Evaluation of Renal Functions in Preterm Infants With Respiratory Distress Syndrome

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Background: The aim of this prospective study was to evaluate urinary glutathione S transferases π (GST- π), beta-2-microglobulin (B2-MG), and N-acetyl- β -D-glucosaminidase (NAG) levels as markers revealing the effect of respiratory distress syndrome (RDS) on renal function in preterm infants. **Methods:** The study was performed with 76 preterm infants whose gestational ages were between 28 and 32 weeks. Twenty-six preterm infants with RDS (cases) and 50 preterm infants without RDS (controls) enrolled in the study. Blood and urine samples were obtained on postnatal (PN) day 3 and 30. Urinary GST- π levels were measured by enzyme-linked immunosorbent assay (ELISA), and urinary B2-MG levels were determined by nephelometric method. **Results:** There was no

significant difference in urinary B2-MG and GST- π levels between RDS and non-RDS groups on PN day 3 ($P > 0.05$ for each). However, preterm infants with RDS had significantly higher urinary B2-MG and GST- π levels than the control group on PN day 30 ($P = 0.0001$ and $P = 0.031$, respectively). Urinary NAG levels were higher in RDS group than those of the controls on both PN day 3 and 30, but these findings were not statistically significant ($P > 0.05$, for each). **Conclusion:** Preterm infants with RDS had increased levels of both GST- π and B2-MG levels on PN day 30, suggesting subclinical tubular dysfunction, probably secondary to hypoxic stress. J. Clin. Lab. Anal. 28:310–314, 2014. © 2014 Wiley Periodicals, Inc.

Key words: beta-2-microglobulin; glutathione S-transferase π ; N-acetyl- β -D-glucosaminidase; preterm infants; renal function; respiratory distress syndrome

INTRODUCTION

Although considerable progress has been made in fetal/maternal patient cares, the frequency of preterm deliveries remains high and practically appears to be increasing (1). Preterm delivery is responsible for 70–85% of fetal, neonatal, and infant deaths, due to several complications. Respiratory distress syndrome (RDS) is the most frequent cause of morbidity and mortality in preterm infants (2). Despite advances in perinatal and neonatal care in RDS prevention and treatment, a large number of infants with RDS suffer from acute kidney injury (3–6).

Glutathione S transferases (GST) are detoxifying isoenzymes that catalyze the conjugation of glutathione to a range of electrophilic compounds (7, 8). The GST is present in all organs, including adrenal glands, liver, kidney, small intestine, testis, and ovary. Three major classes of human GST isoenzymes are α , π , and μ forms (GST- α , GST- π , and GST- μ). Human kidney contains the GST- α

and GST- π forms in relatively large amounts. GST- α is present only in the proximal tubular system, whereas the GST- π is secreted in distal tubules and collecting duct (9). Cytoplasmic GST could be abundant (as much as 2% of the total cytoplasmic proteins) but increased cell membrane permeability and cell disruption result in appearance of these molecules in the extracellular spaces and urine. Therefore, urinary GST may serve as a diagnostic marker for renal disease and damage. Currently, urinary GST- α /and - π have been used as a marker of nephrotoxicity induced by drugs and chemicals (10–12)

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and acute transplant rejection (10, 11). Measurement of urinary GST- α and GST- π may allow differentiating cell damage between proximal and distal tubules. Moreover, urinary excretion of GST- π , but not GST- α , is inversely correlated with creatinine (Cr) clearance in patients with renal impairment (13, 14).

Several other markers have also been used to investigate tubular cell damage in infants. The best-known markers are low molecular weight proteins such as α_1 -microglobulin and beta-2-microglobulin (B2-MG; 15, 16) and cellular enzymes such as *N*-acetyl- β -D-glucosaminidase (NAG; 17). Unfortunately, most of these markers mainly reflect proximal tubular cell damage. Reliable markers of distal tubular damage are required for the corresponding population.

The aim of the present study was to evaluate urinary excretion of π isoenzyme of GST and to understand its relationship with the other urinary markers and with RDS in preterm infants. Our study measured four urinary markers: GST- π (a marker of distal tubular damage, 18) and NAG (a marker of proximal tubular damage, 17). Proximal tubular reabsorption was investigated by assessing urinary B2-MG (16, 19), and distal tubular reabsorption capacity was investigated by fractional excretion of sodium (FENa, 20). Glomerular function was assessed by measuring of serum creatinine (SCr).

MATERIALS AND METHODS

Patient Selection

The study group consisted of preterm infants between 28 and 32 gestational weeks (GW) who were admitted to neonatal intensive care units of our hospital. Seventy-six preterm infants were enrolled in this prospective case-control study. Of those, 26 preterm infants with RDS (RDS group) were matched by same GW and sex, and 50 preterm infants who were not developed RDS (control group) during hospitalization. Clinical and laboratory characteristics of both groups were compared. Any infant who had suspected or culture-proven sepsis, congenital heart disease (such as patent ductus arteriosus), severe perinatal asphyxia or hypotension, urinary tract infections; and those having received exchange transfusion or nephrotoxic medications (such as aminoglycosides, indomethacine, or ibuprofen, etc.) and preterm infants with renal or any other congenital anomalies were excluded. In addition, preterm infants whose mothers have had chronic or pregnancy-induced hypertension, gestational diabetes, hypotension, urinary tract infections, clinical chorioamnionitis, preeclampsia/eclampsia were also excluded.

Clinical Data Collection

Demographic and perinatal characteristics including GW, gender, birth weight (BW) and length, small for gestational age status, mode of delivery, intubation at birth, and Apgar scores at 1 and 5 min were recorded. In infants with RDS, additional parameters such as blood gases levels at the admission, oliguria (urine output <0.5 ml/kg per hour for more than 8 hr after birth), use of mechanical ventilation, and surfactant treatment were also recorded. Urine output was collected in sterile bags or urine catheters for 8 hr. GW was determined by calculation and/or antenatal ultrasonography, and were confirmed by Ballard score at postnatal (PN) period. RDS was diagnosed according to clinical and radiographic criteria.

Estimated glomerular filtration rate (GFR; ml/min/1.73 m²) and FENa levels were also calculated on PN day 3 and 30. GFR was estimated using Schwartz Formula. Schwartz's estimated creatinine clearance (estimated GFR): $k \times \text{height}/\text{SCr}$, the constant k was used as 0.33 for infants who were born before GW of 34. FENa (%) was calculated by the formula; urinary sodium/serum sodium divided by urinary Cr (UCr)/SCr (20).

Sampling Measurements

Peripheral blood sample collected for blood urea nitrogen (BUN), SCr, uric acid; and spot urine sample collected for the evaluation of GST- π , B2-MG, NAG, urine sodium, and UCr were taken on PN day 3 and 30. Biochemical parameters, urine sodium, UCr, and B2-MG were immediately studied. For GST- π and NAG measurement, the urine sample was centrifuged at 4,000 rpm for 8 min and supernatant was stored at -70°C until GST- π and NAG measurement according to the manufacturer's protocol.

Urine B2-MG was studied by nephelometric method using N latex B2-MG kit (Dade Behring Marburg, GmbH, Marburg, Germany) and was expressed in milligrams per liter. For urine GST- π , immunodiagnostic micro ELISA kit was used (Brio Seac Radium Company 50041, Florence, Italy) and was expressed in micrograms per liter. NAG was studied using calorimetric method with Diazyme kit at 505-nm wave length calibrator (Shimadzu UV-1201V Spectrophotometer, Siemens, Columbia, USA). NAG levels were expressed in units per liter.

The procedures were performed according to the Declaration of Helsinki for the ethical standard for human experimentations. The study was approved by the Ethics Committee of Inonu University and written consent forms were signed by the parents before participating in the study.

TABLE 1. Demographic and Perinatal Characteristics of the Preterm Infants With and Without Respiratory Distress Syndrome

	RDS group (n = 26)	Control group (n = 50)	<i>P</i> ^{a,b}
Gestational age (weeks)	29.0 (28.0–29.0)	31.0 (29.7–32.0)	0.0001 ^a
Birth weight (g)	1,206 (1,100–1,396)	1,519 (1,335–1,656)	0.0001 ^a
Birth length (cm)	37.0 (36.0–39.0)	40.0 (37.7–41.0)	0.002 ^a
Male sex (n, %)	12/26 (46.1%)	22/50 (44%)	0.524 ^b
Small for gestational age (n, %)	1/26 (3.8%)	3/50 (6.0%)	0.764 ^b
Vaginal delivery (n, %)	6/26 (23.0%)	15/50 (30.0%)	0.597 ^b
Apgar score 1 min	4.0 (3.0–5.0)	7.5 (7.0–8.0)	0.0001 ^a
Apgar score 5 min	6.0 (5.0–7.0)	9.0 (9.0–10.0)	0.0001 ^a
pH upon admission	7.12 (7.05–7.15)	7.35 (7.32–7.38)	0.0001 ^a

Data were presented as median (25–75% interquartile range), *P*-value is for comparison between controls and patients.

^aMann–Whitney *U* test.

^bChi-square test or Fisher's exact test, *P* < 0.05 is significant.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences program (SPSS) for Windows version 16.0 (SPSS, Inc., Chicago, IL). Distribution of quantitative variables was assessed by Shapiro–Wilk test. The variables did not show a normal distribution (*P* < 0.05). Results are expressed as median (25–75% interquartile range) for quantitative data. Differences between groups were assessed by using Mann–Whitney *U* test. Categorical variables in proportions or percentages were analyzed by Chi-square test or Fisher's exact test, when appropriate. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

The study involved 76 preterm infants. Of those, 12 males/14 females were from RDS group and 22 males/28 females were from control group. There was a significant difference in GW, BW, and height between RDS and control group. No significant difference in terms of gender and mode of delivery was found between the both the groups (*P* > 0.05, for each). In addition, there was a significant difference in Apgar score and blood pH levels at the admission between both the groups (*P* = 0.0001, for each). The demographic and perinatal characteristics are summarized in Table 1.

GST- π levels did not show any significant difference between two groups on PN day 3 (*P* > 0.05). However, GST- π levels were significantly higher in infants with RDS compared to those having no RDS on PN day 30 (*P* = 0.031).

TABLE 2. Biochemical Characteristics of the Preterm Infants With and Without RDS on PN Day 3 and 30

	Day	RDS group (n = 26)	Control group (n = 50)	<i>P</i> ^a
BUN (mg/dl)	3	18.0 (7.0–33.5)	14.0 (7.0–22.0)	0.143 ^a
	30	14.0 (5.0–21.0)	6 (4.0–12.5)	0.010 ^a
SCr (mg/dl)	3	0.99 (0.89–1.12)	0.96 (0.85–1.08)	0.194 ^a
	30	0.52 (0.46–0.65)	0.48 (0.38–0.61)	0.490 ^a
Uric acid (mg/dl)	3	5.1 (3.3–8.4)	4.1 (3.3–6.7)	0.759 ^a
	30	1.1 (1.0–2.1)	2.0 (1.2–2.5)	0.874 ^a
Estimated GFR (ml/min/1.73 m ²)	3	13.2 (11.6–14.4)	13.4 (11.7–15.6)	0.071 ^a
	30	25.3 (21.8–30.4)	29.5 (21.3–38.9)	0.161 ^a
GST- π (mcg/ml)	3	6.6 (4.8–7.7)	6.5 (4.1–8.1)	0.708 ^a
	30	12.0 (8.8–151.6)	8.7 (4.2–22.2)	0.031 ^a
B2-MG (mg/l)	3	4.3 (2.9–7.3)	5.5 (3.6–7.5)	0.501 ^a
	30	10.6 (3.6–23.2)	3.7 (2.1–7.3)	0.0001 ^a
NAG (U/l)	3	3.6 (2.2–4.1)	2.38 (1.2–4.5)	0.620 ^a
	30	8.7 (3.7–11.8)	4.7 (3.3–7.3)	0.223 ^a
FENa (%)	3	4.8 (2.3–6.7)	4.8 (2.5–6.9)	0.041 ^a
	30	1.4 (1.1–2.9)	0.9 (0.5–1.5)	0.040 ^a

Data were presented as median (25–75% interquartile range). *P*-value is for comparison between control and patients.

^aMann–Whitney *U* test, *P* < 0.05 is significant.

In addition, preterm infants with RDS had significantly higher FENa levels on PN day 3 and 30 than the controls (*P* = 0.041 and *P* = 0.040, respectively). Biochemical characteristics of both groups are given in Table 2.

SCr and estimated GFR levels did not show any significant difference between both groups on PN day 3 and 30 (*P* > 0.05). However, serum BUN levels were significantly higher in RDS group on PN day 30 (*P* = 0.010) but not on PN day 3 compared to the controls (*P* > 0.05). Serum uric acid was higher in RDS group than the controls on PN day 3 and 30, there was no significant difference (*P* > 0.05, for each).

There was a trend of higher urinary NAG levels in RDS group than those of the controls both on PN day 3 and 30, but this finding was not statistically significant (*P* > 0.05, for each). However, median B2-MG levels were significantly higher in preterm infants with RDS compared to the control group on PN day 30, but not on PN day 3 (*P* = 0.0001 and *P* > 0.05, respectively).

In addition, other biochemical parameters were compared between PN day 3 and 30 in RDS group, separately. The BUN, SCr, uric acid, and FENa levels were significantly higher on PN day 3 than PN day 30 for the RDS group (*P* = 0.0001, for each). However, estimated GFR, GST- π , B2-MG, and NAG levels were significantly lower on PN day 3 compared to these levels on PN day 30 (*P* = 0.0001, *P* = 0.0001, *P* = 0.006, and *P* = 0.0001, respectively). The results are shown in Table 3.

TABLE 3. Comparison of the Serum Biochemical Parameters Between PN Day 3 and 30 for RDS Group

	PN day 3	PN day 30	<i>P</i> ^a
BUN (mg/dl)	18.0 (7.0–33.5)	14.0 (5.0–21.0)	0.0001 ^a
SCr (mg/dl)	0.99 (0.89–1.12)	0.52 (0.46–0.65)	0.0001 ^a
Uric acid (mg/dl)	5.1 (3.3–8.4)	1.1 (1.0–2.1)	0.0001 ^a
Estimated GFR (ml/min/1.73 m ²)	13.2 (11.6–14.4)	25.3 (21.8–30.4)	0.0001 ^a
GST- π (mcg/l)	6.6 (4.8–7.7)	12.0 (8.8–151.6)	0.0001 ^a
B2-MG (mg/l)	4.3 (2.9–7.3)	10.6 (3.6–23.2)	0.006 ^a
NAG (U/l)	3.6 (2.2–4.1)	8.7 (3.7–11.8)	0.0001 ^a
FENa (%)	4.8 (2.3–6.7)	1.4 (1.1–2.9)	0.0001 ^a

Data were presented as median (25–75% interquartile range). *P*-value is for comparison between PN day 3 and 30 in RDS patients.

^aWilcoxon signed rank test, *P* < 0.05 is significant.

DISCUSSION

Early detection of renal damage is important in the clinical care of preterm infants (21). In this unique population, due to immature glomerular and tubular renal function, adaptation to any external stress may be more difficult and the kidney may also have to work longer for the compensation (22). We compared the urine biomarkers in preterm infants with RDS to those in healthy controls. According to our results, preterm infants with RDS had significantly increased levels of both GST- π and B2-MG levels on PN day 30, suggesting subclinical tubular dysfunction, probably secondary to underlying hypoxic stress. In addition, significantly lower BUN, SCr, uric acid, and FENa levels were observed in the preterm infants with RDS on PN day 30, compared to early days of life. Taken together, results of GST- π and B2-MG urine biomarkers indicated that preterm infants may suffer from acute tubular injury in RDS and associated hypoxic stress.

Gibey et al. (23) reported that term infants have better sodium reabsorption than the preterm infants, and it is resulted in deterioration of distal tubular functions in preterm infants characterized by poor sodium reabsorption. In our study, FENa levels on PN day 3 were significantly higher in the preterm infants with RDS than the preterm infants without RDS, supporting that very premature infants are unable to retain sodium when they are exposed to hypoxic stress even under sodium depletion (24). This failure may be attributable to enzymatic immaturity of the renal tubules, a relatively high extracellular fluid volume, or a relative insensitivity of the tubules to aldosterone (25). In our study, we also observed a significant decrease in FENa on PN day 30, regardless of their RDS status. We think changes in FENa levels between PN day 3 and 30 should be associated with maturation in renal function.

NAG is a glycolytic enzyme found in lysozymes of proximal tubular cells, and increased urinary concentrations

of NAG correlate with proximal tubular damage as its high molecular weight prevents it from filtrating through glomerulus. Therefore, it is used as a noninvasive diagnostic tool to demonstrate the renal damage, especially at proximal tubules caused by toxins (26). It is shown that urinary NAG levels correlate with the severity of damage and indicate pathological changes in kidneys early than routine renal function tests (27). In our study, there was a trend for higher urinary NAG levels in RDS group than the controls on PN day 3 and 30, but it was not statistically significant. According to our results, proximal tubular damage was not a problem in the RDS group.

B2-MG is a low molecular weight protein (molecular weight 12-kDa) that is expressed as major histocompatibility complex class I protein on the surface of most nucleated cells. B2-MG is freely filtered at the glomerulus, but almost totally reabsorbed by the renal tubule in physiological circumstances. Therefore, it is not detected normally in the urine. Elevation of B2-MG in serum reflects renal glomerular dysfunction while its elevation in urine reflects tubular dysfunction (inability to reabsorption) or tubular damage (19, 28). Previous studies have also reported that urinary B2-MG levels are higher in most preterm infants (29, 30). It was reported that the urinary B2-MG levels is only half to one-third on PN day 1 compared to B2-MG levels on PN day 5 or 7 (31, 32). This situation could be explained by better maturation of renal tubular function at PN 1 week or recovery from subclinical transient renal tubular dysfunction. In our study, urinary B2-MG levels increased on PN day 30 in RDS group, which might indicate the existence of subclinical tubular dysfunction, likely secondary to hypoxic stress.

GST- α , $\alpha\beta$ GST- π enzymes are highly stable and retain their antigenic properties. Therefore, these enzymes can be easily and reliably analyzed in urine (9). In normal conditions, the urinary excretion of GST is low. However, the excretion of GST- α increases in conditions affecting proximal tubular function (13–15). Excretion of GST- π increases in acute transplant rejection and acute kidney injury (10, 11, 13, 14). Such indicators could help to differentiate proximal and distal tubular damage in preterm infants. According to our findings, there was no difference in urinary GST- π levels between RDS and control groups on PN day 3, but we found increased urinary GST- π levels on PN day 30 in RDS group, suggesting distal renal tubular damage in this population.

In our study, preterm infants with RDS requiring supplemental oxygen and mechanical ventilation showed higher levels of urinary GST- π , B2-MG, also FENa and NAG levels were high on PN day 30 compared to other preterm infants without RDS. Urinary GST- π , urinary B2-MG, and FENa levels were significantly higher in infants with RDS on PN day 30. NAG levels showed a similar trend but the results were not significant. It is

well known that renal tubules undergo hypoxic injury in various nephropathies. It is not clear that whether distal tubular cells have mechanisms to attenuate this form of injury. According to our results, we suggest that proximal and distal tubular reabsorption are impaired in preterm infants with RDS. We propose that further studies with larger participants should be conducted to explore better the effect of RDS on expression of GST- π , B2-MG, FENa, and NAG levels in the kidney with preterm infants.

In conclusion, the present results demonstrate that urinary excretion of GST- π levels increase in preterm infants with RDS in comparison with the healthy preterm infants without RDS on PN day 30. Our results also showed the potential effect of RDS on the expression of urinary GST- π in preterm infants' kidneys. Therefore, we believe that urinary GST- π levels can be employed as markers of distal tubular damage in preterm infants.

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