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Original Article

Paediatrics Section

Role of Serum Cystatin C Levels in Preterm Neonates with Respiratory Distress Syndrome in Diagnosing Neonatal AKI

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ABSTRACT

Introduction: Despite major advances in perinatal and neonatal care in Respiratory Distress Syndrome (RDS) prevention and treatment, a considerable number of these neonates suffer from RDS along with Acute Kidney Injury (AKI), and concomitant occurrence of RDS and AKI in neonates is associated with poor outcome.

Aim: To determine Serum Cystatin C (sCysC) level and Serum Creatinine (sCr) level in preterm neonates with RDS, and to find correlation, if any, between the sCysC level and sCr level in diagnosing neonatal AKI in them.

Materials and Methods: After dividing the preterm neonates into three groups based on Gestational Age (GA): (24-27 weeks; five each in cases and controls; 28-33 weeks; 15 each in cases and controls; and 34-37 weeks; 20 each in cases and controls), two case-control analyses were conducted. In the first one, sCysC levels were compared between neonates with RDS and the control group. In the second one, sCysC levels were compared between neonates having RDS and AKI (RDS-AKI subgroup), neonates having RDS but no AKI (RDS-no AKI subgroup), and the healthy control group. Student's Independent t-test was used for statistical analyses.

Results: Out of 80 preterm neonates, 40 had RDS and 40 were healthy without RDS taken as controls. There were 10 neonates with AKI (RDS-AKI sub-group), and 30 neonates with RDS without AKI (RDS-no AKI sub-group). No significant differences in sCr levels were found among the RDS and control subgroups on day 3rd and day 30th of life (p=0.151 and 0.658). Serum Cystatin C levels in the RDS-AKI subgroup were significantly higher than in both the RDS-no AKI subgroup and the control group on day-3 (p<0.001). Statistically significant differences in birth weights were observed among the RDS-AKI, RDS-no AKI and control subgroups as depicted in (p=0.025).

Conclusion: sCysC is an independent predictor of AKI in preterm neonates with RDS.

Keywords: Creatinine, Kidney injury, Neonatal intensive care unit, Respiratory failure

INTRODUCTION

Renal functions in neonates are unique as their GFR may vary depending on the degree of renal development at birth [1]. In preterm neonates' renal development is still ongoing and renal function is accordingly immature and are potentially susceptible to injury during the early postnatal period [2]. These preterm neonates when hospitalised in Neonatal Intensive Care Units (NICUs) are at high risk of developing the AKI, which in most cases is prerenal in origin [3,4]. The predominant risk factors for impaired renal function in neonates are: low gestational age, low birth weight, postnatal administration of vasopressors, indomethacin, antibiotics, postnatal illness and use of positive pressure ventilation [5].

One of the common risk factors remains the lung immaturity and subsequent development of RDS [6]. Among the risk factors, low-birth weight and prematurity stand the most important ones for the lung injury. One of the most frequent forms of lung injury in preterm neonates is RDS, which is a common cause of neonatal morbidity and mortality [7]. It is estimated that almost 50% of neonates with Birth Weight (BW) under 1,500 g are affected by RDS [8].

Despite major advances in perinatal and neonatal care in RDS prevention and treatment, a considerable number of these neonates suffer from RDS along with AKI. Concomitant occurrence of RDS and AKI in neonates is associated with poor outcome [9].

Although sCr is the most commonly used marker of the kidney function, but its levels are influenced by muscle mass, sex, age, diet and body composition, as well as its transplacental transport during the fetal life [10]. Due to its transplacental passage and dynamic GFR in preterm neonates' alternatives like serum Cystatin C (sCysC), is presumed to be the better marker of kidney function in neonates. Cystatin C is a 13 -KDa protein, easily filterable and completely metabolised by proximal renal tubules implying an ideal marker for estimation of GFR [11].

In newborns, the sCysC levels are independent of sex, GA, length, muscle mass, bilirubin level, maternal health status [12]. It has been observed that sCysC levels in fetus do not bear any relationship to maternal sCysC levels, hence considered as good marker of GFR in neonates [13]. Moreover, sCr interpretation in preterm neonates makes it more difficult to achieve a consensus regarding renal damage. Keeping in view all these difficulties, in assessing the renal function in hospitalised preterm neonates with RDS, the new biomarkers like Cystatin C is expected to be of greater importance in these situations.

We therefore, undertook this study to compare Cystatin C and Creatinine as markers of GFR in preterm neonates with RDS.

MATERIALS AND METHODS

The present study was a case-control study which was conducted from April 2016 to September 2017, at Post Graduate Department of Paediatrics, GB Pant Hospital, an associated hospital of Government Medical College, Srinagar, Jammu and Kashmir, India. Our study was approved by the Ethical Committee of Government Medical College Srinagar via communication No. 130/ETH/GMC/ICMR; dated 19-03-2016 and written consent forms were signed by the parents before participating in the study.

It Include preterms neonates (Gestational age <37 weeks) with RDS and healthy preterm neonates without RDS admitted in Neonatology unit of the Department of Pediatrics, GB Pant Hospital. GA was assessed by first trimester USG/Last Menstrual Period (LMP) and New Ballard Score System. Exclusion criteria was- Preterm neonate whose mothers were having renal failure, autoimmune disease,

sepsis, chronic or pregnancy induced hypertension, or were on drugs that could have interfered with fetal renal function. In addition, following class of preterm neonates were kept off from the study group: Newborns with major kidney malformation, hepatic, heart or kidney failure, hemolytic disease, neonatal hyperbilirubinemia. Based on available literature about the incidence of the neonatal AKI [14], we took a sample size of 80 neonates, with 40 cases and controls each.

Methods: Preterm neonates with RDS and healthy preterm neonates without respiratory distress syndrome underwent detailed background history and thorough clinical examination. The history included GA, sex, birth order, consanguinity, place of delivery, mode of delivery, perinatal history (asphyxia, meconium aspiration syndrome, resuscitation at birth, mechanical ventilation), obstetric history, family history which was followed by thorough clinical examination. Findings were recorded in predesigned proforma.

Neonates were classified based on GA: (24-27 weeks; 5 each in cases and controls; 28-33 weeks; 15 each in cases and controls; and 34-37 weeks; 20 each in cases and controls). The analysed variables were GA, weight, gender and administration of nephrotoxic drugs. Two case-control analyses were conducted. In the first one, sCysC levels were compared between neonates having RDS and the control group. In the second one, sCysC levels were compared between neonates having RDS and AKI (RDS-AKI subgroup), neonates having RDS but no AKI (RDS-no AKI subgroup), and the healthy control group. Urine output was collected and measured during an 8-h period by collecting into bags or through a urinary catheter.

Sampling and Laboratory Measurements: All study neonates underwent peripheral blood sampling for biochemical parameters such as Blood Urea Nitrogen (BUN), sCr, and sCysC on post-natal day 3 and 30. Serum Cr levels were monitored in all preterm neonates who had AKI. All biochemical parameters except sCysC were immediately analysed. For sCysC, serum was taken from the blood and separated by centrifugation at 4,000 rpm for 8 minutes and stored at -70°C. Storage and assays were performed as per the manufacturer's protocol. sCysC levels were expressed in mg/l. Neonatal AKI diagnosis was based on neonatal RIFLE (nRIFLE) criteria, as per KDIGO 2012 [15] as is described in [Table/Fig-1]. Serum Cr levels were monitored in all preterm neonates who had AKI.

Stage	S. Creatinine (SCr)	Urine output
0	No change in SCr or rise <0.3 mg/dL	≥0.5 mL/kg/h
1	S Cr rise ≥0.3 mg/dL within 48 h or SCr rise ≥1.5-1.9×reference SCr within 7 d	<0.5 mL/kg/h for 6-12
2	SCr rise ≥2.0-2.9×reference SCr	<0.5 mL/kg/h for ≥12h
3	SCr rise ≥3×reference SCr or SCr ≥2.5 mg/dLb or Receipt of dialysis	<0.3 mL/kg/h for ≥24h or anuria for ≥12h

[Table/Fig-1]: Neonatal AKI Staging-KDIGO 2012.
Reference SCr defined as the lowest previous SCr value; SCr value of 2.5 mg/dL represents <10 mL/min/1.73 m^2.

Diagnosis of RDS, was made using the following criteria: 1) PaO_2 <50 mmHg in room air, central cyanosis in room air, a requirement for supplemental oxygen to keep pulse oximetry saturation >85%, or surfactant administration in the first 48 hours of life; and 2) a chest x-ray with a reticulogranular appearance to the lung fields with or without low lung volumes and air bronchograms within the first 24 hours of life [16].

STATISTICAL ANALYSIS

The data was analysed by using the Statistical Package for Social Sciences program (SPSS) for Windows version 16.0. Normal distribution was represented by mean and Standard Deviation (SD), whereas skewed distribution was expressed by median. For groups of more than two, one-way analysis of variance (ANOVA) was used. Categorical variables in proportions or percentages were analysed by Chi-square test or Fisher's-exact test or student's t-test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Our study consisted of a total of 80 preterm neonates, among whom, males and females comprised of 46, and 34 respectively. Neonates were classified into three groups based on GA as shown in [Table/Fig-2].

PMA (Weeks)	RDS group		Control group		p-value@
	No.	%age	No.	%age	
24-27	5	12.5	5	12.5	1.000
28-33	15	37.5	15	37.5	
34-37	20	50.0	20	50.0	
Total	40	100	40	100	

[Table/Fig-2]: Distribution of study population as per Post Menstrual Age (PMA).
@p-value by Chi-square test

Out of 80 preterm neonates, 40 had RDS and 40 were healthy without RDS as the control group. Details of the results are shown in [Table/Fig-2-7]. Following are the noteworthy points summarised as under:

Neonates with RDS; with and without AKI, and controls

Out of 40 neonates with RDS, 10 fulfilled the criteria for AKI during the first seven day of life. Among whom, oliguria was present in four neonates and their Cr levels rose to 0.97 and 1.1 mg/dL on 3rd day of life. Six neonates developed AKI between day 3rd and day 7 of life [Table/Fig-3-7]. There was no oliguria in any neonates in the control group and neonates with RDS but no AKI.

Serum Creatinine (sCr) levels:

No significant differences in sCr levels were found among the RDS and control subgroups on day 3rd and day 30th of life as depicted in [Table/Fig-5].

Serum Cystatin C levels:

Serum Cystatin C levels in the RDS-AKI subgroup were significantly higher than in both the RDS-no AKI subgroup and the control group on day-3 (p<0.001) as shown in [Table/Fig-6].

Birth weight

Statistically significant differences in birth weights were observed among the RDS-AKI, RDS-no AKI and control subgroups as depicted in [Table/Fig-7].

AKI	RDS group		Control group		p-value@
	No.	%age	No.	%age	
Present	10	25.0	0	0	<0.001*
Absent	30	75.0	40	100	
Total	40	100	40	100	

[Table/Fig-3]: AKI in study population.
@p-value by Fisher's-exact test

Group	Urine output <0.5 mL/kg for 6-12 hours	<0.5 mL/kg/h for ≥12 h	<0.3 mL /kg/h for ≥24 h or anuria for ≥12 h	p-value@
Control	-	-	-	0.015
RDS-no AKI	-	-	-	
RDS-AKI	3	1	-	

[Table/Fig-4]: Urine output among studied patients on day 3rd of life.
p-value by Fisher's-exact test

DISCUSSION

Depending on the severity of kidney injury and associated co-morbid conditions, neonatal AKI may vary from a minimal kidney insult to complete renal shutdown that requires renal replacement therapy. It has been reported from various studies that the incidence of neonatal AKI ranges from 8 to 24% among the

Parameter	RDS-AKI Group		RDS-no AKI Group		Control Group		
	Mean sCr (mg/dL)	SD	Mean sCr (mg/dL)	SD	Mean sCr (mg/dL)	SD	p-value@
Day 3 Creatinine	0.98	0.374	1.12	0.45	0.94	0.324	0.151
Day 30 Creatinine	0.51	0.223	0.56	0.335	0.58	0.164	0.658

[Table/Fig-5]: Comparison of creatinine day 3rd and day 30th among the studied neonates.
@p-value by Analysis of Variance (ANOVA)

Parameter	RDS-AKI Group		RDS-no AKI Group		Control Group		
	Mean sCr (mg/L)	SD	Mean sCr (mg/L)	SD	Mean sCr (mg/L)	SD	p-value@
Day 3 Serum Cystatin C level	1.43	0.232	1.13	0.316	1.25	0.153	0.003
Day 30 Serum Cystatin C level	1.41	0.262	1.28	0.241	1.22	0.246	0.093

[Table/Fig-6]: Comparison of serum cystatin C levels on day 3rd and 30th among studies neonates.
@p-value by Analysis of Variance (ANOVA)

Group	Mean	SD	p-value@
Control	2.41	0.221	0.025*
RDS-no AKI	1.61	0.193	
RDS-AKI	1.93	0.156	

[Table/Fig-7]: Comparison of birth weight among the studied population.
*Statistically significant difference (p-value<0.05); @p-value by Analysis of variance (ANOVA)

NICU patients and almost one-third of whom are from preterm neonates [17-19].

Our study comprised of 80 preterm neonates, among whom 46 were males and 34 were females. Out of 80 preterm neonates, 40 were having RDS, and 40 were healthy neonates. In our study the mean birth weight was significantly lower in neonates with RDS-AKI sub group than the control group (p=0.025). Similar observations were made by Bansal SC et al., where in, out of 1745 NICU admissions, 74 (4.24%) were diagnosed having AKI, with a male: female ratio of 1.69:1 [20, 21]. In an another elegant study by Stojanović V, et al., where records of 195 patients were analysed, among whom 175 were having RDS. Neonatal AKI was observed in 85 (44%) neonates [22]. Neonates with AKI had significantly lower gestational age (27.6 vs. 30.3 weeks, p<0.001) and birth weight (1047 vs. 1469 g, p<0.001) in comparison to neonates without AKI, all these observations were quite similar to our results.

In our study population, sCysC levels among the RDS-AKI neonates were significantly higher than in RDS-no AKI and the control group on 3rd day of life (p=0.003) as shown in [Table/Fig-6], which is similar to study conducted by Armangil D et al., [23]. In a parallel situation results of our study are matching with the studies conducted by Cataldi L et al., Nova Ac et al., [3,24].

Preterm neonates are at increased risk of AKI because of ongoing and incomplete nephrogenesis and other co-morbid condition like RDS [23,24]. In our study, the incidence of AKI in preterm neonates with RDS was 25%, which is similar to earlier studies where it ranges between 25 to 66% [25].

From our results AKI in preterm neonates is characterised by high sCysC levels in the RDS-AKI group, which supports the hypothesis that sCysC level rises days earlier than sCr hence helpful in detecting AKI earlier than traditional biomarker sCr. This result is in congruency with the other studies [4,26].

Previous studies have demonstrated that reduced GFR leads to increased sCysC and sCr levels. However, sCysC levels are superior or comparable to sCr for detecting AKI in critically ill children and adults [27-29]. In addition to this, sCysC cannot cross the placenta and reflects the true level from the neonate, it could be considered as a reliable indicator of renal function in the first week of life [30]. By comparing sCysC levels in neonates having RDS with AKI and neonates with RDS without AKI, we found that preterm neonates with RDS and AKI had significantly increased levels of sCysC on day 3rd of life which is indicative of low GFR, which was not true for sCr levels in preterm neonates with RDS and AKI on day 3rd of life which is quite in congruent with the study done by [4,26,31]. By this we infer that sCysC is predictive of subsequent development of AKI in neonates with RDS.

LIMITATION

These include: a) sCr and sCysC levels were not measured every day for all patients, thus AKI could have been missed; b) Using neonatal AKI definition where oliguria is one of the defining criteria which in first 48 hours is a normal phenomenon, can overestimate the neonatal AKI; c) It is difficult to determine which neonates are truly healthy, although we tried to classify patients according to the clinical condition of the neonates.

CONCLUSION

Since neonatal serum creatinine reflects maternal creatinine, hence alternative real time kidney function biomarker like sCysC is considered superior. Because sCysC cannot cross the placenta and remains stable after day 3rd of life, it could be considered as a reliable indicator of renal function in neonates, particularly in the first week of life especially in neonates with RDS.

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