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RHEOLOGICAL BEHAVIOUR OF NEONATAL BLOOD AT TERM WITH OR WITHOUT POLYCYTHEMIA: A STUDY IN 0.38 mm DIAMETER TUBES

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ABSTRACT. The intrinsic resistance of red blood cell (RBC) is a determining factor of the blood viscosity. Hematocrit (Ht) greater than 60% significantly contributes to an increase in this factor. This study determines the viscosity of the intracellular content of RBC in neonates with and without polycythemia. The viscosity is measured in two neonate groups, according to the absence of illness and their hematocrit values, using capillary tubes with a 0.38 mm diameter at 25° C. The internal viscosity was found to be lower in polycythemic neonates together with lower plasma viscosity and mean corpuscular hemoglobin levels. The relative viscosity was significantly greater in the polycythemic group. We draw the conclusion, that viscosity changes due to increased hematocrit are compensated by decreases in the plasma and internal viscosities of the red blood cells thus minimizing their effects on tissue blood flow.

Key words: Hemoglobin, Viscosity, Polycythemia, Newborn, Blood rheology.

INTRODUCTION

Polycythemia is the commonest cause of hyperviscosity in the neonatal period [1] and has been related with the time of cord clamping [2,3,4]. Clinical repercussions and changes in blood viscosity in polycythemic neonates have been studied by several authors [5,6,7,8,9] but the contribution of the internal viscosity RBC to development of neonatal hyperviscosity syndrome is still unknown.

Blood hyperviscosity is responsible for several pathological phenomena during the neonatal period [1,10] and results from either the negative or positive influence of a number of hemorheological factors of which the most studied is hematocrit [11,12]. The pathological manifestations of hyperviscosity result from the decreased blood flow brought about by the acute condition [1,13]. In its chronic form, changes in blood viscosity could play a regulatory role in arterial vasodilation [14].

Studies carried out in capillary tubes enabled us to confirm that blood viscosity decreases with capillary diameter (Fahraeus-Lindqvist effect), but this is non-proportional to decreases in hematocrit [15]. This observation, related to biological membrane interaction and a greater or lesser tendency for cellular aggregation, suggests that the same rheological blood properties can lead to differential effects depending on the distinct flow conditions present. In this study the viscosity characteristics of the intracellular content of RBC and plasma are tested using 0.38 mm diameter capillary tubes in neonates with and without polycythemia.

MATERIAL AND METHODS

Blood samples from 52 newborns were allocated to one of two groups. The first group (n=41) analytically and clinically normal in the medical examination, with gestational ages ranging from 261 to 293 days (277 $\pm$ 9 days) and birthweights of 3150 $\pm$ 490 g. The second group (n=11) were classified according to the presence of polycythemia (hematocrit in the vena greather than 60%). These neonates had a gestational age between 259 and 296 (277 $\pm$ 14 days) and a birthweight of 3100 $\pm$ 290 g. In all cases birthweights were suitable for the gestational age and in agreement with records of fetal growth in our area [16]. The project was approved by the Hospital Ethical Committee and parent or guardian consent was obtained in each case.

Preparation of cytoplasmic content of RBC.- From each of the 52 neonates 3 ml of blood was obtained before second day of life from the antecubital vein and anticoagulated with EDTA (1 mg/ml). The red blood cells (RBC) were separated by centrifugation at 2,000 x g for 10 min and the plasma was decanted off. The cellular fraction was resuspended in Ringer's solution and centrifuged again at 2,000 x g for 10 min. The supernatant was discarded and the operation repeated three times. An equal volume of toluene (Sigma Chem., Co.,) was added to the packed cellular fraction and the mixture was vigorously shaken for 10 min. The hemolysate was transferred to nitrate cellulose tubes and centrifuged at 30,000 x g for 30 min. After discarding the toluene, samples were frozen at -20°C. Samples were defrosted at room temperature and the lower phase was aspirated using a glass tube.

Techniques used.- Red blood cell count, cell volume and hemoglobin concentration were determined with a Coulter Counter (Coulter Electronics, Herts., UK).

Viscosity measurement.- Viscosity measurements (η) were based on those previously described by Stadler et al. [17]. The horizoum aspiration pump at 100 kPa pressure (P) or 1000 cm of H_2O. Capillary tubes were 20 cmntally positioned capillary was loaded with the sample at a 25° C and connected to a vacu long (L) with an internal diameter (D) of 0.38 mm. Applied shear stress was 0.475 Pa (4.75 dynes/cm^2), which was calculated according to the following formula:

$$Shear\ stress = \frac{0.25\ P\ D}{L}\ \left(\frac{dynes}{cm^2}\right) \quad (1)$$

Calculations.- The viscosity (η) of Newtonian fluids in capillaries is expressed by the Hagen-Poiseuille law [18]:

$$\eta = \frac{Pr^4\pi}{8QL} = 0.39\ \frac{Pr^4 t}{VL} \quad (2)$$

where P is the pressure exerted on the fluid; V is the flow volume and t is the flow time (Q=V/t). Since P and V are both constants, the previous formula becomes:

$$\eta = C\ t \quad (3)$$

where C is a constant representing the solution viscosity used as a reference (NaCl 36 g/dl) at 25°C. The internal viscosity (η_{Hb}) can be calculated by:

$$\eta_{Hb} = 0.943\ \frac{t_{Hb}}{t_{ref}} \quad (4)$$

t_{Hb} is the flow time of intracellular content of RBC and t_{ref} is that of the reference solution. The relative viscosity (η_r) is calculated by the equation described by Taylor [19]:

$$\eta_r = [(1-(1-Ht))\ Ht\ T]^{-2.5} \quad (5)$$

where (1-Ht) is the plasma volume after removing the cellular fraction, Ht is the cellular volume and T is the Taylor coefficient [19] calculated from the following equation:

$$T = \frac{p + 0.4}{p + 1} \quad (6)$$

where p is the ratio of the internal viscosity of RBC to the plasma viscosity.

Statistics.- Statistical analysis was carried out using BMDP Statistical Software (University California Press, Berkeley). The Shappiro and Wilk's test was applied followed by correlation and regression analysis and a comparison of non-paired means test (t-test).

RESULTS

As shown in Table I, internal viscosity of RBC in the polycythemic group was significantly lower than viscosity in the control group ($t = 2.01; p<0.05$). However, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) did not differ in the two groups although mean corpuscular hemoglobin concentration (MCHC), hemoglobin concentration and hematocrit were significantly different.

In neonates with polycythemia the viscosity was significantly related to the MCV ($r=-0.79$; $p<0.01$) and hematocrit levels ($r=-0.69; p<0.02$); this relationship was not observed in the control. Plasma viscosity in neonates with polycythemia was significantly lower than in the control. The ratio of the internal RBC viscosity to tha plasma viscosity (Taylor's coefficient) was similar in both groups although the relative viscosity (η_r) was greater in neonates with polycythemia.

TABLE I

Hematological and hemorheological parameters in the two neonate groups, where t is the result of t-test (Student) and p is the level of statistical significance.

	Control group (n=41)	Polycythemic group (n=11)	t	p
pH	7.34 ± 0.05	7.33 ± 0.05	0.68	0.24
Internal viscosity of RBC (mPa.s)	3.53 ± 0.99	2.90 ± 0.58	2.01	0.02
Plama viscosity (mPa.s)	1.17 ± 0.08	1.11 ± 0.06	2.22	0.015
Taylor coefficient	0.84 ± 0.02	0.83 ± 0.02	1.55	0.063
Relative blood viscosity (mPa.s)	51.06 ± 5.26	61.43 ± 7.16	5.35	0.0000
RBC ($10^6/\mu l$)	4.5 ± 0.57	5.68 ± 0.27	6.63	0.0000
Hemoglobin (g/dl)	16.48 ± 1.96	19.26 ± 2.67	3.85	0.0001
Hematocrit (%)	48.63 ± 6.32	63.17 ± 1.93	7.48	0.0000
MCV (fl)	108.11 ± 5.50	101.46 ± 3.08	1.36	0.08
MCH (pg)	36.37 ± 2.51	34.98 ± 2.72	1.60	0.057
MCHC (g/dl)	33.82 ± 1.94	32.13 ± 1.65	2.63	0.005
Ratio VI/CHCM	0.10 ± 0.02	0.09 ± 0.01	1.58	0.059

DISCUSSION

Neonatal polycythemia is directly related with hyperviscosity syndrome and its related pathological phenomena [1,7,8,9] has been the focus of many studies in recent years. The contribution of RBC characteristics such as their deformability and intracellular fluid are however less well known.

Blood viscosity depends on the effective cellular volume and the acceleration to which the fluid is submitted. In turn, effective cellular volume depends on erythrocyte aggregation, erythrocyte deformability and hematocrit [20]. In the case of neonatal polycythemia, the increase in red blood cell number is accompanied of an increase in hemoglobin level and

changes in the cell volume (Table 1). According to several authors [21,22] internal RBC viscosity, directly related to the MCHC is lower in neonates with polycythemia where the MCHC is also lower. Plasma viscosity in neonates with polycythemia is lower than that obtained for the control, though plasma protein level was not determined in our assays because of limited sample volume. This parameter however, was studied by Linderkamp et al. [4] and Uberos J. and Muñoz A. [23,24] who observed no differences in plasma protein levels in neonates with hematocrit greater than 60%. We thus assume that changes in plasma viscosity of neonates with polycythemia are mainly due to changes in water compartmentation. Thornton et al. [25] report small increases in intracellular and interstitial body water volume in neonates with polycythemia although not at statistically significant levels. Dintenfass L. [26] has proposed the autoregulatory control mechanism for the viscosity of blood. We consider that increased hematocrit and relative viscosity at a low shear rate in neonates with polycythemia induce a compensatory decrease in plasma and internal RBC viscosities. These changes could be explained by a slower blood flow in the case of polycythemia with lower tissue and renal perfusion, responsible for an increase in plasma renin activity and an increase in renal water reabsorption. We believe that these viscosity changes could be secondary to an increase in the body water of the interstitial and intracellular intravascular compartments.

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