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Research Article

Tissue Plasminogen Activator as an Early Predictor of Neonatal Idiopathic Respiratory Distress Syndrome Severity

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Abstract

Background: Activation of clotting and fibrinolysis takes place in advanced respiratory distress syndrome (RDS) of preterm infants. Tissue plasminogen activator (tPA) is an essential component of the fibrinolysis that can reflect the degree of its activation.

Objective: This work aimed to study early changes in plasma tissue plasminogen activator level and its relation to the severity and outcome of idiopathic respiratory distress syndrome in preterm infants.

Methods: The present study was conducted on 25 preterm babies with respiratory distress syndrome, 15 males and 10 females with a mean gestational age of (31.5 ± 2) weeks. The control group included 25 healthy preterm infants, 15 males and 10 females with a mean gestational age of (34.4 ± 0.82) weeks. All babies were admitted to Neonatal Intensive Care Unit of El-Matariah Teaching Hospital. All babies were subjected to thorough clinical and radiologic examination, laboratory tests including; full blood count, C-reactive protein, blood culture and sensitivity to exclude infants with septicemia. Estimation of tPA concentration (tPA ELISA Kit) in the plasma was done twice for the cases, in the 1st and 2nd day postnatal (tPA1 and tPA2) and once for the control group.

Results: The present study showed that plasma tPA levels (tPA_1, tPA_2) were significantly higher in infants with RDS (6.32 ± 3.47, 9.37 ± 4.62) ng/ml compared to the controls (4.44 ± 1.56) ng/ml ($P < 0.01$, $P < 0.05$). Infants with severe RDS had significantly higher tPA_1 and tPA_2 (8.86 ± 2.13, 12.71 ± 3.54) ng/ml compared to those with moderate (5.05 ± 3.92, 8.49 ± 4.41) ng/ml ($P < 0.05$, $P < 0.01$) and mild RDS (4.53 ± 2.12, 5.14 ± 2.51) ng/ml ($p < 0.05$, $P < 0.01$). The present study also showed a significant negative correlation between Apgar score at one minute and tPA_1 among cases ($p < 0.05$) ($r = -0.453$). There were significant positive correlations between radiological score and tPA_1 ($p < 0.01$, $r = 0.609$) and tPA_2 ($p < 0.01$), ($r = 0.678$) and as well between clinical score and tPA_1 ($p < 0.05$), ($r = 0.499$) and tPA_2 ($p < 0.01$) ($r = 0.651$). Moreover, tPA_1 and tPA_2 were significantly higher in the non-surviving infants (8.32 ± 3.08, 12.82 ± 3.02) ng/ml compared to the surviving infants (4.74 ± 2.98, 6.66 ± 3.79) ng/ml ($p < 0.01$). The recorded sensitivity, specificity and positive predictive value of tPA_1 as a diagnostic test were 44%, 96% and 91% respectively.

Conclusion: The diagnosis as well as the degree of neonatal RDS severity and outcome could be predicted early in the preterm infants through tPA estimation.

Keywords: *Tissue Plasminogen Activator; RDS; Fibrinolysis*

Introduction

Respiratory distress syndrome is characterized by the presence of fibrin rich exudate in the alveoli [1]. Both intravascular and intra-alveolar fibrin depositions are thought to contribute to acute lung injury and respiratory insufficiency, which might explain the

association between systemic clotting activity and respiratory distress syndrome severity [2]. Intravascular fibrin deposition increase alveolar capillary membrane permeability, thus contributing to the formation of protein rich pulmonary edema, whereas, intra-alveolar fibrin disturbs surfactant function [3].

Term and preterm newborn infants have poor defense to fibrin formation because of insufficient fibrinolytic activity [4]. The key event of fibrinolysis is the conversion of plasminogen into plasmin, which is mediated by tissue type plasminogen activator antigen in the presence of fibrin. Plasmin in turn causes lysis of fibrin and production of fibrin degradation products [5]. In healthy preterm infants, low plasminogen plasma concentration and activity occur in the face of normal to high plasmin inhibitor concentration and activity thus creating a hypofibrinolytic state. In preterm infants with severe RDS, increased plasma tPA concentrations have been found. However, fibrinolytic activity is probably insufficient for the amount of intravascular and intraalveolar deposition of fibrin in the lungs of these infants [6].

This work aimed at evaluation of plasma tissue type plasminogen activator antigen as a marker of severity in the early course of neonatal respiratory distress syndrome and consequently initiating early aggressive intervention.

Methods

The present study was conducted on 25 preterm babies with respiratory distress syndrome, 15 males and 10 females with a mean gestational age of (31.5 ± 2) weeks and a mean birth weight of (1.73 ± 0.24) kg. All babies were admitted to Neonatal Intensive Care Unit of El-Matariah Teaching Hospital.

Inclusion criteria:

1) No maternal infection, amnionitis or prolonged rupture of membranes > 24 hours.
2) Gestational age between 27 and 33 weeks.
3) Birth weight appropriate for gestational age.
4) No major congenital malformation.
5) No evidence of infection 3 days after study completion.

These infants were divided into 3 groups according to the severity of respiratory distress based on clinical and radiologic criteria:

Group I: Babies with mild respiratory distress comprised 5 babies, 4 males and 1 female with a mean gestational age of 33 ± 2 wks and a mean birth weight of (1.840 ± 0.564) kg. The clinical manifestations of this group are consistent with grade 2 or 3 of the Down scoring system and the radiologic findings are consistent with grade I (fine reticulogranular appearance).

Group II: Babies with moderately severe respiratory distress comprised 11 babies, 5 males and 6 females, with a mean gestational age (31.7 ± 1.79) weeks and mean birth weight of (1.9 ± 0.36) kg. The clinical manifestations of this group are consistent with score 4 or 5 of the Down scoring system and the radiologic criteria are consistent with grade II (mottling with air bronchogram).

Group III: Babies with severe respiratory distress comprised 9 babies, 6 males and 3 females with a mean gestational age of (30.3 ± 1.73) weeks and a mean birth weight of (1.48 ± 0.241) kg. The clinical manifestations of this group were consistent with score 6 or more of the Down scoring system and the radiologic findings are consistent with grade III or IV (white lung).

The control group comprised 25 preterm infants with uneventful postnatal period and with normal radiologic findings. They were 15 males and 10 females with a mean gestational age (34.4 ± 0.82) weeks, a mean birth weight (2.35 ± 0.356) kg.

All babies were subjected to the following:

1) Full medical history with particular emphasis on:
   - Maternal history particularly maternal age, parity, disease and medications during pregnancy.
   - History of current pregnancy particularly antenatal care and cause of prematurity.
   - Mode of delivery.
   - Need for resuscitation and Apgar score at 1 and 5 minutes.
2) Thorough clinical examination with particular emphasis on:
   - Birth weight.
   - Assessment of gestational age using the criteria of New Ballard score.
   - Assessment of the severity of respiratory distress using clinical RDS scoring system of Down (1980) [7].
3) Routine laboratory investigations:
   - Complete blood count using (Coulter counter T660 – Coultronics France).
   - C-reactive protein (Latex agglutination test kit, Biotec Laboratories Ltd, UK).
   - Analysis of arterial blood gases (IL Model 1312 Blood Gas Manager).
   - Blood culture and sensitivity (blood agar media).
4) Radiologic investigation:
1) Chest X-ray postero-anterior view with determination of the grade of RDS according to the radiologic score of Halliday (1998) [8].
5) Estimation of tissue type plasminogen activator antigen in the plasma of patients and controls using commercial kit, IMU-Bind® tPA ELISA Kit, distributed by NiBSCY Blanch Lane, South Mimms, Potter Bar, Hert Fordshire, England.

Two blood samples were taken for each baby. The first one was taken on the first day and within 6 - 12 hours after birth (tPA_1), while the other was taken on the second day (tPA_2), and for the control group only one blood sample was obtained.

Data analysis

The results were analyzed by commercially available software package (Statview 40, Abacus Concepts, Inc, Berkley, CA, USA). Student t-test was used to compare between means of two independent groups. Paired t-test was used to compare between mean of two different variables in the same group. Pearson correlation (r) was used to correlate less than 0.05 were considered as statistically significant.

Results

Plasma tPA levels (tPA_1, tPA_2) were significantly higher in infants with RDS (6.32 ± 3.47, 9.37 ± 4.62) ng/ml compared to the controls (4.44 ± 1.56) ng/ml ($P < 0.01$, $P < 0.05$) (Figure 1). Infants with mild RDS had no significant elevation of plasma tPA levels (tPA_1 = 4.53 ± 2.17, tPA_2 = 5.14 ± 2.51) ng/ml in comparison to the control Infants ($p > 0.05$). In moderate RDS group tPA had no significant early rise (tPA_1 = 5.05 ± 3.39) ng/ml but later during the second day there was a significant increase (tPA_2 = 8.49 ± 4.41) ng/ml compared to the controls ($p < 0.05$). Only, in infants with severe RDS a significant rise of plasma tPA was recorded early during the first day (tPA_1 = 8.86 ± 2.13) ng/ml and further rise in the second day (tPA_2 = 12.80 ± 3.53) ng/ml ($p < 0.01$). Infants with severe RDS had significantly higher tPA_1 and tPA_2 (8.86 ± 2.13, 12.71 ± 3.54) ng/ml compared to those with moderate (5.05 ± 3.92, 8.49 ± 4.41) ng/ml ($P < 0.05$, $P < 0.01$) and mild RDS (4.53 ± 2.12, 5.14 ± 2.51) ng/ml ($p < 0.05$, $P < 0.01$).

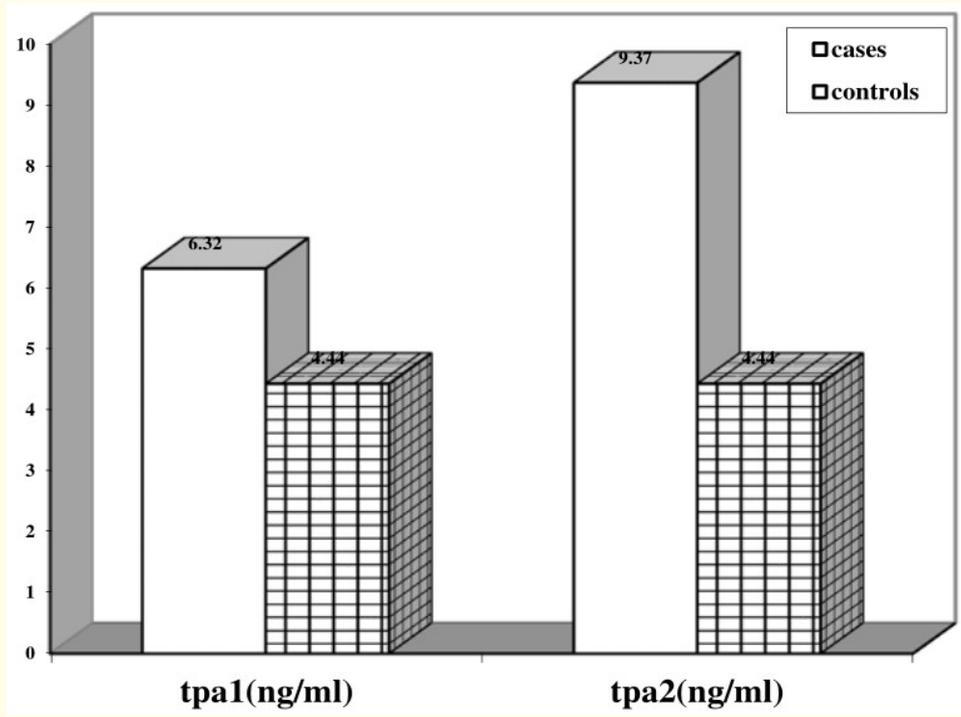

Figure 1: *Comparison between cases and control group as regard plasma tPA_1 and tPA_2.*

The present study also showed a significant negative correlation between Apgar score at one minute and tPA1 among cases ($p < 0.05$) ($r = -0.453$), (Figure 2). Apgar score at one minute had also, significant negative correlations with the clinical score ($r = -0.515$, $p < 0.01$) and radiologic score ($r = -0.627$, $p < 0.01$).

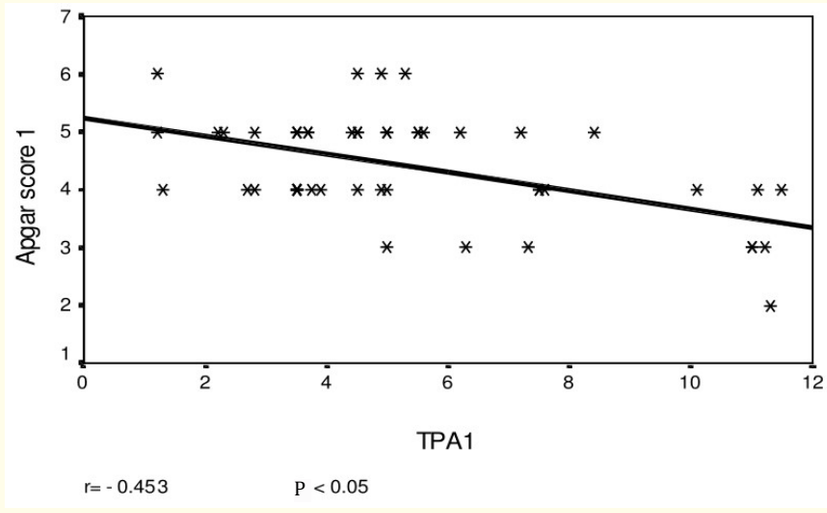

Figure 2: *Correlation between Apgar score at one minute and tPA_1 (ng/ml) in RDS cases.*

There were significant positive correlations between clinical score and tPA_1 ($p < 0.05$) (Figure 3), ($r = 0.499$) and tPA2 ($p < 0.01$) ($r = 0.651$) and as well between radiological score and tPA_1 ($p < 0.01$, $r = 0.609$) (Figure 4) and tPA_2 ($p < 0.01$), ($r = 0.678$).

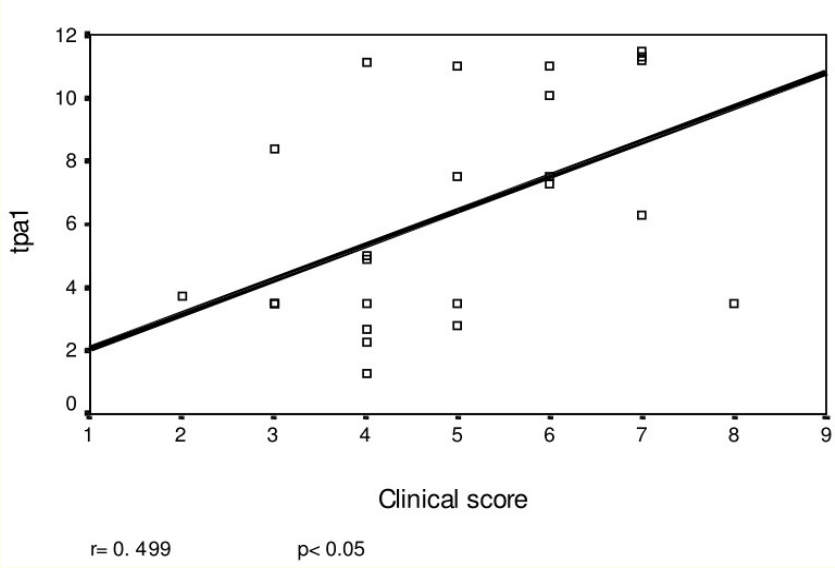

Figure 3: *Correlation between clinical score and tPA_1 (ng/ml) among infants with RDS.*

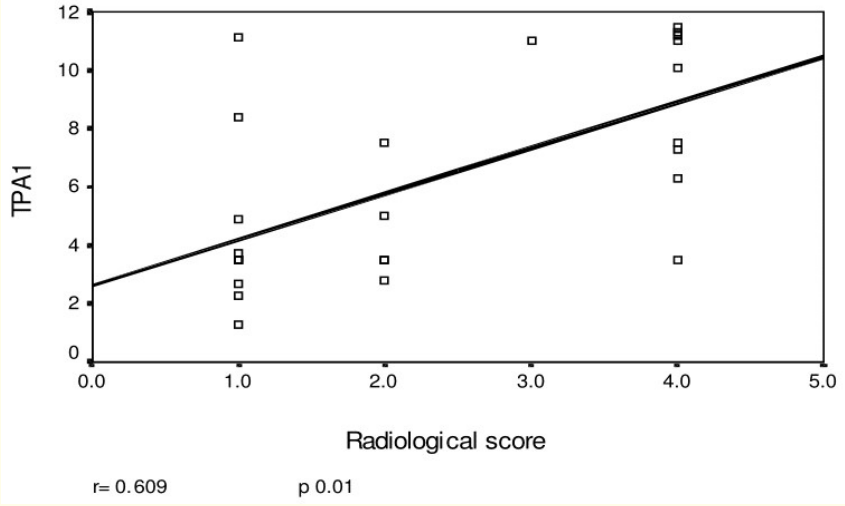

Figure 4: *Correlation between radiological score and tPA_1 (ng/ml) among the studied infants.*

Moreover, tPA_1 and tPA_2 were significantly higher in the non-surviving infants (8.32 ± 3.08, 12.82 ± 3.02) ng/ml compared to the surviving infants (4.74 ± 2.98, 6.66 ± 3.79) ng/ml ($p < 0.01$) (Table 1).

	GROUP	N	MEAN (NG/ML)	SD (NG/ML)	T	P
TPA_1	SURVIVING	14	4.739	2.98	2.94	HS
	NON-SURVIVING	11	8.32	3.075		
TPA_2	SURVIVING	14	6.66	3.79	- 4.38	HS
	NON-SURVIVING	11	12.8	3.02		

Table 1: *Comparison between surviving and non-surviving regarding tPA_1 and tPA_2.*

S: Significant ($p < 0.05$); HS: Highly Significant ($p < 0.01$)

The recorded sensitivity, specificity and positive predictive value of tPA_1 as a diagnostic test was 44%, 96% and 91% respectively compared to 64%, 96% and 94% with tPA_2 (Table 2).

	SENSITIVITY	SPECIFICITY	PPV
TPA_1	44%	96%	91%
TPA_2	64%	96%	94%

Table 2: *Test of sensitivity, specificity and positive predictive value of tPA_1 and tPA_2.*

PPV: Positive Predictive Value

Cut off value of tPA = Mean ± 2SD = 7.4 ng/ml

Discussion and Conclusion

In preterm infants with advanced respiratory distress syndrome, there is a systemic activation of clotting and fibrinolysis [9]. Activation of clotting is characterized by fibrin deposits in the pulmonary microcirculation and in the small airways of preterm infants with severe respiratory distress syndrome [10]. This activation process likely contributes to respiratory insufficiency in neonatal RDS [6].

The fibrinolytic system is the principle effector of clot removal and enzymatic degradation of fibrin. Its action is coordinated through plasminogen-plasmin system, activators, and inhibitors [11].

In preterm infants with severe RDS, increased plasma tPA concentrations have been found. However, fibrinolytic activity is probably insufficient for the amount of intravascular and intra-alveolar fibrin deposition of the lungs of these infants [3].

The presumptive relation between tPA plasma level and respiratory distress syndrome was the stimulus for this work to study changes in plasma tPA levels during the course of RDS in premature infants.

The present study showed that plasma tPA levels were significantly higher in infants with RDS compared to the controls. Previous research discovered that both stressed preterm and term infants are able to increase tPA levels [12]. The activation of clotting with fibrin formation strikingly enhances the activation rate of plasminogen by increasing the level of tPA triggering the fibrinolytic process [13]. The results were comparable with those reported by Brus., *et al*. [3]. They reported a mean level of tPA1, 8.9 ng/ml in infants with severe RDS compared to 8.8 ng/ml in this study, and a mean plasma level of tPA2 6.4 ng/ml compared to 12.7 ng/ml in this study. The higher tPA_2 value in the current study could be explained by the difference in sample timing as tPA_2 was measured on the second day postnatal during the peak of clinical and pathological status, while their tPA_2 was measured on the third day postal, as the cases might show some improvement.

The present study showed significant elevation of plasma tPA in infants with severe RDS than in infants with mild and moderate RDS. Brus., *et al.* [6] reported that activation of clotting, fibrinolysis and kinin-kallikrein is associated with aggravation of the clinical manifestations in preterm infants with RDS. Also, Sarnaik and Lichlait [14] concluded that products of clotting and fibrinolysis are considered to be important determinants of lung injury in RDS because they can damage pulmonary vascular endothelium directly or indirectly by neutrophil and platelet activation.

Further evidences of the link between tPA and the disease morbidity were emerging as there was a significant positive correlation between clinical score of the disease severity and tPA_1 and tPA_2. Also, a significant positive correlation was found between radiological score and tPA_1 and tPA_2. It was clear that estimation of tPA as early as 6 hours after preterm infant delivery could predict the degree of the disease severity.

Not only the disease morbidity that could be predicted by tPA estimation but also the case fatality and outcome. tPA1 and tPA2 were significantly higher in the non-surviving infants as compared to the surviving. The higher incidence of complications in severe RDS cases, whether due to the disease process itself or its management were the apparent direct explanation of increased mortality. This is in agreement with Greenough., *et al*. [15], who reported that patients with severe RDS had a high death rate than others with less severe forms of the disease. This can be explained by many factors affecting the severity of RDS including sepsis, disseminated intravascular coagulation (DIC) or intracranial hemorrhage (ICH).

On the first day, tPA_1 had a sensitivity of 44%, specificity of 96% and positive predictive value of 91% while, tPA_2, taken during the second day, had a sensitivity of 64%, specificity of 96% and positive predictive value of 94%. The first sample could detect nearly half of the cases with the same specificity as the second sample. Repetition of tPA on the second day can discover more cases, up to nearly two thirds of the poor outcome infants, guiding to modify the treatment into a more aggressive one.

The present study showed a significant negative correlation between plasma tPA_1 and Apgar score at 1 minute in babies with RDS. This finding could be explained by the fact that babies with lower Apgar score at one minute tend to have more severe RDS and consequently higher tPA_1. This is in agreement with Brus., *et al*. [3], who reported that higher tPA levels were associated with lower Apgar score and lower arterial umbilical pH values at birth in infants with severe RDS. Nemerson [16] reported that hypoxemia and acidosis at birth are able to induce release of tissue factor and tPA by the activated endothelial cells. So, Apgar score could be used together with tPA as prognostic indices for the morbidity and mortality in infants with RDS.

To conclude, tPA can be used for early detection of morbidity and mortality of respiratory distress syndrome in the predisposed neonates.

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