

## Research Communication

# Serum Endothelin-1 and Transforming Growth Factor- $\beta$ Levels in the Newborns With Respiratory Distress

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The purpose of this present study was to evaluate the serum levels of ET-1 and TGF- $\beta$  in the newborns with respiratory distress. In this study, newborns with respiratory distress hospitalized into the Newborn Intensive Care Unit were included. The highest values of ET-1 and TGF- $\beta$  were obtained from newborns with diagnosis as meconium aspiration syndrome ( $5.70 \pm 5.87$  pg/mL and  $3.75 \pm 1.94$  pg/mL, resp) in the sample obtained in the first six hours after birth, and these are statistically different from control group ( $P < .05$ ). Also, same results were obtained for newborns with respiratory distress syndrome ( $3.37 \pm 1.59$  pg/mL and  $2.05 \pm 0.98$  pg/mL, resp). After oxygen treatment, ET-1 values obtained in the first six hours of life were decreased regularly in the following days ( $P < .05$ ). In the differentiating diagnosis of the respiratory distress of newborns, the investigation of ET-1 and TGF- $\beta$  levels is meaningful. The ET-1 levels investigated in the first six hours is more useful in determining the prognosis, and repeating ET-1 levels in the following days is more meaningful to determine clinical response.

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## INTRODUCTION

Respiratory distress continues to account for significant mortality and morbidity in the Neonatal Intensive Care Unit. At birth, pulmonary vascular resistance decreases with initiation of ventilation. Respiratory distress syndrome (RDS) in premature infants is caused by a structural immaturity of lungs and the insufficient production of surfactant and its incidence is inversely related to gestational age [1]. The problems concerning to the respiratory system prolong the hospitalization period in the premature infants [2].

Endothelin (ET) is a peptide of 21 amino acids in chain with two disulfide bonds with three distinct isoforms: ET-1, ET-2, and ET-3 [3]. Endothelin causes isolated contraction of pulmonary veins, vascular smooth muscle mitogenesis, myocardial cell hypertrophy, positive inotropic and chronotropic effects, bronchoconstriction, mucous secretion, cellular proliferation, and inflammatory reactions [4]. Hypoxia, stress, antidiuretic hormone, and the secretion of some mediators stimulate its synthesis. Clinical investigations have shown increased plasma concentrations of ET-1 during RDS and in case of pulmonary hypertension of other origins. But it is unclear whether ET-1 is actually responsible for the pulmonary hypertension, or the increased ET-1

plasma concentration is a result of the pulmonary hypertension that originated otherwise [5–8].

Also transforming growth factor beta (TGF- $\beta$ ) is a family of three isoforms that regulate cell growth and differentiation, extracellular matrix synthesis cytokines production, and vascular neogenesis [9]. The increase in TGF- $\beta$  precedes the development of pulmonary hypertension which increases circulating ET-1 levels in animals. TGF- $\beta$  induces ET-1 gene expression and ET-1 peptide synthesis in bovine pulmonary artery endothelial cells [10, 11]. The cells responsible for increased ET-1 synthesis during hypoxia are unclear, and short-term effects of hypoxia raise plasma ET-1 levels in animal models; whether chronic hypoxia would lead to different results is unknown [12].

Experimental studies have suggested that ET-1 plays an important role in pulmonary vascular reactivity in neonatal RDS. There is also an elevation of ET-1 in tracheal aspirates from these infants [13]. TGF- $\beta$  showed the strongest stimulatory effect on ET-1 and gene transcription in vascular smooth muscular cells [14]. There are few studies measuring ET-1 and no study measuring TGF- $\beta$  by enzyme immunoassay with a very small number of human premature newborns suggesting that ET-1 is elevated in RDS.

TABLE 1: Demographic characteristics of the patient and control groups.

	RDS	RDS-2	MAS	Total	Control group
Gestational age	—	—	—	—	—
Premature	62	8	3	73	9
Mature	—	16	11	27	11
Total	62	24	14	100	20
Gender	—	—	—	—	—
Female	24	11	7	42	6
Male	38	13	7	58	14
Total	62	24	14	100	20
Birth weight (g)	1367 ± 368	2633 ± 577	2989 ± 914	1898 ± 864	2575 ± 863

The purpose of this present study is to evaluate the serum levels of ET-1 and TGF- $\beta$  in the newborns with respiratory distress (diagnosis as RDS, as transient tachypnea (RDS-2), and as meconium aspiration syndrome (MAS)), to investigate the meaningfulness of the repetitive values of ET-1 in the followup of these diseases, and to determine the reflection of serum ET-1 level on the mortality at the first six hours after birth.

## MATERIALS AND METHOD

In this study, newborns with respiratory distress hospitalized into the Newborn Intensive Care Unit were included. The study group was evaluated by 100 newborns, 62 diagnosed as RDS, 24 as RDS-2, and 14 as MAS within the last six months. Moreover, a control group was evaluated with 20 healthy newborns. For the study, written permits were taken from the parents of each newborn, as well as an approval of the regional Ethics Committee.

A detailed history of each infant was obtained. The gestational age of the newborns was determined according to the New Ballard Score [15]. According to the gestational age, newborns younger than 38 weeks were classified as premature and newborns between 38–42 weeks as mature. After a detailed physical examination, the newborns were investigated with respect to their blood gases, complete blood count, full blood biochemistry, and C-reactive protein levels, and their culture samples were taken.

Moreover, first blood samples were obtained from all premature and mature sick or healthy newborns in the first six hours after birth. The serum was collected into polypropylene tube and centrifuged immediately and was stored at  $-70^{\circ}\text{C}$ . In addition, repetitive blood samples were obtained at the third, 7th, 14th, and 28th day from the patients whom oxygen supply continued. These serums were also stored at  $-70^{\circ}\text{C}$ . Then, the samples were resolved and they were studied with ELISA method in the Immunology Laboratory.

Plasma TGF- $\beta$  level was determined by capture ELISA according to the instructions of R & D Systems using monoclonal antihuman TGF- $\beta$ , R & D Systems, Inc (USA). In brief, 100  $\mu\text{L}$  of the capture antibody was transferred to an ELISA plate and incubated overnight at room temperature. Each well was then washed three times with wash buffer. After removal of the buffer, the plates were blocked by adding

300  $\mu\text{L}$  of PBS containing 5% tween 20, 5% sucrose, and 0.05%  $\text{NaN}_3$  to each well and incubated at room temperature for a minimum of 1 hour. 100  $\mu\text{L}$  of blood plasma sample per well was added, the ELISA plate was covered with an adhesive strip and incubated 2 hours at room temperature. 100  $\mu\text{L}$  of streptavidin HRP (R & D Systems, Catalog # DY998, 1/200 in appropriate diluent) was added to each well; the plate was covered and incubated for 20 minutes at room temperature. After subsequent addition of substrate solution and stop solution (both from R & D Systems, Inc), the optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

All samples for ET-1 measurement were tested in duplicate. ET-1 was determined by an enzyme immunoassay (QuantiGlo Human ET-1, R & D Systems, Inc, Minneapolis, Minn, USA). The minimum detectable dose of ET-1 was 0.16 pg/mL, with intra- and interassay coefficients of variation of 2.5 and 5%, respectively.

Statistical evaluations were made by means of SPSS 11.0 package program. All the results were given primarily as medium and standard deviation. Moreover, in the differential diagnosis the meaningfulness of the ET-1 and TGF- $\beta$  values was investigated using the Scheffe and Tukey post hoc tests, in the followup, the differences of the ET-1 levels were investigated using the Kruskal-Wallis variance analysis. In all of the results  $P < .05$  was accepted as meaningful.

## RESULTS

The newborns comprising the study group had a gestational age between 28–42 weeks. From all of the patients 62 (62%) newborns were diagnosed as RDS and all of them were premature. Of 24 newborns with RDS-2 diagnosis 16 (67%) were mature and of 14 newborns with MAS diagnosis 11 (79%) were mature. In the control group, only 11 (55%) newborns were mature (Table 1). Male predominance with a percentage of 61% was determined in the group with RDS, and weight results were considerably lower due to prematurity (1367 ± 368 g).

Plasma endothelin-1 and TGF- $\beta$  concentrations of the mature newborns in the control group were measured as 0.77 ± 0.56 pg/mL and 0.25 ± 0.41 pg/mL, respectively, and did not show a significant difference according to the gestational age ( $P > .05$ ) (Figure 1).

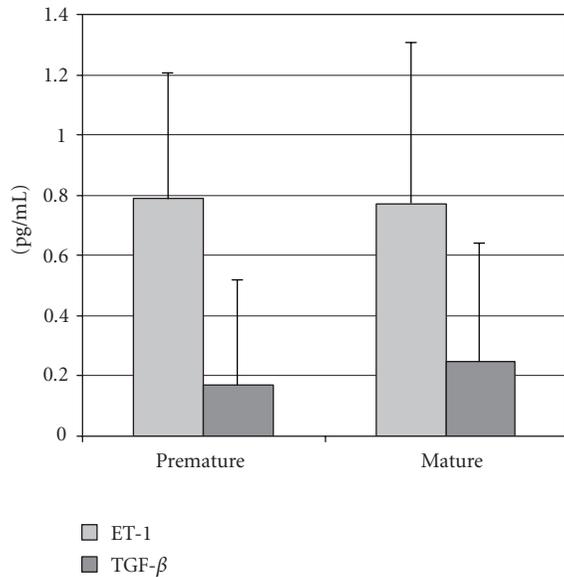


FIGURE 1: Plasma ET-1 concentrations of the healthy premature and mature newborns were  $0.79 \pm 0.44$  and  $0.77 \pm 0.56$  pg/mL, respectively. This difference was not significant ( $P > .05$ ). Also TGF-β concentrations in this group were  $0.17 \pm 0.37$  and  $0.25 \pm 0.41$  pg/mL, respectively, and was not significantly different ( $P > .05$ ).

Plasma endothelin-1 and TGF-β concentrations of the newborns with different diagnosis measured in the first six hours of life are summarized in Figure 2. In the description of the effectiveness of the treatment and in the early determination of the prognosis plasma ET-1 and TGF-β concentrations in newborns with MAS diagnosis were determined as  $5.70 \pm 5.87$  pg/mL and  $3.75 \pm 1.94$  pg/mL, and with RDS diagnosis were determined as  $3.37 \pm 1.59$  pg/mL and  $2.05 \pm 0.98$  pg/mL. According to the control group, plasma ET-1 and TGF-β concentrations of the newborns with respiratory distress were determined to be statistically higher ( $P < .05$ ).

With the oxygen supply, it was observed that plasma ET-1 concentrations of the newborns obtained in the first six hours of life are desposed to decrease regularly in the following days (Figure 3). In the same way, plasma ET-1 concentrations obtained in the first six hours and repetitive plasma ET-1 concentrations in the following days were statistically higher in the newborns who died later compared to the survivors ( $P < .05$ ) (Figure 4).

**DISCUSSION**

Respiratory distress is a major problem in the newborns and different reasons cause this problem. Among those, the most frequent ones are RDS; a problem of premature newborns, and the others are RDS-2 and MAS; a problem of mature newborns. In this study, diseases causing respiratory distress after birth in the early period were evaluated, and RDS was the most frequent (62%).

Endothelin-1 and TGF-β levels, the vascular factor, and material of respiratory distress were investigated. In the

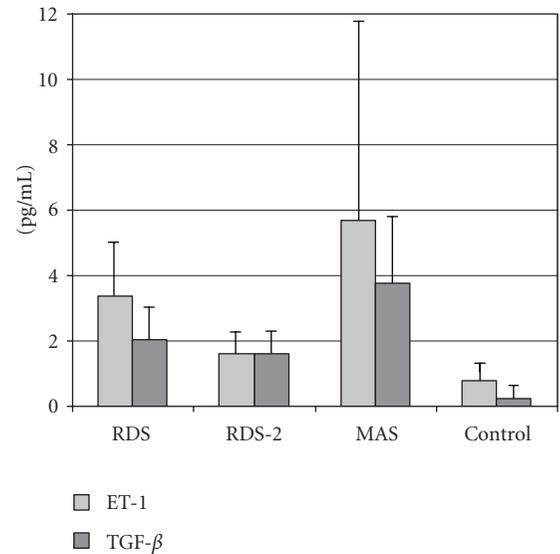


FIGURE 2: ET-1 concentrations according to diagnosis in the sick newborns at the sixth hour after birth were  $3.37 \pm 1.59$  pg/mL in RDS,  $1.60 \pm 0.66$  pg/mL in RDS-2,  $5.70 \pm 5.87$  pg/mL in MAS, and  $0.78 \pm 0.50$  pg/mL in healthy group. Only the concentrations of ET-1 in RDS and MAS groups were significantly different ( $P < .05$ ). Also plasma TGF-β concentrations were  $2.05 \pm 0.98$  pg/mL in RDS,  $1.59 \pm 0.66$  pg/mL in RDS-2,  $3.75 \pm 1.94$  pg/mL in MAS and  $0.22 \pm 0.39$  pg/mL in healthy group. Only the difference in RDS and MAS group was significant ( $P < .05$ ).

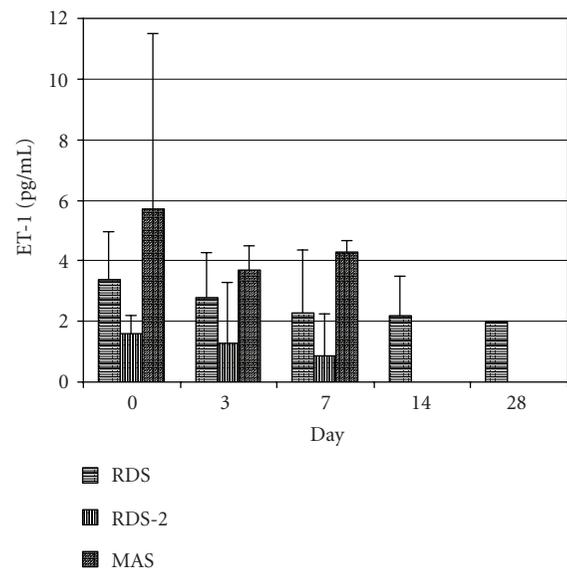


FIGURE 3: Changes of the plasma ET-1 ( pg/mL) concentrations during the oxygen treatment days in the different dignostic newborns tended to decrease. The plasma ET-1 concentrations in the sixth hour of life were decreased at third day as  $2.80 \pm 1.49$  pg/mL in RDS,  $1.28 \pm 0.84$  pg/mL in RDS-2, and  $3.69 \pm 2.13$  pg/mL in MAS group. This values also was significantly different as ( $P < .05$ ). Values at the seventh and simultaneous days were also decreased.

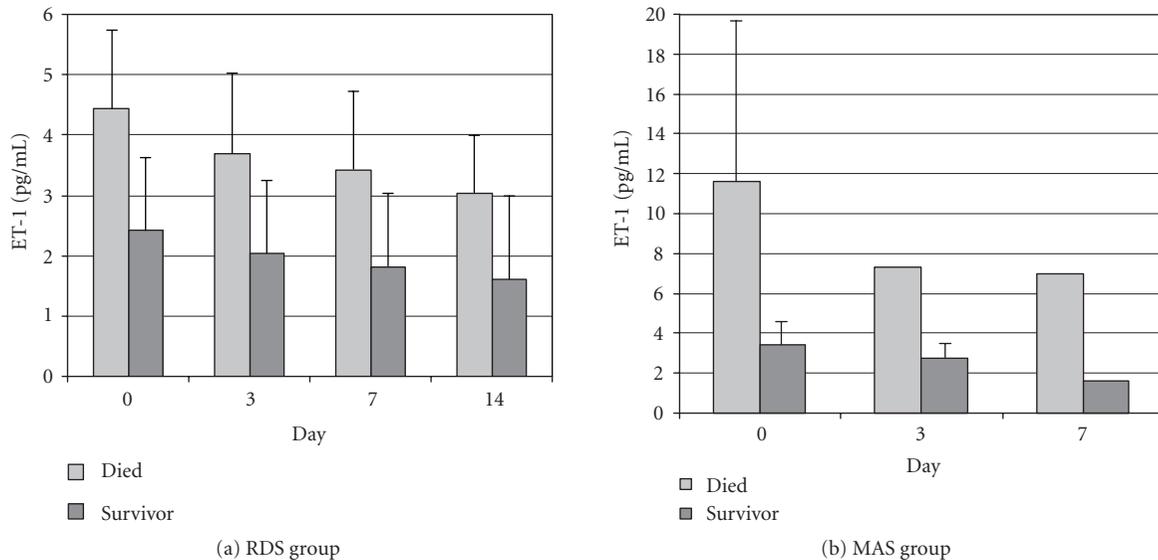


FIGURE 4: ET-1 concentrations in the newborns who died were  $4.44 \pm 1.26$  pg/mL in RDS and  $11.60 \pm 8.97$  pg/mL in MAS groups ( $P < .05$ ) and in those who survived were  $2.42 \pm 1.19$  pg/mL in RDS and  $3.41 \pm 1.31$  pg/mL ( $P < .05$ ) at the sixth hour of life. ET-1 concentrations in the newborns who died were  $3.70 \pm 1.31$  pg/mL in RDS and  $7.30 \pm 0.00$  pg/mL in MAS group ( $P < .05$ ) and in those who survived were  $2.04 \pm 1.20$  pg/mL in RDS and  $2.77 \pm 0.73$  pg/mL ( $P < .05$ ) at the third day of life. ET-1 concentrations in the newborns who died were  $3.42 \pm 1.27$  pg/mL in RDS and  $7.00 \pm 0.00$  pg/mL in MAS group ( $P < .05$ ) and in those who survived were  $1.82 \pm 1.15$  pg/mL in RDS and  $1.60 \pm 0.00$  pg/mL ( $P < .05$ ) at the seventh day of life. ET-1 concentrations in the newborns who died were  $3.03 \pm 0.95$  pg/mL and in those who survived were  $1.60 \pm 1.35$  pg/mL in RDS ( $P < .05$ ) at the 14th day of life.

control and patient groups' plasma, ET-1 and TGF- $\beta$  concentrations were statistically different. The highest value was obtained in the newborns with MAS and the others were ordered as RDS, RDS-2, and the healthy newborns.

Kaapa et al [16] in a similar study found that plasma ET-1 concentrations were not correlated with the pulmonary pressure but that high plasma concentrations of ET-1 reflected severe pulmonary damage. In another study, there was a significantly higher ET-1 concentration in newborns with pulmonary hypertension than healthy newborns or newborns with RDS [17]. In contrast of our study, Kuo et al [18] determined the highest values of plasma ET-1 concentrations in the first six hours of life in the newborns diagnosed as RDS, and the newborns diagnosed as MAS had a second highest values of ET-1 concentrations. Like our study, Kojima et al [19] found out that plasma ET-1 concentrations in newborns with RDS were higher compared to the newborns with RDS-2. A study of Benjamin et al [20] demonstrated that infants with and without RDS had similar umbilical cord ET-1 concentrations, whereas ET-1 concentrations were higher in RDS than in control newborns 18–40 hours after birth. The increased vascular resistance in RDS may be related to high plasma ET-1 concentrations.

In an experimental model of RDS in the newborn lamb, the ET-1 concentration was increased after induction of RDS concomitant with the development of pulmonary hypertension, from an early time point onwards. Increased ET-1 concentration during RDS appeared to be reached in the early phase of pulmonary hypertension development. Also increased circulating levels of ET-1 were correlated with the severity of pulmonary hypertension [21].

Whereas, TGF- $\beta$  is secreted from the alveolar macrophages in the lungs, and in case of damage it is responsible to the organization of the fibrosis growth, inflammatory response, and the recovery of the tissue [22]. For this reason, the TGF- $\beta$  studies were realized in the patients with bronchopulmonary dysplasia, where fibrosis was dominated [23, 24]. In our study, according to the results of plasma ET-1 levels, the first highest levels of TGF- $\beta$  were in newborns with MAS and the second were in newborns with RDS.

Starting from the moment of the diagnosis, it was observed that ET-1 concentrations of the patients who received surfactant and mechanic ventilator supply were decreased. Kuo et al [18] and Niu et al [25] emphasized those plasma ET-1 concentrations of the newborns with and without bronchopulmonary dysplasia did not show any difference. The endothelium modulates vascular tone by releasing endothelium-derived vasodilators, including nitric oxide, prostacyclin, bradykinin, and vasoconstrictors such as ET-1 and angiotensin II, in response to a number of biochemical and physical stimuli. Recent studies have suggested that an imbalance between nitric oxide and ET-1 may contribute to changes in vascular tone observed in these diseases. A number of vasculopathies associated with an impaired bioavailability of nitric oxide have been found to be linked to enhanced synthesis of ET-1 [26].

In our study, plasma ET-1 concentrations might have a best indicator of the prognosis in the first six hours of life, but TGF- $\beta$  concentrations did not have the same effect, because it was a significant difference between the survivors and dead newborns, in whom ET-1 concentrations were higher in the first six hours of life. These newborns presented severe

damage in the lungs, starting from the first hour. We did not see any other study emphasizing this subject in the literature.

As a result, it was decided that, in the differentiating diagnosis of the RDS, RDS-2, and MAS, which are a significant problem of premature and mature newborns, the investigation of ET-1 and TGF- $\beta$  concentrations is meaningful, but that in wider groups, it is required to determine the borderline values. It was observed that the ET-1 levels investigated in the first six hours are more useful in determining the prognosis, and the ET-1 concentrations investigated in the following days are more meaningful presenting clinical recovery. In the determination of prognosis, TGF- $\beta$  concentration investigated in the first six hours does not seem meaningful. Since the results are still contradictory, it was emphasized that it is required to carry out new researches.

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