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Factor VIII related antigen in term and preterm newborns with severe neonatal haemorrhage

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Plasma factor VIII related antigen (VIIIR:Ag) concentrations were studied in term and preterm newborn infants. The control population was a group of normal children 2 to 12 years of age without any manifest disorder. The aim of the present investigation was to follow the change of VIIIR:Ag in the newborn and to study its level in sick preterm infants with severe bleeding disease. The VIIIR:Ag level in the control children was $86.2 \pm 20.5\%$. The lowest concentration was measured in term infants between 48—72 hours of life. The highest VIIIR:Ag level ($129.1 \pm 8.1\%$) was found in those preterm newborns who died of pulmonary and intracranial bleeding in the early neonatal period. VIIIR:Ag is a useful marker of endothelial cell damage in the perinatal period.

Haemorrhage, both localized and generalized, is a significant cause of mortality and morbidity in the neonatal period [11, 13, 17, 20]. Numerous studies have demonstrated deficiencies of various blood coagulation factors in the normal newborn and more severe ones in preterm infants [5, 8, 9, 18]. The endothelial cells and platelets have an important role in the maintenance of vascular integrity and their factors, besides ensuring the normal balance, are useful markers of pathological conditions. Endothelial cell injury is probably a primary event in the pathology of a number of diseases affecting blood vessels. Plasma level of factor VIII related antigen (VIIIR:Ag) may be a sign of intimal damage [1, 4, 7].

Factor VIII/von Willebrand factor complex (FVIII/vWF), being a glycoprotein of high molecular weight, has distinct constituents, i.e.: factor VIII procoagulant activity protein (VIII:C), the antigenic expression of VIII:C (VIII:CAg), von Willebrand factor protein, the antigenic expression of von Willebrand factor (VIIIR:Ag), and the ristocetin cofactor (VIIIR:RCo) [22]. VIIIR:Ag and VIIIR:RCo are closely related. Both are synthesized by vascular endothelial cells and possibly by megakaryocytes and platelets [14, 16, 19).

Estimation of VIIIR:Ag in plasma by precipitation with rabbit antiserum is a simple and useful parameter of endothelial cell injury.

The aetiological factors that have been suggested as the basis of endothelial cell damage in the perinatal period are the respiratory distress syndrome, asphyxia, acidosis, infec-

tions, hypoglycaemia, hypercapnia, icterus, congenital heart disease, fetal distress and the mode of delivery [10]. In these disorders, oxygen radicals are produced in large quantity in hypoxia and hyperoxia, especially during the rapid change from hypoxia to hyperoxia [2]. This state may lead to severe damage of cells and tissues, mainly in preterm infants whose endogenous antioxidant activity is weak [3]. The endothelial cell injury then affects the microcirculation, leading to generalized neonatal bleeding syndrome with massive pulmonary and intracranial haemorrhage.

The aim of the present investigation was to study the plasma VIIIR:Ag level in normal children, term and preterm infants and in those sick preterm infants who died in consequence of pulmonary and intracranial haemorrhage.

Material and Methods

The VIIIR:Ag level was estimated in children hospitalized at our Department. Blood samplings were carried out only when blood was needed for other purposes, too. A total of 67 children were examined; their grouping was as follows.

Group 1. Thirty control children 2 to 12 years of age, without any manifest disorder.

Group 2. Twelve term newborn infants of 37 to 41 (mean 39.3 ± 1.1) weeks gestational age and 3203 ± 378 g mean birthweight, without any adaptational problems.

Group 3. Eleven preterm newborn infants of 26 to 36 (mean 31.2 ± 2.7) weeks gestational age and 1457 ± 354 g mean birthweight, without any risk factor considered to be important in the development of endothelial cell damage.

Group 4. Fourteen preterm newborn infants of 26 to 36 (mean 30.5 ± 2.6) weeks gestational age and 1358 ± 389 g mean birthweight who then died at 1 to 8 (mean 3.6 ± 2.5) days of age with severe pulmonary and/or intracranial haemorrhage confirmed at necropsy.

The first blood sample was drawn of these infants within the first four hours, the second between the 48—72 hours of life. In the fourth group, in 6 infants we could not measure the second VIIIR:Ag level, because they died earlier.

VIIIR:Ag was determined in citrated venous plasma. Blood was mixed 9:1 with 3.8% trisodium citrate solution and plasma was prepared by centrifuging at 3000 *g* at 4°C for 15 minutes and stored at −30°C. VIIIR:Ag was measured by the Laurell rocket immunoelectrophoretic technique using rabbit antiserum (Behring Diagnostics, Germany). Serial dilutions of the pooled and test plasma were prepared for each plate and run simultaneously; the amount of VIIIR:Ag in the patients' plasma was compared to that of the pooled plasma and the result was expressed in percents [21].

Statistical analysis was performed by Student's *t* -test.

Results

The VIIIR:Ag level of the control children in Group 1 was 86.2 ± 20.5% of the normal adult level (Fig. 1). In Group 2 the protein concentration was higher (106.7 ± 15.8) in the first hours of life than in Group 1, but on the third day the level fell significantly by 23.7% and reached the normal level. Group 3 showed shortly after birth a level (89.6 ± 12.2%) lower than in Group 2, but it increased 3 days later. The highest VIIIR:Ag concentration was measured in Group 4, where in the first hours the VIIIR:Ag concentration (119.8 ± 18.0%) was significantly higher than in Group 3, and in those 8 children who survived the first three days, the level ($128.1 \pm \pm 8.1$%) was the highest between 48 and 72 hours of age.

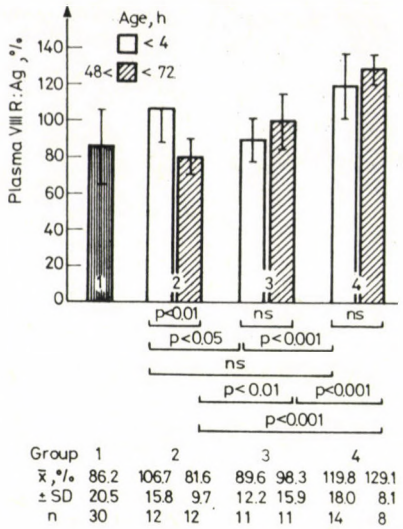

FIG 1. Plasma VIIIR:Ag levels in the four groups. Group 1: control children; Group 2: term newborns; Group 3: preterm newborns; Group 4: preterm newborns with bleeding disorder. Blood samples were obtained within the first four hours, and between the 48 — 72 hours of life

DISCUSSION

The aim of the present study was to follow the circulating VIIIR:Ag level in the neonatal period and especially in preterm infants. The VIIIR:Ag value of control children 2—12 years of age was found to be lower than the normal adult level. The reason for this might be a decreased synthesis of VIIIR:Ag, or an increased resistance of the endothelial cells of children against damaging effects. The only fact supporting the latter assumption is that thromboembolism and angiopathies are less frequent in children than in adults.

The stress during delivery might be the reason for the high concentration of VIIIR:Ag in term newborn infants. The placenta can also be the source of VIIIR:Ag, because obliterations may occur during delivery. In term infants the decrease of VIIIR:Ag concentration three days after birth points to improvement in the microcirculation and in the condition of endothelial cells.

The initial low value of VIIIR:Ag in preterm babies increases with time, indicating the sensitivity of endothelial cells. The cardiovascular system is also immature and the stress situations impair the repair mechanism. The phenomenon is reflected in the VIIIR:Ag concentration measured in preterm infants suffering from generalized bleeding syndrome; the highest values were observed in that population. Sometimes, the damage to endothelial cells is further increased by medical treatment; one of such factors is the toxicity of oxygen. Thus, VIIIR:Ag is a good marker of endo-

thelial cell damage in the perinatal period. We found the highest concentrations in that population where generalized bleeding had developed in the early neonatal period.

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