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Neonatal acute kidney injury and serum cystatin C as its early predictor: An original research

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***Abstract*---**Aim: To assess cystatin-C as an early biomarker for identifying AKI in newborns at risk for the condition. Methodology: Prospective cohort study in neonates with preterm and term admitted to NICU. The study group comprised of 24 Neonates with risk of AKI & 24 Neonates with no risk of AKI. The Biochemical investigations were done. Collected data was subjected to statistical analysis using SPSS. Results: In this study, there is no significant correlation between gestational age and Creatinine & Cystatin C levels of day 1 & day 5/ death.There is no significant correlation between birth weight and Creatinine day 1, Creatinine & Cystatin C levels of day 5/ death. But positive correlation between birth weight and Cystatin C levels of day 1. Serum Cystatin C can predict development of AKI in at cutoff point of >1.41 mg/l, AUC= 1.000 with sensitivity of 93.3 % and specificity of 97.4 %. Conclusion : The rate of rise of serum cystatin C was earlier and the absolute number was also higher than serum creatinine. Cystatin C levels can predict AKI earlier than serum

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creatinine value. It can predict AKI atleast 1 or 2 days earlier than serum creatinine.

***Keywords*---**neonates, AKI, cystatin C, GFR.

Introduction

Acute Kidney Injury (AKI), is defined as an abrupt (within seven days) reduce in kidney function currently defined as an absolute increase in serum creatinine of more than or equal to 0.3 mg/dL (⩾ 26.4 μmol/L), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline) or a reduction in urine output (oliguria of <1 mL/kg per hour over 24 hours)1,2 The prevalence of AKI varies greatly depending on the research population, ranging from 1% to 56%.3 More than 65% of newborns with AKI are just 7 days old.4 AKI causes a number of difficulties because nitrogenous waste builds up in the body. Multi-organ dysfunction could result from this in turn. Despite their known limitations, serum creatinine level and urine output are currently the accepted indicators of AKI. Their utility in the early identification of AKI is constrained by their low sensitivity and specificity, as well as the fact that renal disease delays the change in creatinine level. Therefore, it is crucial to have a reliable biomarker that can predict the emergence of AKI following renal injury.5 Numerous research are examining the effectiveness of more recent biomarkers for the detection of AKI, including neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and cystatin C.1 The fact that it is produced at a largely constant rate, released into the plasma, and filters through glomeruli with a filter efficiency of more than 99% makes cystatin C one of them and an excellent biomarker of kidney function. A 13 kDa endogenous cysteine proteinase inhibitor is called cystatin-C.5 Cystatin C (cys-C), a protein with a low molecular mass, was formerly known by the names g-trace, post-g-globulin, and gamma-CSF, among others. The term "cystatin" was first used in 1981 by Barrett, who was conducting research on a group of cysteine proteinase inhibitors.6 Simonsen et al.7 initially demonstrated in 1985 that there was a strong inverse relationship between the concentration of this protein in serum and glomerular filtration rate (GFR). Cystatin C is an inhibitor of cystine protease and is a member of the cystatin superfamily of cystine protease inhibitors.8,9 Numerous studies have examined cystatin-C as an endogenous marker of kidney function in populations at risk for or suffering from AKI, and they have found that cystatin-C is more accurate than serum creatinine levels at differentiating between normal and impaired kidney function. Urine cystatin C – as a marker of tubular dysfunction. In renal tubular cells, cytostatin C is rapidly reabsorbed and degraded. This is disrupted in tubular dysfunction cases, and cystatin C is eliminated in the urine as a result. Consequently, cystatin C levels in urine can serve as a sign of tubular dysfunction. Only the kidneys can get rid of cytostatin C by glomerular filtration. There is no additional renal excretion pathway. In this investigation, we assessed cystatin-C as an early biomarker for identifying AKI in newborns at risk for the condition. Additionally, we evaluated how well serum creatinine levels and cystatin-C performed as diagnostic indicators of AKI.

Methodology

The research was conducted at the NICU and Post-natal wards of Government medical college, Nizamabad, Telangana. Institutional Ethical Committee clearance was obtained before starting the study. The parent was given an explanation of the study's goal in their native language. Parental, caregiver, or legal guardian consent was obtained in writing after consultation. At the start of the study, family members and caregivers (a parent or both parents) were assured of the study's confidentiality.
Study design: Prospective cohort study.
Study population:
The study group comprised of 24 Neonates with risk of AKI & 24 Neonates with no risk of AKI
Study duration:1 year (September 2018 to July 2019).
Inclusion criteria: Neonates with preterm and term admitted to NICU.
Exclusion criteria: Neonates with prenatal diagnosis of renal diseases.

Sampling technique

For cases, 1st Sample collected at 0-2nd day and 2nd sample at time of 5th or death. 1st sample: cord blood, 2nd sample: 2ml and For controls, 1st sample collected at zeroth day. 1st sample: cord blood.

Investigations

Cystatin C measured by Particle Enchanced Nephelometric Immune Assay (PENIA). Cystatin C GFR was estimated by Filler's equation [log GFR=1.962+ [1.123xlog (1/cystatin C)]. Creatinine is measured by jaffe method. Creatinine based GFR by Schwartz equation [GFR=k× height/cr, k=0.33]

Reference values

Normal Serum Creatinine level in new-born: 0.8–1.0 mg/dL at 1 day, 0.7–0.8 mg/dL at 3 days, and <0.6 mg/dL by 7 days of life.10 Serum Cystatin C level in new-born – 1.15 to 2.24 mg/dl.11

Statistical analysis

The SPSS 22 version of software was used to analyse the data, which was entered into a Microsoft Excel data sheet. Data that was categorical was displayed as frequencies and proportions. Mean and standard deviation were used to depict continuous data. Using SPSS, the Pearson's correlation was examined. The serum levels of cystatin C and creatinine were plotted against the ROC curve.

Results

In this study, 54 % males & 46 % females in control group, 55 % males & 45% females in AKI risk group and 33% males & 77 % females in AKI group. 21% preterm & 79% term under control group, 66% preterm & 34% under AKI risk group and 83 % preterm & 17 % term under AKI group. 96% birth weight were

2.5 to 3.5 kg & 4% were > 3.5 kg under control group, 28 % were < 2.5 kg .50% each were primi & multipara in control group. 55% were primi & 45% multi in AKI risk group and 50% each primi & multi in AKI group. the maternal risk factors were 22% PIH, 22% GDM & 27% UTI in AKI risk group. 50% PIH, 17% PPROM & 17% UTI in AKI group. 100% were APGAR above 6 at 1st min & 5th min in control group. 5% APGAR below 6 & 95% APGAR above 6 at 1st min and 100% APGAR above 6 at 5th min in AKI risk group. In AKI group 100 % APGAR below 6 at 1st & 5th mins as shown in table 1. The symptoms, conditions affecting morbidity and Nephrotoxic drugs during NICU stay distribution are depicted in table 2.

Assessment of renal injury

Assessment of renal injury was done by measuring Creatinine & Creatinine GFR levels at Day 1 and at day 5/ death and Cystatin C & Cystatin C GFR levels – Day 5/death. The measures were as shown in table 3 and table 4 respectively. In this study, there is no significant correlation between gestational age and Creatinine & Cystatin C levels of day 1 & day 5/ death.There is no significant correlation between birth weight and Creatinine day 1, Creatinine & Cystatin C levels of day 5/ death. But positive correlation between birth weight and Cystatin C levels of day 1. Similarly there is no significant correlation between length and Cystatin C levels of day 1, Creatinine & Cystatin C levels of day 5/ death. But positive correlation between length and Creatinine levels of day 1.But there is positive correlation between Serum Creatinine and Serum Cystatin C levels of Day 1 & Day 5/ death as shown in table 5. Receiver operating characteristic (ROC) curve, table 6 and figure 1 showed that D1 Serum Cystatin C can predict development of AKI in at cutoff point of >1.41 mg/l, AUC= 1.000 with sensitivity of 93.3 % and specificity of 97.4 %.

Discussion

Cystatin-C is the optimal GFR marker. It is entirely and readily filtered by glomerular filtration, and no tubular secretion occurs. Cystatin C is a protein that is not glycosylated. Since it does not bind to plasma proteins, it can only be removed through glomerular filtration. Cystatin C is produced at a steady rate by all nucleated cells in the body. 12 This is because the gene that regulates the production of cystatin C is a housekeeping gene that is continuously expressed. Furthermore, inflammatory processes have little effect on the synthesis of cystatin C.13 Cystatin C is rapidly reabsorbed and destroyed in tubular cells of the kidney. In cases of tubular dysfunction, this process is disrupted and cystatin C is eliminated in the urine in its native form. Consequently, cystatin C levels in urine can serve as an indicator of tubular dysfunction. Hence this study was carried out aimed to determine the specificity and reliability of Serum Cystatin C and Serum Creatinine as markers of GFR in Neonates with risk of Acute Kidney Injury. Out of 24 cases, six (25%) developed AKI in this study which is in line with study conducted by Tarek Mohey El- Gammacya et al., in 2018 14 where 26% developed AKI. Gestational age in this study, 21% preterm & 79% term under control group, 66% preterm & 34% AKI risk group and 83 % preterm & 17 % term under AKI group was in line with Satvik Chaitanya Bansal et al15 study 71% term & 29 % preterm in AKI risk group and 53% preterm & 47% term in

control group. In this study, 100% were APGAR above 6 at 1st min & 5th min in control group. 5% APGAR below 6 & 95% APGAR above 6 at 1st min and 100% APGAR above 6 at 5th min in AKI risk group. In AKI group 100 % APGAR below 6 at 1st & 5th mins. In study conducted by Ahmet Taner Elmas et al,16 Apgar scores at 1 and 5 min were also significantly lower in neonates with RDS compared to the control group regardless of AKI status ($p<0.0001$). In this study, there is no significant correlation between gestational age and Creatinine & Cystatin C levels of day 1 & day 5/ death.There is a positive correlation between birth weight and Cystatin C levels of day 1, length and Creatinine levels of day 1 & Serum Creatinine and Serum Cystatin C levels of Day 1 & Day 5/ death which is in contrast to the study conducted by Milena Treiber et al (2006)17, where no significant correlation between birth weight, Gestational age and creatinine, cystatin C levels. Receiver operating characteristic (ROC) curve showed that D1 Serum Cystatin C can predict development of AKI in at cutoff point of >1.41 mg/l, AUC= 1.000 with sensitivity of 93.3 % and specificity of 97.4 %. D1 Serum Creatinine can predict development of AKI in at cutoff point of >0.61 mg/l, AUC= 0.852 with sensitivity of 66.7 % and specificity of 44.4 %. In study conducted by Mohamed Abdelaziz El-Gamasy et al 18, Cystatin-C had cut off value ≥ 0.6 (mg/L), sensitivity of 85% with specificity 80%, PPV 83.6%, NPV 76.3% and accuracy 82.9%. On other side, serum creatinine had at cut off value ≥ 0.5 (mg/L), sensitivity of 41.5%, specificity 52.7%, PPV 66.3%, NPV 70.3% and accuracy 69.5% and Milena Treiber et al (2014) conducted a study which revealed that, the ROC curves, analyzing the AS versus the control group, showed the biggest AUC for cysC umb, followed by cysC-3, Cr-umb and Cr-3 (0.918; 0.698; 0.692; 0.660).

Conclusion

The incidence of AKI in this study was found to be 25% out of 24 neonates with AKI risk admitted in NICU. There was a higher incidence of AKI in babies born preterm. The risk factors for AKI included oliguria, CCF, nephrotoxic drugs, low APGAR, respiratory distress, birth asphyxia, sepsis and placental abruption. Serum creatinine was significantly higher in babies after day 3. The rate of rise of serum cystatin C was earlier and the absolute number was also higher than serum creatinine. Cystatin C levels can predict AKI earlier than serum creatinine value. It can predict AKI atleast 1 or 2 days earlier than serum creatinine.

References

1. Abrahamson M, Mason RW, Hansson H, Buttle DJ, Grubb A, Ohlsson K. Human cystatin C. Biochem J 1991; 273: 621- 631
2. Abrahamson M. Human cysteine proteinase inhibitors. Isolation, physiological importance, inhibitory mechanism, gene structure and relation to hereditary cerebral hemorrhage. Scand J Clin Lab Invest 1988; 48: 21- 31
3. Ahmet Taner Elmas & Yilmaz Tabel & Özlem Nalbantoğlu Elmas : Serum cystatin C predicts acute kidney injury in preterm neonates with respiratory distress syndrome. Pediatr Nephrol (2013) 28:477–484
4. Bagga A, Sinha A, Gulati A. Protocols in Pediatric Nephrology, 1st e, CPS publishers and Distributors Pvt Ltd.
5. Barrett AJ. Cystatin, the egg white contribution inhibitor of cysteine

proteases. Method Enzymol 1981; 80: 771- 8
6. Grubb A: Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 1992; 38(suppl 1):S20-S27
7. Grubb Ao. cystatin C. properties and uses as diagnostic marker. Adv Clin chem.. 2000;35:63-99.
8. Karel Allegaert, Djalila Mekahli, and John van den Anker. Cystatin C in newborns: a promising renal biomarker in search for standardization and validation. J Matern Fetal Neonatal Med. 2015 ; 28(15): 1833–1838.
9. Milena Treiber, Breda Pec˘ovnik Balon, and Maksimiljan Gorenjak. Cystatin C versus creatinine as a marker of glomerular filtration rate in the newborn. Wien Klin Wochenschr (2006) 118 [Suppl 2]: 66–70.
10. Mohamed Abdelaziz El-Gamasy. Early Predictors of Acute Kidney Injury (AKI) in a Sample of Egyptian Full Term Neonates. Medical & Clinical Reviews, ISSN 2471- 299X Vol.3 No.3:12, 2017
11. Mosby's Diagnostic lab test references; 14th edition 2019.
12. Nada A, Bonachea EM, Askenazi Dj. Acute kidney injury in the fetus and neonates. Semin Fetal Neonatal Med. 2017; 22:90-97
13. Randers E, Erlandsen EJ: Serum cystatin C as an endogenous marker of the renal function—A review. Clin Chem Lab Med 1999; 37:389-395.
14. Satvik Chaitanya Bansal, Archana Somashekhar Nimbalkar, Amit R Kungwani, Dipen Vasudev Patel, Ankur Rajinder Sethi, Somashekhar Marutirao Nimbalkar. Clinical Profile and Outcome of Newborns with Acute Kidney Injury in a Level 3 Neonatal Unit in Western India. Journal of Clinical and Diagnostic Research. 2017 Mar, Vol-11(3): SC01-SC04.
15. Simonsen O, Grubb A, Thysell H. The blood serum concentration of cystatin C (gamma-trace) as a measure of the glomerular filtration rate. Scand J Clin Lab Invest 1985; 45: 97- 101
16. Tarek Mohey El-Gammacya, Dina Mohamed Shinkara, Noha Refaat Mohamedb and Amany Rasmy Al-Halagc: Serum cystatin C as an early predictor of acute kidney injury in preterm neonates with respiratory distress syndrome. Scandinavian journal of clinical and laboratory investigation 2018. https://doi.org/10.1080/00365513.2018.1472803.
17. Vachvanichsanong P, McNeil E, Dissaneevate S, Dissaneevate P,Chanvitan P, Janjindamani W. Neonatal acute kidney injury in a tertiary centre in a developing country. Nephrol Dial Transplant 2012; 27: 973-7.
18. Zappitelli M,Ambalavanan N, Askenazi Dj,et al. Developing a neonatal acute kidney injury research definitation: a report from the NIDDK neonatal AKI workshop. Pediatr Res. 2017;82:569-573.

Tables and graphs

Table 1: distribution of study participants according to demographic data

DEMOGRAPHIC DATA		Controls (24)	Percent	AKI risk (18)	Percent	AKI (6)	Percent	Overall percent
Gender	Male	13	54%	10	55%	2	33%	52%
	Female	11	46%	8	45%	4	77%	48%
Gestational age	Preterm	5	21%	12	66%	5	83%	46%
	Term	19	79%	6	34%	1	17%	54%

	Post term	0	0%	0	0%	0	0%	0%
Birth weight in kgs	< 2.5	0	0%	5	28%	4	67%	18%
	2.5 – 3.5	23	96%	13	72%	2	33%	80%
	>3.5	1	4%	0	0%	0	0%	2%
Mode of delivery	Normal	4	17%	6	33%	1	17%	23%
	Breech	0	0%	0	0%	1	17%	2%
	LSCS	20	83%	12	77%	4	66%	75%
Parity of mother	Primi	12	50%	10	55%	3	50%	52%
	Multi	12	50%	8	45%	3	50%	48%
Maternal risk factors	PIH	0	0%	4	22%	3	50%	29%
	PPROM	0	0%	0	0%	1	17%	4%
	GDM	0	0%	4	22%	0	0%	17%
	UTI	0	0%	5	27%	1	17%	25%

Table 2: Distribution of study participants based on their symptoms, conditions affecting morbidity and nephrotoxic drugs administered during their NICU stay

Various parameters		AKI risk (18)	Percent	AKI (6)	Percent
Symptoms	Respiratory distress	11	61%	3	50%
	Poor perfusion	0	0%	4	66%
	Abdominal distension	2	11%	1	16%
	Apnea	1	5%	2	33%
	Temp. instability	2	11%	0	0%
	Shock	2	11%	1	16%
	Oliguria	0	0%	6	100%
	Anuria	0	0%	0	0%
	Seizure	5	27%	2	33%
	Hypoglycemia	5	27%	4	66%
Conditions affecting morbidity	Respiratory distress	11	61%	3	52%
	Sepsis	7	39%	1	16%
	CCF	0	0%	1	16%
	Placental abruption	0	0%	1	16%
Nephrotic drugs during NICU stay	Aminoglycosides	13	72%	5	83%
	Ibuprofen	0	0%	0	0%
	Indomethacin	0	0%	1	17%

Table 3: Assessment of Renal injury on day 1

Day 1 renal injury assessment	Controls (24)		AKI risk (18)		AKI (6)	
	Mean	Range	Mean	Range	Mean	Range
Creatinine (mg/dl)	0.72	0.46- 0.88	0.64	0.46-0.88	0.95	0.6-1.50
Creatinine GFR (ml/min/1.73 m2)	27.96	22-40	30.17	22-40	21.33	12-31

Cystatin C (mg/dl)	1.74	1.21-1.69	1.61	1.34-1.73	2.12	1.89-2.56
Cystatin C GFR (ml/min/1.73 m2)	42.96	44-59	45.44	42-52	35.50	29-39

Table 4: Assessment of Renal injury on day 5

Day 5 renal injury assessment	AKI risk (18)		AKI (6)	
	Mean	Range	Mean	Range
Creatinine (mg/dl)	0.41	0.21-0.67	0.92	0.30-1.70
Creatinine GFR (ml/min/1.73 m2)	51.44	28-89	28.33	11-62
Cystatin C (mg/dl)	1.41	1.25-1.68	1.87	1.57-2.31
Cystatin C GFR (ml/min/1.73 m2)	51.67	44-57	40.17	32-46

Table 5 : Pearson's correlation – AKI risk group & AKI group

	Creatinine day 1	Cystatin C day 1	Creatinine Day 5	Cystatin C Day 5
Gestational Age	-.052	-.364	-.283	-.234
Sig. (2-tailed)	.809	.080	.181	.271
Birth weight	-.365	-.422*	-.340	-.324
Sig. (2-tailed)	.080	.040	.104	.122
Length	-.432*	-.355	-.287	-.281
Sig. (2-tailed)	.035	.089	.174	.184

Table 6: ROC curve distribution - Cystatin C day 1, Creatinine day 1, Cystatin C day 5 & Creatinine day 5

Biochemical test	Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
S. Creatinine day 1	.852	.097	.011	.662	1.000
S. Cystatin C day 1	1.000	.000	.000	1.000	1.000
S. Creatinine day 5/ death	.806	.127	.028	.556	1.000
S. Cystatin C day 5/ death	.935	.049	.002	.838	1.000

Figure 1: ROC curve