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HEMOSTATIC SYSTEM IN EARLY RESPIRATORY DISTRESS SYNDROME: REDUCED FIBRINOLYTIC STATE?*

*Murat Yurdakök MD**, Şule Yiğit MD****

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Previous studies suggest that there is a systemic activation of clotting and fibrinolysis in preterm infants with advanced respiratory distress syndrome (RDS). However, there are no data on the hemostatic status in the early stages of the disease; therefore, we studied some of the hemostatic parameters in these patients and made several studies at different times in preterm infants who did or did not develop RDS, using similar protocols. We found normal plasma fibrinogen, protein C, protein S, C4b-binding protein, thrombomodulin, antithrombin III, thrombin-antithrombin III complex, prothrombin fragment 1.2, plasminogen, tissue plasminogen activator, alpha-1 antitrypsin, alpha-2-macroglobulin and protein Z. However, lower D-dimer and higher plasminogen activator inhibitor and von Willebrand factor antigen levels were found within six hours of life in infants who later developed RDS compared to the control group. These findings suggest that disseminated intravascular coagulation is not prominent in the early stages of RDS. Moreover, reduced D-dimer and increased plasminogen activator inhibitor and von Willebrand factor antigen levels are probably related to the abnormalities in the fibrinolytic mechanism due to lung damage in RDS, but further studies are needed to show their pathogenic significance in RDS. *Key words: disseminated intravascular coagulation, fibrinolysis, hemostatic system, newborn infants, respiratory distress syndrome.*

Clinical and animal studies demonstrate abnormalities in the coagulation systems in adult respiratory distress syndrome (ARDS). Common ARDS-related coagulation disorders include disseminated intravascular coagulation (DIC) and inhibition of fibrinolysis. Disruption of the alveolocapillary membrane integrity in RDS results in leakage of coagulation factors into the alveolus. The combination of high levels of alveolar-activated procoagulant factors and inhibited fibrinolysis leads to alveolar fibrin deposition and hyaline membrane formation in RDS. DIC may not only be associated with RDS, but it, may be a predisposing factor for developing RDS. Since fibrinogen and fibrin-degradation products (FDP) are known to be potent inhibitors of surfactant and thrombin, FDP and plasmin may increase pulmonary damage by inducing chemotaxis and aggregation of neutrophils. Therefore, it has been suggested that attenuation of lung injury may occur and increased survival may be achieved by interrupting the coagulation cascade early in RDS[1].

* From the Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

*** Assistant Professor of Pediatrics, Hacettepe University Faculty of Medicine.

It has been reported that there is a systemic activation of clotting, fibrinolysis, and kinin-kallikrein in infants with RDS within 12 to 24 hours of birth[2]. However, there are no data on the hemostatic status in the early stages of the disease. Therefore, we studied some of the DIC parameters in these patients. The main limitation in these studies is the ethical dilemma of attaining enough blood samples in the first few hours of life to evaluate the various parameters of the coagulation and fibrinolytic systems simultaneously. For this reason, we mad several studies in preterm infants at different times using similar protocols. All neonates included in the study received vitamin K_1 (1 mg) intramuscularly upon delivery. Infants who were in a stable clinical condition served as the control group. The study group comprised infants who developed RDS, which was considered to be present if all the following diagnostic criteria were fulfilled: symptoms of respiratory distress within one hour after birth and present for at least 24 hours, respiratory support including mechanical ventilation, and typical findings on lung x-ray and arterial blood gas analysis. None of the infants with RDS had any other disease. All blood samples for hematological testing were obtained from a peripheral vein within six hours after birth.

In developed DIC, reduced fibrinogen, protein C and antithrombin III (AT-III) levels, as well as elevated levels of FDP, including D-dimers (D-D), are found[3]. Although decreased levels of AT-III have been described in preterm infants who developed RDS[4], we found normal AT-III and protein C levels within six hours after birth in preterm infants who developed RDS[5].

Protein S, a vitamin K-dependent cofactor for activated protein C, exists in plasma in a free active form and in an inactive form bound to C4b-binding protein (C4b-BP). The increased protein S anticoagulant activities (i.e. free protein S levels) compared with low total protein S levels are probably due to low or undetectable levels of C4b-BP in newborn infants[6]. On the other hand, C4b-BP is an acute phase reactant protein and present a strong increase in inflammation. The increased levels of C4b-BP can affect the distribution of protein S in plasma, producing a shift in protein S to the complex inactive form[7]. Therefore, we studied total protein S and C4b-BP in pretem infants with or without RDS in the first few hours of life. Although the free protein S levels could not be determined, we found normal total protein S and C4b-BP levels (unpublished data).

Thrombomodulin is a high-affinity thrombin receptor present on endothelial cells. It binds and inactivates thrombin and simultaneously activates protein C. Thrombomodulin is also present in the circulation, and this soluble form of thrombomodulin appears to be active in its endothelial form[8]. Hypoxemia[9], adult RDS[10] and DIC[11] may cause an increase in plasma thrombomodulin levels; however, we found normal plasma thrombomodulin levels in preterm infants who developed RDS in the first six hours of life[12].

Fibrin-degradation products, including D-D, are the end products of fibrinolysis which result from the conversion of an inert plasma proenzyme (plasminogen) into a proteolytic enzyme (plasmin)[3]. Therefore, evaluation of plasminogen levels is important in the biological interpretation of serum FDP in cases of DIC. If the concentration of plasminogen is very deficient, a low level of FDP cannot exclude DIC. We found normal plasma plasminogen levels in preterm infants who developed RDS[13]. Therefore, the reduced D-D levels in preterm infants who developed RDS could not be explained by normal plasminogen levels. Reduced D-D levels may also be due to increased clearance of FDP or overconsumption during DIC. The finding of normal plasma fibrinogen, AT-III and protein C, but of reduced D-D levels within six hours after birth in preterm infants who developed RDS suggests that DIC is not prominent in the early stages of RDS[5].

Theoretically, plasma protein C, AT-III, and even D-D levels are not sensitive indicators of DIC. The specific detection of thrombin should be suitable for use in the active state of DIC. However, thrombin is very rapidly bound and thereby inactivated by its main physiological inhibitor, AT-III. For this reason, a more direct method to evaluate the thrombin level is to measure the thrombin-antithrombin complex (TAT) formed with AT-III[3]. Prothrombin fragment 1.2 (PF1.2), which is released from prothrombin during its activation to thrombin, is also important in the diagnosis of active DIC[3]. However, we found normal TAT and PF1.2 levels in the first few hours of life in preterm infants who developed RDS[14]. These relatively more specific parameters (i.e., TAT and PF1.2) of DIC support our hypothesis, as DIC is not a prominent event in early-stage (or developing) RDS[5].

We found normal tissue plasminogen activator (tPA) and higher plasminogen activator inhibitor (PAI) levels in preterm infants who later developed RDS compared to the control group[4]. Both tPA and PAI are isolated from many cells, including the endothelium[15]. The increased levels of PAI in developing RDS may be related to endothelial damage, because in the early stages of RDS we found increased levels of von Willebrand factor antigen (vWF-Ag)[16], which is a sensitive marker for systemic and/or lung endothelial damage[17]. However, the reason for increased PAI levels without any increase in tPA levels remains to be elucidated.

Alpha-2-macroglobulin and alpha-1-antitrypsin are produced and secreted by various cells, including endothelial cells. Therefore, increased levels of these slow thrombin inhibitors may be expected in RDS; we found normal levels in these patients in the earlier stages of the disease[18,19].

Although the physiological function of protein Z, a vitamin K-dependent protein, is still unknown, the observation that thrombin associates with phospholipid surfaces in the presence of bovine protein Z has prompted the suggestion that this phenomenon may provide a mechanism whereby thrombin is kept from

diffusing into the vascular lumen and away from the site of injury[20]. However, we found normal plasma protein Z levels in the first few hours of life in preterm infants who developed RDS[21].

Increased TAT formation, increased tPA plasma concentrations and increased plasma kallikrein activity within 12 to 24 hours of birth in infants with RDS suggested that there is a systemic activation of clotting, fibrinolysis, and kinin-kallikrein[2]. However, the abnormalities in clotting and fibrinolysis in the early stages of RDS are presumably different from that seen in the later stages of the disease. We found, for example, normal plasma fibrinogen. AT-III, protein C, thrombomodulin, protein S, C4b-BP, plasminogen and tPA, but lower D-D and higher PAI levels within six hours of life in preterm infants who later developed RDS (Table I). Therefore, activation of clotting is not prominent in the early stages of RDS and a reduced fibrinolytic system is more prominent. Further studies will clarify the changes in the hemostatic system in earlier and later stages of RDS will and show their pathogenic significance in developing RDS.

Table I: Some Hemostatic Parameters in Infants with RDS in the First Few Hours of Life (mean ± SD)

	Controls	Infants with RDS	Ref.
Fibrinogen (mg/dl)	170.3 ± 16.4	74.4 ± 20.9	5
Protein C (%)	40.3 ± 46.7	45.5 ± 56.0	5
Total protein S (%)	40 ± 8	37 ± 11	Up
C4b-binding protein (%)	123 ± 163	141 ± 193	Up
Thrombomodulin (ng/ml)	18.3 ± 22.8	8.1 ± 10.2	12
Antithrombin III (mg/dl)	18.4 ± 1.1	18.9 ± 4.4	5
Thrombin-antithrombin III complex (µg/L)	78.1 ± 72.8	64.8 ± 69.9	14
Prothrombin fragment 1.2 (nmol/L)	9.0 ± 7.4	9.9 ± 5.7	14
D-dimer (µg/ml)	4.0 ± 0.9	2.1 ± 0.8*	5
Plasminogen (%)	55.3 ± 33.1	41.4 ± 19.2	13
Tissue plasminogen activator (mg/ml)	17.1 ± 8.8	19.2 ± 11.4	5
Plasminogen activator inhibitor (IU/ml)	7.1 ± 6.0	34.8 ± 14.4*	5
von Willebrand factor antigen (%)	65.9 ± 11.6	73.3 ± 12.5**	16
Alpha-1 antitrypsin (g/dl)	1.1 ± 0.4	0.9 ± 0.4	18
Alpha-2-macroglobulin (mg/dl)	116.7 ± 30.5	104.8 ± 34.4	19
Protein Z (µg/ml)	0.28 ± 0.14	0.34 ± 0.20	21

Mann-Whitney U test: * $p < 0.001$, ** $p < 0.05$

RDS: respiratory distress syndrome; UP: unpublished.

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