DISCUSSION

Studies of neonatal hemorheology classically relate blood viscosity with the moment of umbilical cord clamping (7), in an attempt to explain neonatal polycythemia during the first days of life, and the pathogenesis of neonatal hyperviscosity syndrome. Blood viscosity is, for these reasons, not comparable in the fetal and neonatal periods (7,11,13). Hematocrit, although important, is not the only criterion that should be considered in evaluating fetal and neonatal blood viscosity (14).

Plasma viscosity, in contrast to blood viscosity, does not undergo significant changes during birth, hence its rheological characteristics in the fetus and neonate are thought to be similar. The relative contribution of plasma to fetal blood rheology was investigated by Gross and Hathaway (4) in cross-suspensions of umbilical cord plasma and erythrocytes. These authors found that the filterability of fetal red blood cells in adult plasma was similar to that of adult red blood cells in adult plasma, ie, the type of plasma had significant effect on erythrocyte filterability. The rheological behavior of adult erythrocytes in fetal plasma was similar to that of fetal cells in fetal plasma.

Few studies have examined plasma viscosity in umbilical cord blood (7,9,14, 15,16), and the differences in sampling strategy and methodology make it difficult to compare their results. Our plasma viscosity values were lower than those reported in the literature (5,7,9,14) and we found no significant differences between the umbilical artery and vein. We measured with a capillary viscosimeter, as has recommended the International Committee for Standardisation in Haematology (8) and our results for plasma viscosity were similar to those of Stadler and Linderkamp (16), who used a capillary viscosimeter.

Our results show that the protein composition and the viscosity of plasma are similar in both umbilical vessels and do not change in either umbilical vessel during perinatal asphyxia. However, our results show that different plasma protein fractions have different effects on plasma viscosity in each group.

Fletcher et al. (17), have found changes of plasma viscosity in pregnants which are related with an increase in fibrinogen catabolism initiated by action of thrombin, doing fibrinogen-fibrin complexes (high-molecular weight fibrin complexes - HMWFC-). The levels of HMWFC in the newborn are greater than in the adult (17), this shows an increase of the fibrinogenolysis in the fetus. The fibrinogenolysis may be altered by circumstances like hypoxia or acidosis, which may produce two effects on the clearance of HMWFC in the fetus: 1.) Increase of fibrinogenolysis and production of fibrinogen-fibrin complexes. 2.) Decrease in the placental clearance of circulating complexes by a redistribution of blood flow in the hypoxemia (18). Therefore, the newborns with acute fetal distress show similar relations between serum globulins and plasma viscosity of umbilical artery and vein. We didn't find changes of viscosity in the groups studied, although we observed a different relationship between the globulins and plasma viscosity in each group. The increase of the relation in umbilical vein of preterm newborns without acute fetal distress between serum globulin and plasma viscosity may be

explained by clearance in the placenta of the circulatory complexes. The plasma viscosity and serum globulins aren't different in umbilical artery and vein in each group, moreover the relation between serum proteins and plasma viscosity is different in each blood vessel. These findings may be explained by the interference of some plasma components with placental clearance.

Although Nanjii and Blank (19) reported significant correlations between serum sodium levels and plasma viscosity, we were unable to find any correlation between these two parameters in any of the groups or in either of the umbilical vessels.

Hypoxia may cause lactic acidosis and hyperosmolality. This led us to investigate possible modifiers of microcirculatory blood flow, such as osmolality, anion gap and osmol gap, in addition to classical hemorheological variables. Marked variations in plasma osmolality have been reported in neonates (20, 21), with values as high as 320 mOsm/Kg. No significant differences in osmolality were noted between preterm neonates with and without AFD, or between cord arterial and venous values (TABLE I and II).

Schmid-Schönbein et al. (22) reported alterations in erythrocyte morphology and filterability when plasma hyperosmolality or acidosis were induced. Reinhart et al. (9) showed that despite the structural differences between fetal and adult hemoglobin, there was no significant difference in intraerythrocyte viscosity. Hemoglobin suspensions free of membrane lipid contamination show Newtonian rheological behavior, and are not affected by shear rate (10). We, therefore, used a capillary viscosimeter for measuring intraerythrocyte viscosity. With this instrument, we obtained values much lower than those obtained with axial type viscosimeters (9, 23, 24, 25), and our values are corresponding with theoretical values only after applying Huggins' equation (25).

Intraerythrocyte viscosity in our sample of preterm infants did not differ significantly in neonates with or without AFD, neither were differences found between umbilical arterial and venous blood. We conclude that acute asphyxia does not cause significant alterations in plasma and internal viscosity in the fetus.

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