PROSPECTIVE STUDY OF INCIDENCE OF ACUTE RENAL FAILURE IN PRETERM BABIES IN A TERTIARY CENTER IN SOUTH INDIA

A dissertation submitted in partial fulfillment of MD Pediatrics

Examination of the Tamil Nadu Dr. M.G.R Medical

University, Chennai to be held in April 2015

CERTIFICATE

This is to certify that the dissertation entitled "**PROSPECTIVE STUDY OF INCIDENCE OF ACUTE RENAL FAILURE IN PRETERM BABIES IN A TERTIARY CENTER IN SOUTH INDIA**" is the bonafide original work of Dr. Nithya. J. P submitted in partial fulfillment of the requirement for MD Pediatrics Degree Examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2015.

Guide:

Dr. Indira Agarwal

Professor and Head

Department of Child health II

Christian Medical College

Vellore - 632004.

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Co-guide:

Dr. Anil Kurien Kuruvilla

Professor and Head

Department of Neonatology

Christian Medical College

Vellore - 632004.

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HEAD OF DEPARTMENT

Dr. Anna Simon

Professor and Head of Child health

Christian Medical College

Vellore - 632004.

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PRINCIPAL

Dr. Alfred Job Daniel

Christian Medical College

Vellore - 632004.

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CANDIDATE

Dr. Nithya J. P.

PG Trainee

MD Pediatrics

Christian Medical College

Vellore - 632004.

</div>

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ACKNOWLEDGEMENTS

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PROSPECTIVE STUDY OF INCIDENCE OF ACUTE RENAL FAILURE IN PRETERM BABIES IN A TERTIARY CENTER IN SOUTH INDIA

Introduction

Acute kidney injury (formerly known as acute renal failure) is a condition where there is loss of excretory function of the kidney. This leads to various complications due to accumulation of nitrogenous wastes. This in turn may lead to multi-organ dysfunction. Various studies have been done in different populations showing the incidence of acute kidney injury in various conditions and age groups.

Incidence of acute renal failure has been reported in different studies in various countries. The incidence varies from 3.4 to 24% across various countries. Of these, pre-renal type was found to be most common. There is no definite data available on incidence of renal failure in Preterm babies in India. Being a Tertiary care center in South India, our Neonatal intensive care unit is also a referral center and has a high turnover of preterm babies.

Preterm babies were chosen for this study as they are a vulnerable population and are exposed to various risk factors; predisposing them to multi-organ injury. Due to the myriad of complications preterms are exposed to in the early weeks and multiple interventions with prolonged hospitalization, these babies are predisposed to acute kidney injury and its long term consequences. Factors predisposing to acute kidney injury are usually multifactorial. The most common among these being perinatal asphyxia and sepsis. Both these factors are common in preterm babies.

In addition to these, incomplete nephrogenesis also increases the risk of renal failure in preterm babies compared to term babies. Diagnosis of acute kidney injury in this population is difficult as non-

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oliguric renal failure is more common and by the time oliguria sets in, significant renal injury has already taken place. This brings the role novel biomarkers like urine NGAL which has been proved to be useful in diagnosing acute kidney injury in other situations and age groups.

This study was conducted to look at the prevalence of acute kidney injury in preterm babies and also the various risk factors which may pre-dispose to renal injury. Serial monitoring of creatinine and urine output   was done weekly. The usefulness of urinary NGAL as an early marker of renal injury was assessed. Follow up of the   same population to look at residual impaired renal function was planned.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Acute kidney injury (formerly known as acute renal failure) is a condition where there is loss of excretory function of the kidney. This leads to various complications due to accumulation of nitrogenous wastes. This in turn may lead to multi-organ dysfunction. Various studies have been done in different populations showing the incidence of acute kidney injury in various conditions and age groups.

Incidence of acute renal failure in preterm babies has been reported in different studies in various countries. The incidence varies from 3.4 to 24% across various countries. Of these, pre-renal type was found to be most common. (1)

Acute kidney injury (formerly known as acute renal failure) is a complex disorder. Various risk factors predispose different populations to renal injury and the severity also differs depending on the damage that has occurred.

ETIOLOGY OF ACUTE KIDNEY INJURY

Renal failure has been broadly divided into 3 types - pre-renal, intrinsic renal and post-renal causes.

Pre-renal causes include those where there is decreased blood flow to the kidneys. It is either due to volume loss, hypotension and ischemia. These include dehydration, acute blood loss, sepsis, hypoalbuminemia and cardiac failure.

Intrinsic renal causes may be due to a localized condition or as a part of systemic disease. This includes Glomerulonephritis, Hemolytic uremic syndrome, Henoch Schonlein purpura, Acute tubular necrosis, Rhabdomyolysis and Tumor lysis syndrome.

Post-renal causes lead to obstructive uropathy. The obstruction may be at any level and may be

congenital or acquired. These include posterior urethral valve, ureteropelvic/vesicopelvic obstruction, ureterocele, tumour, neurogenic bladder and renal calculi.

Etiology is important as management depends on the cause. In children, the most common cause of renal failure is glomerulonephritis. In neonates, it is commonly pre-renal and most often associated with asphyxia and sepsis.

Studies in Children were done as early as 1970s. Griffin etal described the etiology, clinical features, management and outcome of acute renal failure in 25 children under 2 years of age. Hemolytic uremic syndrome was found to be the common cause. Structural anomalies, septicemia and presumed acute tubular necrosis were the other causes. Mortality rate was 12% and 60% required dialysis as mode of renal replacement therapy. (2)

A Jordanian retrospective study looked at etiology and mortality in neonatal AKI. This was a multi-center retrospective study which considered oliguria along with serum creatinine and blood urea nitrogen levels for diagnosing AKI. Neonatal asphyxia was the most common (40%) cause of mortality and was associated with oliguria. Cumulative mortality rate was 45% in the absence of dialysis. (3)

Mortazavi etal looked at medical records of 151 neonates with AKI. Perinatal asphyxia, sepsis, hyaline membrane diseases were the major causes of AKI. Mortality rate was 20.5%. (1)

Table 1: Causes of renal failure (4)

PRERENAL
Dehydration
Hemorrhage
Sepsis
Hypoalbuminemia
Cardiac failure
INTRINSIC RENAL
Glomerulonephritis
• Postinfectious/poststreptococcal
• Lupus erythematosus
• Henoch-Schönlein purpura
• Membranoproliferative
• Anti-glomerular basement membrane
Hemolytic-uremic syndrome
Acute tubular necrosis
Cortical necrosis
Renal vein thrombosis
Rhabdomyolysis
Acute interstitial nephritis
Tumor infiltration
Tumor lysis syndrome
POSTRENAL
Posterior urethral valves
Ureteropelvic junction obstruction
Ureterovesicular junction obstruction
Ureterocele
Tumor
Urolithiasis
Hemorrhagic cystitis
Neurogenic bladder

Pathophysiology of acute renal failure

Among the different types it is pre-renal causes which commonly lead to renal failure in the acute setting. In the general population, this is mostly seen in sepsis with multi-organ dysfunction; in children with acute gastroenteritis and dehydration and in newborn with asphyxia/sepsis.

Intravascular volume depletion and hypotension
Gastrointestinal tract, renal, dermal losses
Hemorrhage

Decreased effective intravascular volume
Congestive heart failure
Cirrhosis
Nephrosis
Peritonitis

Medications
Cyclosporin A
Tacrolimus
Angiotensin-converting enzyme inhibitors
Nonsteroidal antiinflammatory drugs
Radiocontrast agents
Amphotericin

Hepatorenal syndrome

Generalized or localized reduction in renal blood flow

Acute kidney injury

Sepsis

Renal vascular disease

Large vessel
Renal artery thrombosis
Arterial occlusion during surgery
Renal artery stenosis

Small vessel
Vasculitis
Atheroembolism
Hemolytic uremic syndrome/thrombotic thrombocytopenic purpura
Malignant hypertension
Scleroderma
Preeclampsia
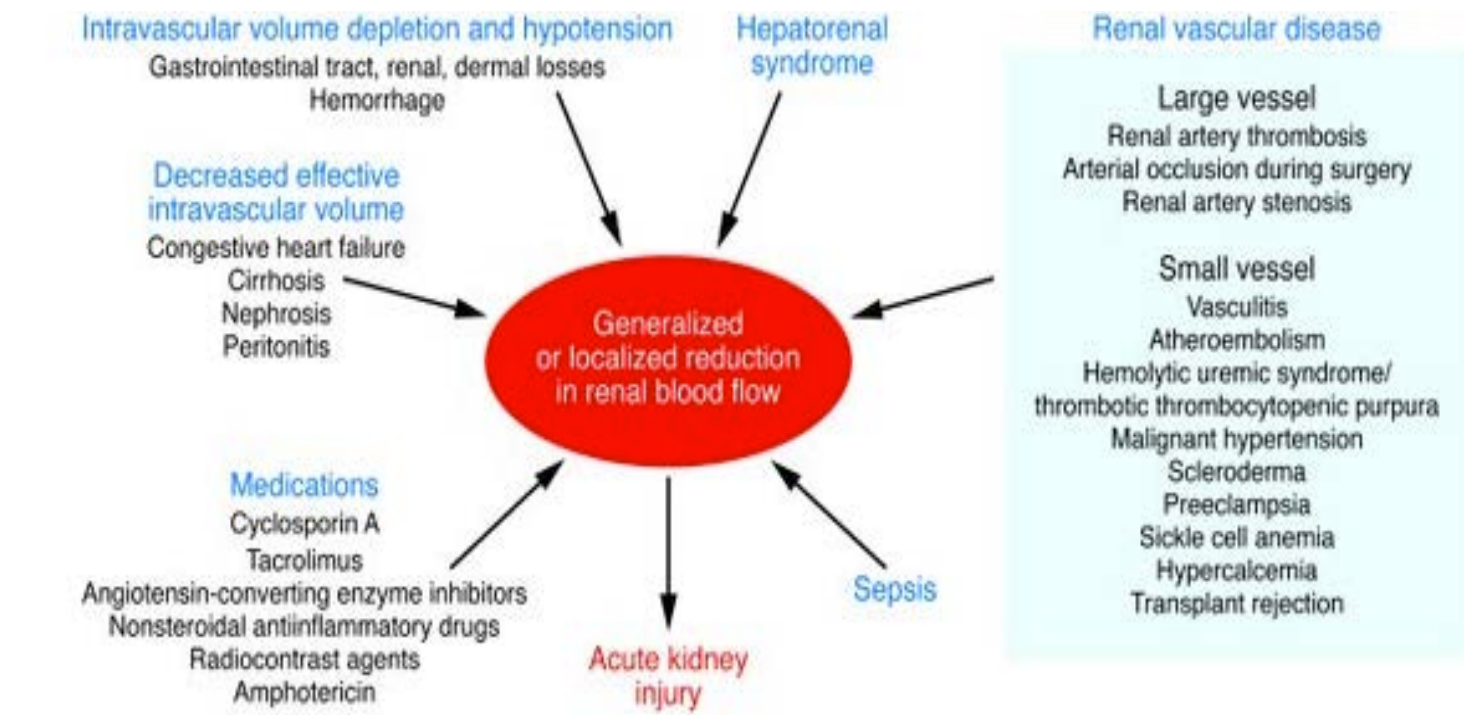Sickle cell anemia
Hypercalcemia
Transplant rejection

Figure 1: Summary of the various conditions which cause either generalized or localized reduction in blood flow to the kidneys. (5)
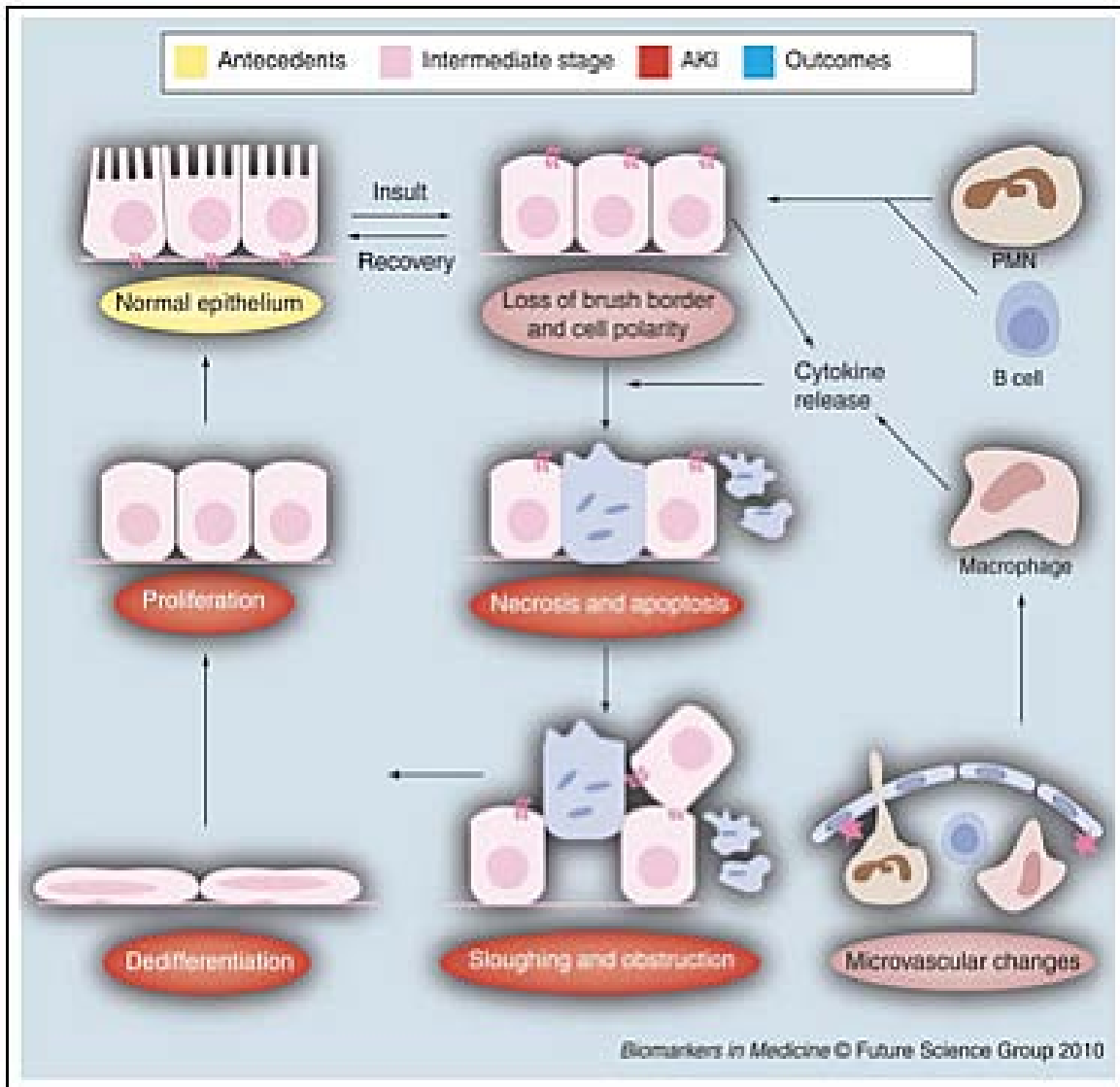
Figure 2: The various stages of kidney injury. Initial reversible injury only leads to loss of brush border. If not treated, there is inflammation leading to necrosis and apoptosis. This may lead sloughing of cells and obstruction. There is also dedifferentiation and proliferation which replaces normal epithelium and affects renal function. (6)

Injury at the cellular level

The endothelial and smooth muscle cells play a major role in acute kidney injury. The renal blood flow has to be affected dramatically to cause acute renal failure. However, what is commonly seen is decreased renal blood flow regionally which leads to impaired function. The role of endothelial cells

is to maintain vascular tone, leukocyte function and smooth muscle responsiveness.

When there is endothelial injury, there is vasoconstriction at arteriolar level in response to the various mediators that are released (endothelin – 1, angiotensin II, thromboxane A2, prostaglandin H2, leukotrienes C4 and D4, adenosine) and the sympathetic nerve stimulation. Acetylcholine, bradykinin and nitric oxide cause decreased vasodilatation. Vasoactive cytokines and endothelin worsen the vasoconstriction worsening the ischemia. Endothelial-leukocyte interaction activates the coagulation cascade and along with vasoconstriction results in regional ischemia (most commonly affects the outer medulla). Proximal tubules are thereby more injured and there is inadequate sodium reabsorption. The macula densa senses this and there is also a functional arteriolar vasoconstriction at the pre-glomerular level. Ultimately, all this leads to decreased glomerular filtration. The endothelial injury also leads to increased microvascular permeability. This in turn leads to interstitial edema.

Tubular cells are passively injured but they also a play a major role in injury by secreting various cytokines and chemokines. White blood cells – neutrophils, monocytes and macrophages aggravate ischemic injury by producing protease, myeloperoxidase, and reactive oxygen species. They increase vascular permeability and reduce cellular integrity of both tubular epithelial and endothelial cells

When there is severe injury, the viable and non-viable cells are desquamated and excreted as casts in the urine. These casts are hallmark findings in acute renal failure. The casts may obstruct the tubules and increase intra-tubular pressure, worsening injury.

When there is AKI, a number of molecules are activated. Of them, KIM-1 (Kidney injury molecule 1) and neutrophil gelatinase associated lipocalin were found to be elevated. Both seem to have a role in limiting immune response in AKI. Their levels increase early in injury and may be useful in diagnosis of AKI.
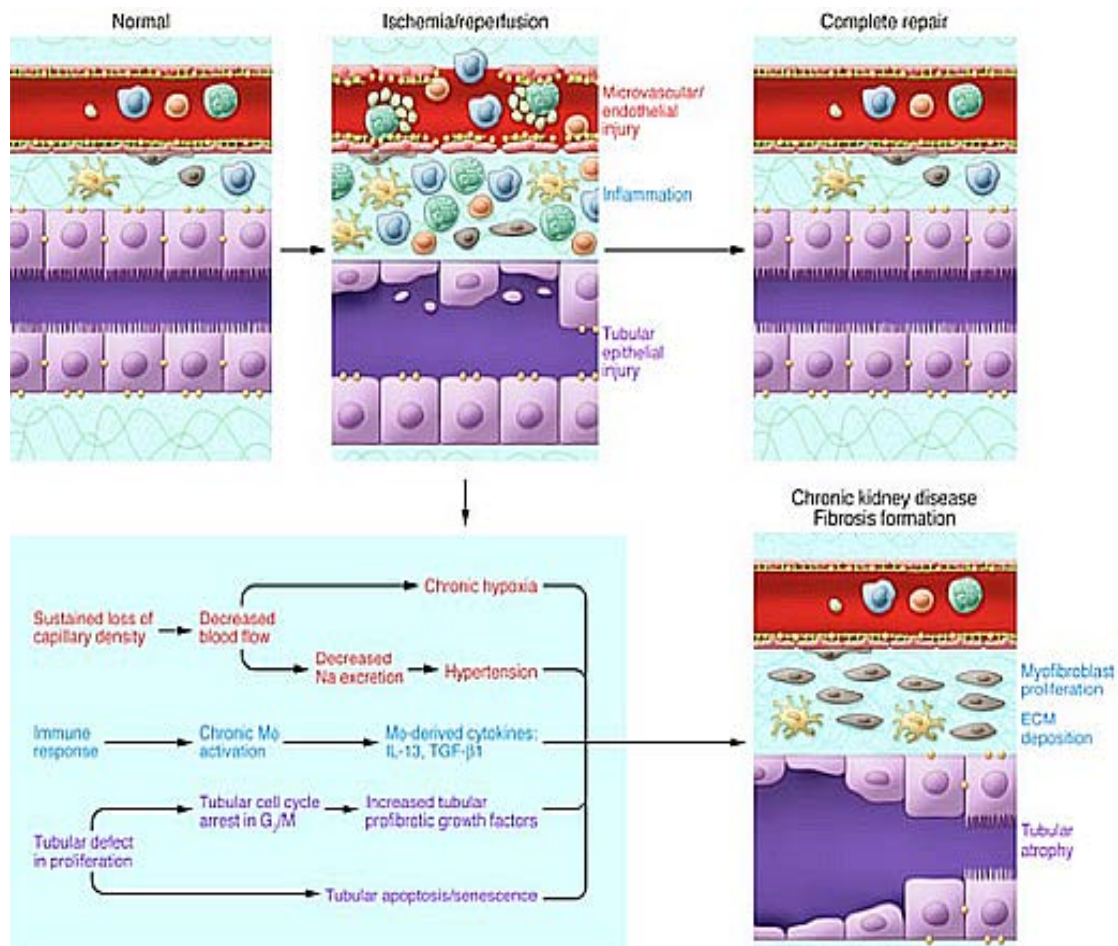
Figure 3: Cellular pathophysiology of acute kidney injury

Repair of epithelium

Normally, when the kidney recovers from the insult, the epithelial cells which were lost will be replaced by surviving cells. Surviving cells dedifferentiate, migrate along the basement membrane, multiply and restore the functional integrity of the nephron.

The normal repair need not occur in every case. In some cases, there is progression of AKI to chronic kidney disease. There are maladaptive changes instead of structured repair leading to fibrosis, incomplete tubular repair, persistent tubulo-interstitial inflammation, fibroblast proliferation and excessive deposition of extracellular matrix. If there is long term hypoxia, there is sustained loss of peri-tubular vessels and chronic fibrosis.

ACUTE KIDNEY INJURY IN PRETERM BABIES

Acute renal failure is characterized by sudden onset of impaired function, which leads to retention of nitrogenous wastes, and there is dysregulation of extracellular fluid, acid base homeostasis and electrolyte imbalance. (7)

ETIOLOGY OF ACUTE KIDNEY INJURY

Factors leading to acute renal failure may be broadly divided into 3 sub-categories – pre-renal, renal and post renal.

Pre-renal azotemia: This is due to decreased blood flow to the kidney.

Decreased blood flow to kidney may be seen due to:

1. Dehydration – increased loss like in diarrhea, increased trans-epidermal loss of free water, post operative in gastro-intestinal surgeries or chest tube losses

2. Blood loss compromising circulatory volume – fetomaternal hemorrhage, perinatal blood loss

3. Capillary leak (infection, hydrops fetalis and low albumin levels)

4. Increased abdominal pressure (post-operatively in gastroschisis, omphalocele, congenital diaphragmatic hernia; ascites and necrotizing enterocolitis)

5. Decreased Cardiac output (cardiac failure)

6. Drugs affecting renal perfusion (Indomethacin and ACE inhibitors)

Renal causes of acute renal failure range from congenital to acquired causes

1. Acute tubular necrosis (may be due to severe ischemia or toxins)

2. Infections (localized to the kidneys or part of severe systemic infections)

3. Vascular causes – renal artery or venous thrombosis

4. Nephrotoxic drugs like Aminoglycosides, Indomethacin, Amphotericin B, radio-contrast dyes and acyclovir

5. Congenital malformations like renal agenesis, dysplastic or polycystic kidneys

Post renal or obstructive causes

These may be congenital or acquired; intrinsic or extrinsic obstruction.

It includes obstruction at various levels – prepuceal, urethral stenosis, posterior urethral valve, pelvi-ureteral junction obstruction.

Extrinsic obstruction may be due to sacrococcygeal teratoma or hematocolpos.

AKI IN PRETERM BABIES

Preterm babies differ from the rest of the pediatric population. They are a more vulnerable population and are prone organ injury. The reason they are more prone to multi-organ injury is due to their immature immune system and incomplete organogenesis.

Higher incidence of asphyxia and occurrence of hyaline membrane disease, intra-ventricular hemorrhage, patent ductus arteriosus, sepsis and effects of nephrotoxic drugs like aminoglycosides and NSAIDS predispose them to multi-organ injury. Nephrogenesis is complete only by 34 weeks. So any preterm baby born before 34 weeks has an immature renal system and being at a higher risk for ischemia, hypovolemia and hypotension; they will be more prone to acute kidney injury. Most common is pre-renal type of renal failure (8)

In the US, premature birth incidence rate was 12.8% of all live births. Risk factors include increasing maternal age, IVF pregnancies, multifetal pregnancies; complications like PIH, use of tobacco and alcohol were some of the contributing to preterm birth in addition to spontaneous preterm labour. (9)

Primiparity, poor socio-economic status, lower educational status and poor maternal nutritional status were found to be some of the predisposing factors to preterm labour by Fathima etal in a study in India (10)

RISK FACTORS IN PRETERM BABIES PREDISPOSING TO AKI

Asphyxia is a common risk factor in preterm babies which leads to hypoperfusion and increases risk of AKI. It accounts for almost one third of all asphyxiated babies and the mortality rates are high in view of other risk factors associated with prematurity. (11) Prematurity – lower the gestational age and birth weight, mode of delivery – Caesarean section are some of the factors associated with increased risk of resuscitation at birth. (12)

Hyaline membrane disease (otherwise known as Respiratory distress syndrome) is the most common complication seen in preterms. There is higher incidence of HMD in Mothers with PIH. (13) With the use of antepartum steroids, non-invasive ventilatory techniques and surfactant use, the incidence of complications following HMD has decreased over the years. The incidence is as high as 28% in a few centers. (14)

Sepsis, both early and late onset sepsis is higher in preterms. With use of intra-partum antibiotics, the infection rates have drastically dropped in the West. Hypotension and hypoperfusion associated with sepsis increases the risk of renal failure. (15)

Necrotizing enterocolitis is a disease of preterm which affects the gut. It is often associated with multi-organ dysfunction. A New South Wales study showed decreasing trend of NEC following the use of antepartum steroids, early enteral feeding and gradual grading up of feeds. The incidence of NEC was 5% in this group. (16)

Other risk factors include use of nephrotoxic drugs like Indomethacin, aminoglycosides, etc. Renal failure is mostly reversible following drugs toxicity. Documented renal failure following Indomethacin is often seen but the long term renal function was found to be normal. (17)

The global incidence of AKI in neonates ranges from 3.4 to 24%. (1) There is no data available on incidence of neonatal AKI in the Indian setting. There were few studies done in the India on asphyxiated neonates. Ashraf etal looked at 80 asphyxiated neonates born during March 2006 and

February 2007. In Srinagar, these 80 babies were thoroughly examined after getting detailed history. Investigations done included urine examination, serum electrolytes, creatinine, urea, uric acid, ultrasonography of the abdomen and cranium. AKI was seen in 45% of them- non oliguric type was seen in 77%. Mortality rate was 11.1% and ultrasonography in the acute setting showed increased echogenicity of kidney in all of them. (18) A case control study done by Gupta etal in Rajasthan where 70 asphyxiated neonates and 28 healthy neonates were recruited. Asphyxia was defined as Apgar score less than 7 at 5 minutes. Urine microscopy, serum creatinine and urea were done for all babies. Oliguria (urine output <0.5 ml/kg/hour), Creatinine > 1mg/dL and urea > 40 were the criteria required to define AKI. AKI was seen in 47.1% and non-oliguric AKI was found in the majority of the (78%). Those with severe asphyxia had oliguria. The mortality rate was 7.1% which were asphyxia related. The survivors were followed up at 1 and 6 months and none of them had residual renal dysfunction. (19)

NEPHROGENESIS

The fetal kidney develops from three successive mesodermal structures which are pronephros, mesonephros and metanephros. Metanephros arises from the ureteric bud (branch of Wolffian duct). Interaction between metanephric mesenchyme and ureteric bud epithelium lead to "branching morphogenesis" which leads to formation of collecting system. Differentiation of metanephros occurs only by 5 weeks. First nephrons are formed only by 8 weeks and nephrogenesis is complete only by 34 weeks. Nephrons grow in size from 35 weeks till birth. (20)

Kidney weight increases with gestational age towards the latter half of pregnancy. Renal architecture may be appreciated as early as 20 weeks. The ratio between renal and abdominal circumference at the level of umbilical vein remains constant (0.27 to 0.30).

As nephrogenesis is incomplete in those born prior to 34 weeks, it has been proposed that they have a

lesser renal volume and the function may also be impaired. In addition to this, the risk factors they are exposed to possibly worsen the renal function. Therefore it is important to detect acute kidney injury in neonatal period. It is also important to follow up these babies and monitor growth, blood pressure and renal function as they may be at risk for chronic kidney disease. (21)
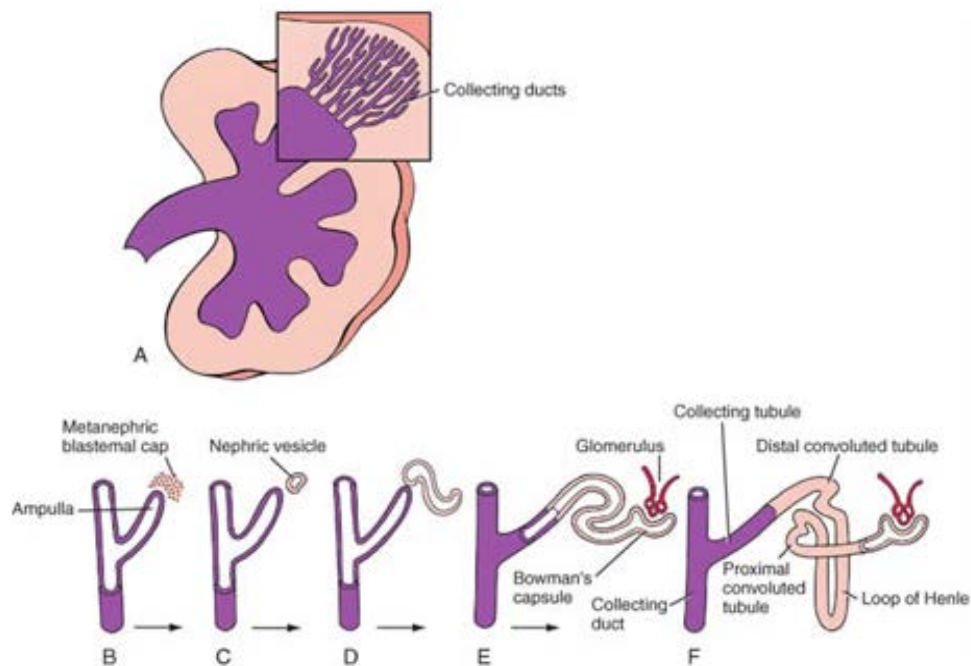

Figure 4: Nephrogenesis

Functional status of the kidneys in older children and adults can be assessed by monitoring urine output and serum creatinine levels. This cannot be done in neonates due to various reasons. Creatinine measured within 72 hours reflects maternal creatinine values. In fact, this may persist as long as 15 days in preterm babies. The other factors which determine creatinine level include muscle mass, age, hydration status and gender. In view of which, it may not be the ideal diagnostic tool for assessing renal functional status.

A recent Serbian study published in 2014 was conducted in University of Novi Sad by Stojanovic etal, retrospectively looked at 150 preterm babies born before 37 weeks of gestational age. All babies had baseline creatinine done on day 3 of life followed by everyday or every other day monitoring of

creatinine for the first 7 days. If there was any deterioration, creatinine monitoring was done for atleast 48 hours following the deterioration. In this study, AKI was defined as creatinine more than 0.3 mg/dL compared to baseline values or compared to the ones done 48 hours prior. Urine output was also monitored. Staging of disease was by modified AKIN criteria into 3 stages. Antenatal factors like PIH, Diabetes, antibiotics and chorioamnionitis in the Mothers were looked at. Gestational age, birth-weight, Apgar score, temperature at admission, serum lactate, serum pH, ventilation, use of nephrotoxic drugs, inotropes and maximum FiO2 requirement was analysed.

AKI incidence was 26%. It was more common in those with birth-weight less than 1500 Gm (94.8%) and gestational age less than 28 weeks (64.1%). Non-oliguric renal failure was common (60%) than oliguric (25%) or anuric (15%) renal failure. Metabolic acidosis was more pronounced in those with AKI. All of them were on assisted ventilation with higher oxygen requirement. Certain complications like intracranial hemorrhage, necrotizing enterocolitis, sepsis and PDA were more common in the AKI group and statistically found to be independent risk factors for AKI. In one third of them, creatinine normalized and they survived. Despite normalized creatinine, 7.7% died. Creatinine remained high in 59%. Mortality rate was found to be high (69.2%) in the AKI group – highest in stages 2 and 3 AKI and in those with birth-weight less than 1500 Gm. They concluded that AKI was a major problem in preterm babies and it is important to identify it early to initiate prompt treatment. (22)

Over the past many years, various biomarkers have been found to be useful in determining acute renal injury. These have been used in different age groups with various risk factors and complications. They have been found to be useful in determining renal damage at the risk and injury level; even before irreversible damage and failure has occurred. Of these, urinary NGAL has been studied extensively in various populations.

DIAGNOSIS OF AKI

CLINICAL HISTORY AND EXAMINATION

A detailed history and thorough clinical examination will help diagnose the type of renal failure and treat appropriately. The age of the patient, drug history, past history and family history give important clues to etiology.

Universal definition of acute kidney injury was not available till 2004 when Nephrologists and critical care groups came up with the RIFLE criteria. RIFLE stands for Risk, Injury, Failure, Loss and End stage renal disease. This is applicable to adult population. (23)

A modified Pediatric RIFLE criterion was proposed based on estimated creatinine clearance. (24)

The modified Pediatric RIFLE criteria may not be helpful in preterm babies. However, the concept of RIFLE helps us understand the importance of detecting kidney damage at risk or injury rather than at failure stage when significant renal damage has occurred and may progress to loss of function and even end stage renal disease. This is depicted in figure 5. This throws light on the existence of biomarkers which help detect kidney damage at earlier stages.
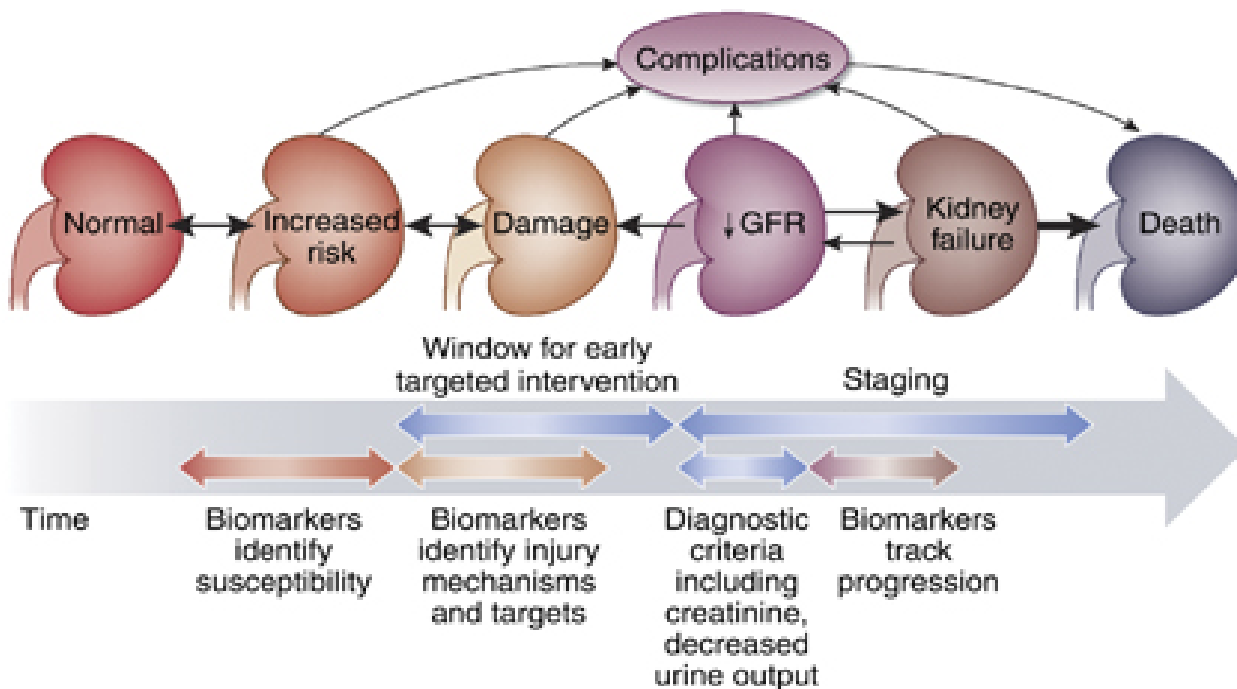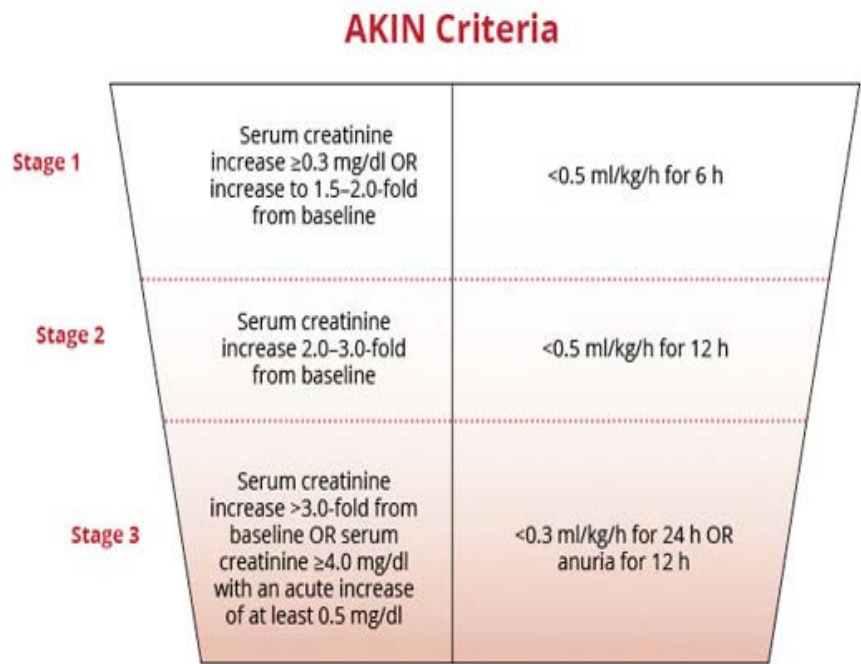

Fig 5: RIFLE criteria

AKIN (Acute Kidney Injury Network) has a criteria similar to the RIFLE which is based on creatinine and urine output. ADQI (Acute Dialysis Quality Initiative) introduced the RIFLE criteria in 2004. (25)

Later in 2008, the AKIN group came with their criteria. This was based on the evidence that small rise in creatinine is associated with adverse outcomes. A rise of more than 0.3 mg/dL and a time constraint of 48 hours was used. (26) It was later found in a multicenter trial which looked at 59 ICUs across

AKIN Criteria

	Serum creatinine	Urine output
Stage 1	Serum creatinine increase ≥0.3 mg/dl OR increase to 1.5–2.0-fold from baseline	<0.5 ml/kg/h for 6 h
Stage 2	Serum creatinine increase 2.0–3.0-fold from baseline	<0.5 ml/kg/h for 12 h
Stage 3	Serum creatinine increase >3.0-fold from baseline OR serum creatinine ≥4.0 mg/dl with an acute increase of at least 0.5 mg/dl	<0.3 ml/kg/h for 24 h OR anuria for 12 h

Australia-New Zealand that there was no advantage of one criteria over the other. (27)

Figure 6: AKIN criteria

Table I. Pediatric-Modified RIFLE (pRIFLE$_{\Delta SCr}$) Criteria

	SCr	Urine output
Risk	SCr increase by 25%	<0.5 ml/kg/h for 8 h
Injury	SCr increase by 50%	<0.5 ml/kg/h for 16 h
Failure	SCr increase by 75%	<0.3 ml/kg/h for 24 h or anuric for 12 h
Loss	Persistent failure >4 weeks	
End stage	End stage renal disease (persistent failure >3 months)	

SCr: Serum creatinine. pRIFLE: Pediatric risk, injury, failure, loss and end-stage renal disease.

Figure: 7 Pediatric – Modified RIFLE criteria

The significance of RIFLE criteria is timely intervention and preventive measures may help salvage renal function. When a vulnerable patient has been identified, avoiding nephrotoxic drugs and volume resuscitation or inotropic support may help prevent renal failure. If adequate measures are not taken, the patient may go into renal failure requiring dialysis or even end stage renal disease.

LAB INVESTIGATIONS

Even before creatinine or novel biomarkers of acute kidney injury, something as simple as urine examination will give us a clue about ongoing kidney injury. Urine analysis showing red blood cell casts is suggestive of glomerulonephritis, while cola coloured urine with high myoglobin levels is suggestive of rhabdomyolysis - which may predispose to acute tubular necrosis if there is no intervention at this point.

Urine indices like urine osmolality, urinary sodium levels and fractional excretion of sodium help differentiate between pre-renal and renal failure. (28)

In case of obstructive causes, it is important that imaging is done (atleast ultrasonography) because just urinary catheterization may relieve the obstruction and help resolve renal failure.

Creatinine as a marker of kidney injury

Creatinine is a breakdown product of creatinine phosphate in the muscle. It is produced at a fairly constant rate in the body and depends on muscle mass. Other factors which influence creatinine levels include age, gender and hydration status.

Creatinine metabolism:

Creatinine is synthesized in the liver by methylation of glycocyamine by S-adenosyl methioinine. It is transported through blood to various organs including muscle and brain. In these organs, it is phosphorylated to form phosphocreatine. (29)

The kidneys excrete creatinine mainly by glomerular filtration and also by proximal tubular secretion.

As creatinine is hardly reabsorbed, it reflects the glomerular function. In other words, blood and urine creatinine levels can be used to calculate creatinine clearance which reflects the glomerular filtration rate (GFR). The GFR measures renal function.

Factors affecting creatinine levels

Men have more muscle mass than women and children. Therefore, tend to have higher creatinine values. Similarly, older children have more muscle mass and creatinine levels tend to be higher. Meat intake may alter the creatinine levels as well. (30)

In term neonates, creatinine levels in the first 72 hours reflect maternal levels. In preterm babies, this may persist even up to 15 days. Gestational age, weight, muscle mass and hydration status determine creatinine levels in this population. Pre-renal causes and non-oliguric renal failure being more common, acute kidney injury may not be easily diagnosed in this population. (31)

Methods of measuring creatinine level

Jaffe reaction was first described in 1886. Max Jaffe found that when mixed with sodium hydroxide solution, creatinine forms a reddish orange colour and the level of creatinine can be measured by colorimetric methods. This test has undergone various phases and currently done after IDMS (isotope dilution mass spectrometry) calibration. (32) Other methods use enzymatic methods - creatinase, creatinine deaminase and isotope dilution mass spectrometry.

Estimation of renal function by creatinine clearance:

The serum creatinine levels depend on various factors. Therefore, an absolute value of creatinine may not reflect the actual renal function. It was later found that creatinine clearance helps estimate the GFR and renal function. Creatinine clearance may be estimated by various formulas. (30)

Schwartz equation:

Cr Cl (ml/min/1.73m²) = [length (cm) x k] / S Cr

This can be used in infants over 1 week to adolescents 18 years of age

K = 0.45 for infants 1 to 52 weeks,

K = 0.55 for children 1 to 13 years,

K = 0.55 for adolescent girls 13 to 18 years

K = 0.7 for adolescent boys 13 to 18 years

CREATININE IN NEONATES

In term neonates, creatinine levels in the first 72 hours reflect maternal levels. In preterm babies, this may persist even up to 15 days. Gestational age, weight, muscle mass and hydration status determine creatinine levels in this population. Pre-renal causes and non-oliguric renal failure being more common, acute kidney injury may not be easily diagnosed in this population (29).

Boer etal looked at creatinine levels done in infants less than one year of age during the period 2003 and 2008 and came up with reference values for creatinine. (33) Different studies have taken different cut-off values of creatinine to define AKI. An Italian case-control study by Cataldi etal studied potential risk factors leading to AKI in preterm babies. Creatinine value of more than 1.3 mg/dL in babies born at less than 33 weeks and more 1 mg/dL in those > 33 weeks of gestational age was used to define AKI. (34)

Kapoor etal reported incidence of AKI in Maulana Azad medical college, New Delhi based on creatinine and oliguria in a retrospective study. Absolute value of creatinine more than 1.5 mg/dL was taken to define AKI and incidence was found to be 9.6%. Most common predisposing factor was sepsis (61.3%) followed by asphyxia (22.7%). Both term and preterm babies were included in the study. Pre-renal failure was identified as most common type of renal failure (50%). Mortality rate was 15.9%. (35)

Stojanovic etal took a rate of rise in creatinine rather than an absolute value to define AKI in preterm babies. A rise of more than 0.3 mg/dL was taken to define AKI (22).

NOVEL BIOMARKERS IN DETERMINING ACUTE KIDNEY INJURY

There are many biomarkers now available which are found to be useful in determining acute renal injury. The urinary biomarkers are found to rise just hours after the insult has occurred and help us to intervene even significant irreversible injury has occurred. Creatinine rise is found to occur 48 hours after significant injury has occurred and intervention may or may not help salvage the renal function.

YEAR	MYOCARDIAL INJURY MARKER	RENAL INJURY MARKER
1960	Serum LDH	Serum creatinine
1970	CPK, myoglobin	Serum creatinine
1980	CKMB	Serum creatinine
1990	Troponin T	Serum creatinine
2000s	Troponin I	Serum creatinine
Outcome	Multiple therapies	Supportive care
	50%↓ in mortality	High mortality rates

Table 2: Gold standard diagnostic tools in myocardial injury versus kidney injury

Discovery of Troponin has improved mortality and morbidity rates in myocardial injury. Timely intervention has helped salvage lives. It is important to have Troponin like markers where timely intervention can prevent irreversible injury in the kidneys. The only marker available was creatinine. Unfortunately creatinine is not a marker that increases so early in injury. Significant injury (25 to 50%) has already occurred by the time creatinine rise is seen. It also depends on the method used to estimate creatinine level. In certain population like newborn, high bilirubin may interfere with estimating creatinine. (29)

Over the past decades, a lot of research has gone into finding other markers for renal injury. Different biomarkers were discovered over a course of time. Urinary biomarkers have been used in the past for detecting renal injury in various situations and age groups including newborn babies post-cardiac surgery.

There are many biomarkers available but extensive research studies are required to prove their usefulness in preterm babies. Some of these include neutrophil gelatinase associated lipocalin (NGAL), Interleukin 18 (IL-18), kidney injury molecule -1 (KIM-1), Osteopontin (OPN), beta 2 microglobulin (B2mG) and Cystatin C (Cys-C).

PHASES OF DEVELOPING A BIOMARKER

There are five phases for development of a biomolecule. Pre-clinical discovery, development of an assay, use of assay retrospectively and compare with existing tests, prospective screening to prove its efficacy and finally disease control. (36)

NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN

Lipocalin -2 (LCN2) also known as oncogene 24p3 or neutrophil gelatinase associated lipocalin is a protein.   It is encoded by LCN2 gene. As the name suggests, it is expressed by neutrophils and in low levels by kidney, prostate and epithelia of the respiratory and gastro-intestinal tract. It is a 25kDa protein covalently bound to neutrophils. Originally, it was isolated from supernatant of activated human neutrophils and therefore the name. It was later found to be elevated in conditions even when neutrophil counts were not high.

The distal tubules and various other organs produce NGAL but it is reabsorbed in the proximal tubules. It is an iron transporting protein. Iron has been hypothesized to protect proximal tubule. NGAL was found to inhibit apoptosis, enhances proliferation and provide functional as well as pathological protection in rat models. It was also found to bind to bacterial siderophobes and rendering innate immunity against bacterial infection.   It limits bacterial overgrowth by sequestering bacterial siderophores. (37)
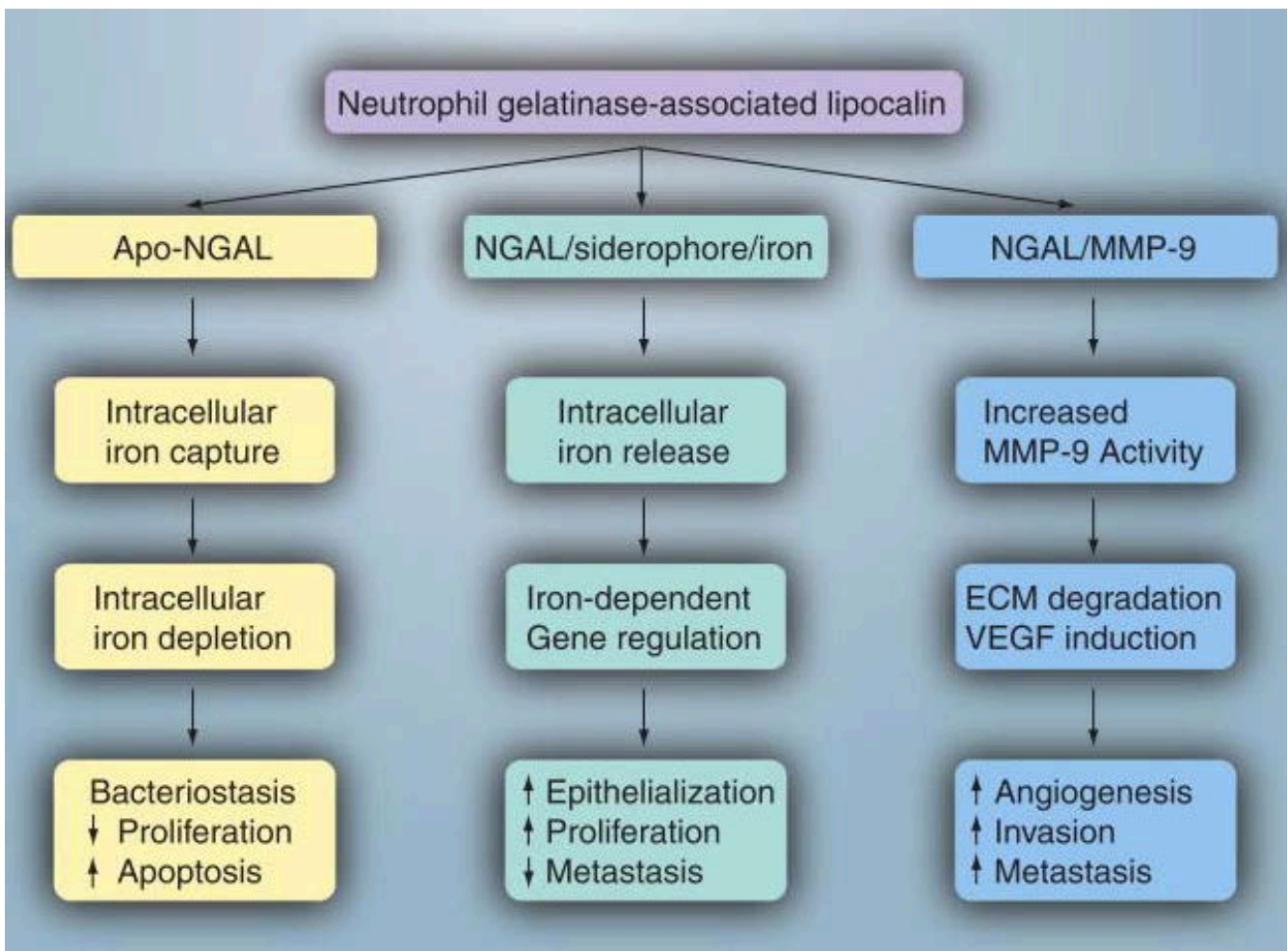
Figure 8: Molecular role of NGAL

ROLE OF NGAL IN ACUTE KIDNEY INJURY

NGAL was found to be elevated in the urine 2 to 4 hours after renal injury. Normally, NGAL reaches proximal tubules after glomerular filtration. It is reabsorbed into the systemic circulation via megalin. Therefore, there is a very low NGAL level in the distal tubules and only trace amounts will be excreted in the urine. When there is acute kidney injury, the proximal tubules are unable reabsorb the high levels of NGAL.

In addition to this, the distal tubules also secrete NGAL. So the urine level of NGAL is elevated in acute kidney injury. (Figure 9) (6)
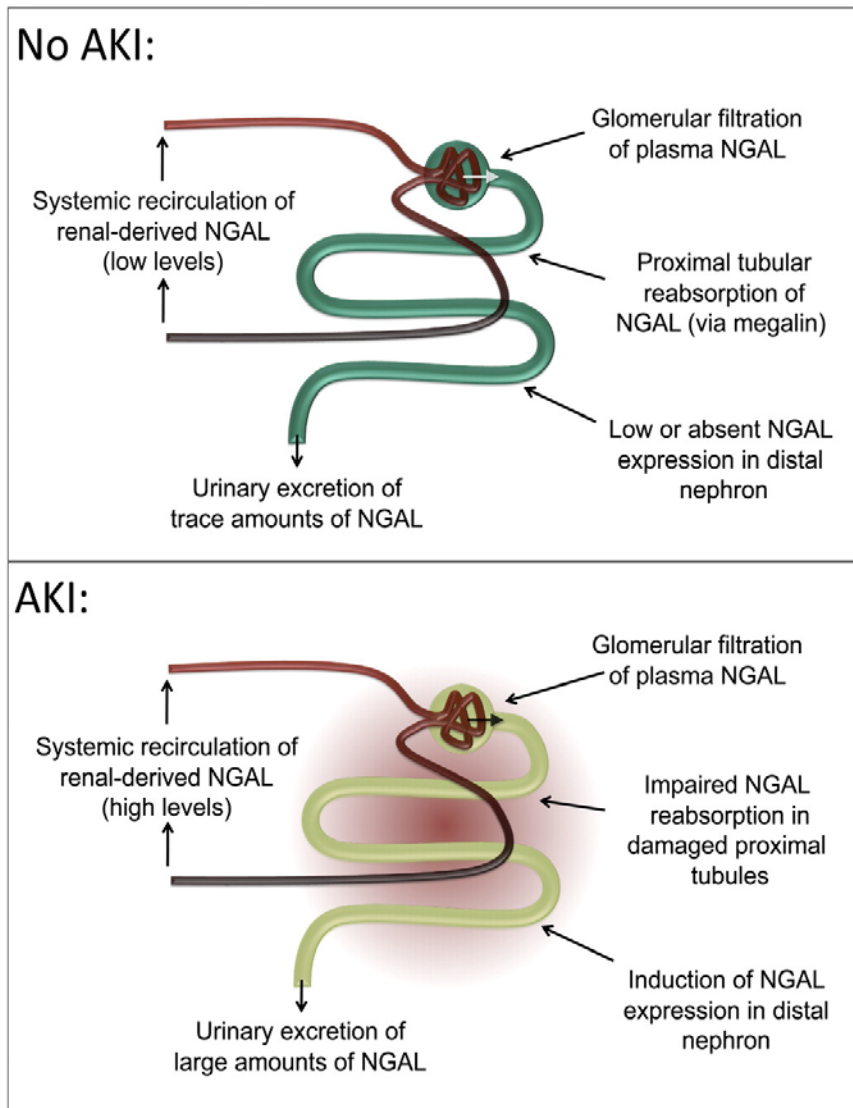
Figure 9: NGAL in acute kidney injury

The reason why NGAL was chosen over other biomarkers is because it fulfills most of the criteria for an ideal test. (6)

Table 3: Properties of NGAL

Biomarker property NGAL	NGAL
Noninvasive to measure, using urine or blood	Yes
Rapid, inexpensive to measure	Yes
Results available while damage is limitable	Yes
Amendable to clinical assay platforms	Yes
Sensitive to establish an early diagnosis	Yes
High gradient to allow risk stratification	Yes
Specific to intrinsic AKI (versus prerenal)	Yes
Discerns AKI from chronic kidney disease	No
Increases proportional to degree of damage	Yes
Associated with a known mechanism	Yes
Identifies primary location of injury within kidney	Yes
Results predict clinical outcomes	Yes
Results predict efficacy of therapies	Yes
Results expedite drug development process	Yes

Studies on NGAL IN AKI:

There are studies carried out at various phases for developing NGAL as a biomarker for AKI. Most of these studies were conducted by Devarajan etal, a Cincinnati group whose pioneering work on this biomarker is extensive and revolutionized approach to AKI.

<u>Preclinical phase</u>

Supavekin etal conducted studies on mouse models to look at differential gene expression following renal ischemia /reperfusion. Mice unilateral renal artery clamping for 45 minutes to induce renal ischemia and were sacrificed at 0, 3, 12 and 24 hours to look for reperfusion. They found tubular cell apoptosis using DNA laddering and TUNEL assays. CDNA microarrays were used to identify genes expressed. Along with pro-apoptotic factors, growth factors signal transduction and transcription factors were also expressed. (38) Kiyoshi etal used a mouse model to show that NGAL rescues kidney from ischemic injury. NGAL was introduced into mice with severe renal injury (during the early stages) and found that it dramatically protects the kidney and mitigates azotemia. (39) Experiments by Mishra etal in-vitro cultured human proximal tubular cells found that NGAL levels increasing in both unilateral as well as bilateral renal injury. It was also found that level of NGAL rise was directly proportionate to duration and dose of renal ischemia. Mild perfusion injury to the cultured human proximal tubular cells also leads to increase in NGAL levels. (40)

<u>Clinical studies</u>

Most clinical studies were done in post-cardiac surgery, critical care and post transplant patients (liver and renal transplant. (41) A cross-sectional study in human subjects admitted in intensive care unit with established acute renal failure. NGAL levels were found to be 10 folds higher in the plasma and 100 folds higher in the urine in these subjects when compared to plasma and urine levels in normal subjects.   This study by Mori etal   high NGAL also correlated with high creatinine. Renal biopsy done showed immuno-reactive NGAL in more than 50% of the cortical tubules. (39)

Zapitelli etal conducted studies on critically ill children (from one month to twenty one years of age). They found that NGAL rise was seen early in AKI in those who manifested a significant rise in creatinine only after 48 hours. They concluded that NGAL was useful in detecting early kidney injury

but not of much use once the creatinine levels have started rising. (42) Children undergoing cardio-pulmonary by pass surgery were studied by Dent etal. In this cross sectional study, multiple samples of NGAL was taken post surgery. AKI was defined as 50% or greater rise of creatinine. It was again found that NGAL rise was seen early (within hours) when compared to creatinine which increased only after 1 to 3 days. (43) Similar study was done by Koch etal in children post cardiac surgery for congenital heart disease showing NGAL as useful biomarker for AKI. (44)

A prospective study on role of NGAL being a biomarker of acute renal injury following cardiac surgery was done by Misra etal where all post cardiac surgery patients during the period January to November 2004 were enrolled. Urine and serum NGAL was monitored in these patients along with urine output and creatinine levels. ELISA and immunoblot techniques were established with commercially available antibodies. It was found that NGAL rise was much earlier during AKI compared to creatinine rise. NGAL level rose hours after an insult whereas a significant rise of creatinine was seen only after 48 hours. (21) Nieman etal described use of NGAL in post liver transplant subjects. It was found that the rate of rise of NGAL rather than a single value was useful in predicting those at higher risk of AKI. Creatinine was monitored daily and RIFLE criteria as used to define AKI. (46) All the mentioned studies used serial monitoring of plasma or urine NGAL and it was found that NGAL is a promising troponin like marker in AKI. This may help decrease morbidity and mortality in patients with AKI.

NGAL IN NEONATAL AKI

Extensive studies on this biomarker have been done by the Cincinnati group (Devarajan etal). Initial studies were to look at the normal trend of NGAL in preterm low birth weight babies. The urine NGAL level was found to be inversely proportional to gestational age and birth weight. (47)

This study did not look at NGAL as a marker of AKI. An Egyptian cohort study on asphyxiated babies showed elevated serum NGAL to be an early marker predicting AKI. (48)

Most studies used creatinine and urine output as gold standard to study NGAL. Creatinine reference values in the first year were extensively studied by Boer et al (33).

There is need for more data to analyse the usefulness of NGAL as a biomarker of AKI in neonates. Preterms are particularly susceptible as they are exposed to several risk factors which may predispose to renal injury and long term consequences. Early detection will help to decrease long term morbidity and adverse outcome. This study was thus planned with the hope that it will provide important guidelines on monitoring of preterm babies for kidney injury.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

The purpose of this study was to determine incidence of acute renal failure in preterm babies born in a tertiary care center and its outcome

AIM:

To study the incidence of acute renal failure in inborn Preterm babies at a tertiary care center in South India

OBJECTIVES

Primary objectives:

1. To determine incidence of acute renal failure in preterm neonates

2. To study risk factors pre-disposing to renal failure

Secondary objectives:

1. To evaluate the usefulness of Biomarkers like NGAL as marker of renal functions and predictors of acute kidney injury in preterm babies.

2. To study renal outcome of preterm babies at 6 months

MATERIAL AND METHODS

MATERIAL AND METHODS

STUDY DESIGN:

Descriptive study looking at incidence of acute renal failure in preterm babies

STUDY SETTING:

Christian Medical College is a tertiary care center situated in Northern part of Tamil Nadu.

This study was conducted in Neonatal intensive care unit as collaboration between Departments of Neonatology and Pediatrics unit II during the period - May2014 and August 2014. During the study period, babies born at less than 32 weeks of gestational age were recruited for the study. Those babies with abnormal antenatal renal scans, major systemic congenital anomalies and chromosomal anomalies were excluded.

SAMPLE SIZE:

Sample size was calculated based on incidence of renal failure. Incidence of renal failure worldwide ranges from 3.4 to 24% as mentioned in an Iranian study. Assuming that incidence of renal failure in our population is 10% with a precision of 6% and confidence interval of 95%, sample size was calculated to be 96.

Estimating single proportion (Absolute precision)

Assumptions

- The outcome variable measure should be binary (success/failure, alive/dead)

- p is probability of success in each trial; (1-p) is probability of failure

- The sampling distribution of the sample proportion (p) is approximated to normal.

Formula

$$n = \frac{Z^2_{1-\alpha/2}\, p\,(1-p)}{d^2}$$

Where,

p : Expected proportion

d : Absolute precision

1- α/2 : Desired Confidence level

Expected Proportion	0.1
Precision (%)	6
Desired confidence level (1- alpha) %	95
Required sample size	96

Note: Based on the incidence (3.4 to 24)% of renal failure among the preterms as quoted in Iranian study.

All those babies who fulfilled the inclusion Criteria and had none of the Exclusion criteria were recruited. After counseling the parents and getting consent from them, the study proformas were filled in. The details included demographic data like hospital number, age, gender, gestational age, birth weight; maternal history- both antenatal and peri-partum period. Neonatal history - perinatal history including Apgar score, resuscitation details, clinical features suggestive of sepsis, routine investigations and procedures which may predispose to renal failure were looked at.

During the hospital stay, weekly monitoring was done. Urine output, clinical deterioration if any, details of interventions – long line, ventilation, any unexpected event and use of nephrotoxic drugs

were noted. Urine output was monitored non-invasively once a week by either urine collecting bags or weighing the cotton pad.

In case of death or the baby being discharged against medical advice, they were considered as case dropouts.
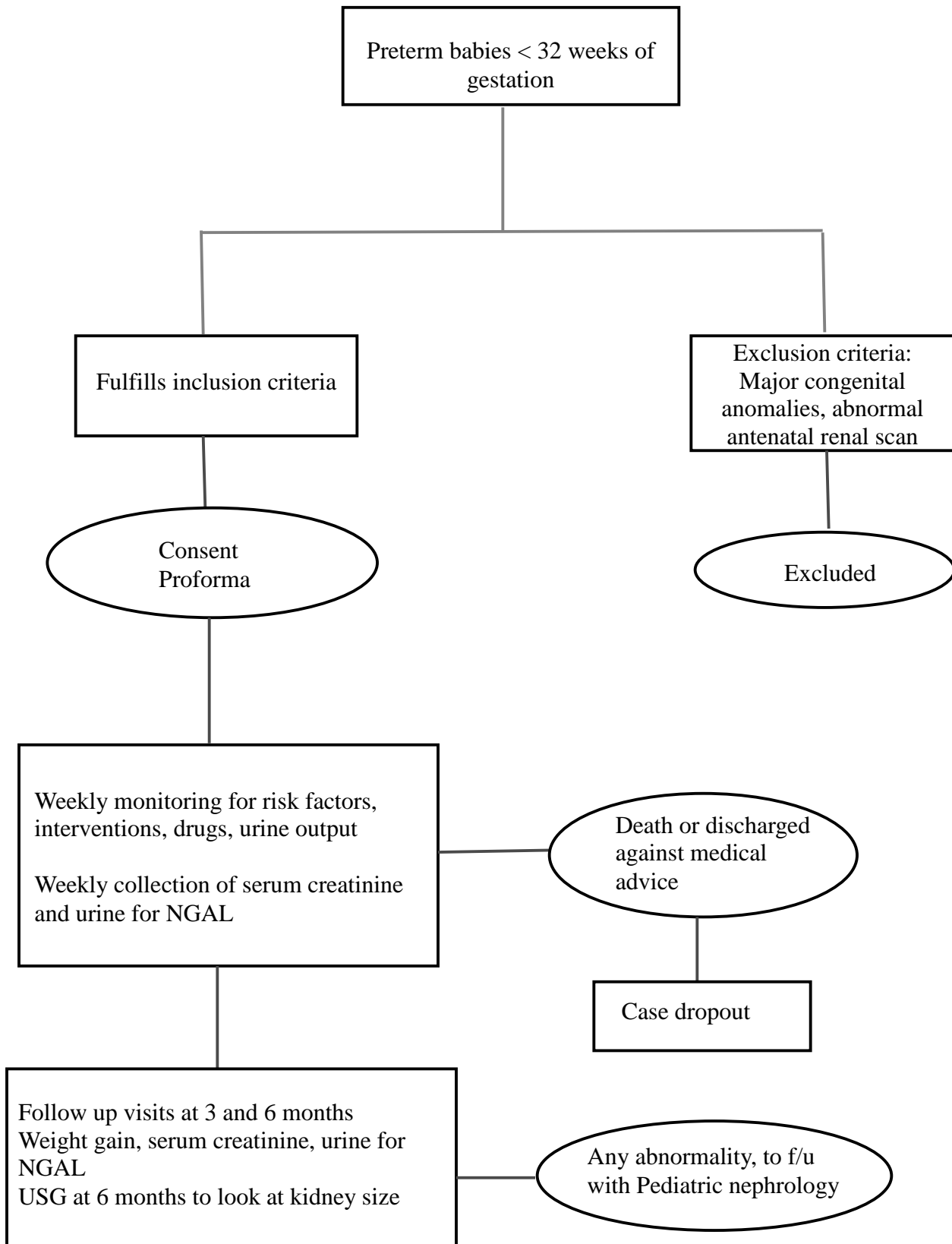
Blood sampling for serum creatinine and urine for NGAL was collected once a week. Serum creatinine was processed in Clinical biochemistry lab of CMC Vellore using standard methods. Serum creatinine more than 1.3 mg/dL or more than 50% rise of creatinine compared to previous value was used to define AKI. Urine for NGAL was collected and stored. Urine NGAL was processed for 30 babies as part of a pilot study to look if it was useful in detecting acute kidney injury in this population. Urine NGAL was processed using a rapid ELISA kit (kit 037).

Following discharge, all babies were planned be followed up at 3 and 6 months. At 3 months, along with routine clinical examination (growth monitoring, daily urine output and feeding), urine microscopy, serum creatinine will be checked and a sample for urine NGAL will be collected and stored. At 6 months, in addition to these, ultrasound abdomen will be done to look at kidney size. At follow up, if there is altered renal function on blood tests or abnormalities in ultrasound scan, these cases will be followed up in Pediatric nephrology.

The data collected was statistically analysed. Descriptive statistics were reported using Mean+/- SD for continuous variables; Median (IQR) for non-normal variables, categorical variables were be reported using n and %. Repeated measures ANOVA were done for the parameters which are measured for various time points. Categorical variables were assessed using Chi square/Fishers exact test.

Incidence was reported using n and %. Risk factor analysis was done using Log binomial to estimate the RR. Survival analysis was done to assess the outcome of renal failure.

FLOW CHART SHOWING ALGORITHM OF THE STUDY

Preterm babies < 32 weeks of gestation

Fulfills inclusion criteria

Exclusion criteria: Major congenital anomalies, abnormal antenatal renal scan

Consent Proforma

Excluded

Weekly monitoring for risk factors, interventions, drugs, urine output

Weekly collection of serum creatinine and urine for NGAL

Death or discharged against medical advice

Case dropout

Follow up visits at 3 and 6 months
Weight gain, serum creatinine, urine for NGAL
USG at 6 months to look at kidney size

Any abnormality, to f/u with Pediatric nephrology

45

RESULTS

RESULTS

This study was carried out in the Neonatology department in collaboration with the Pediatric unit

of Christian Medical College Vellore during the period of May 2014 to August 2014.

Babies born at or less than 32 weeks (up to 32+6 weeks) of gestational age were included. Those with

major congenital anomalies and abnormal antenatal renal scans were excluded.

Weekly monitoring of risk factors, interventions, drugs were noted. First sample of Serum creatinine

and urine for NGAL were collected after 72 hours of age.

The data was statistically analysed using SPSS software, version

The results of our study are depicted below.

There were a total of **4823** live births during this period.

There were **789 preterm deliveries** (less than 37 weeks) **(16.36%).**

Of these, **80 were preterm deliveries < 32 weeks** gestation **(10.14%).**

Of this group a total of 79 were recruited in the study.

A total of 79 babies were recruited. Of these, three died (one within 3 days of life and two were late

neonatal deaths) and two were discharged against medical advice and hence could not be evaluated

completely.

DEMOGRAPHIC DATA

GESTATIONAL AGE

Table 1: Gestational age distribution

Gestational age	Number (n = 79)	Percentage (%)
< 28 weeks	12	15.1
29 to 30 week	26	32.9
30 to 31 weeks	41	51.8

27 wk – 1
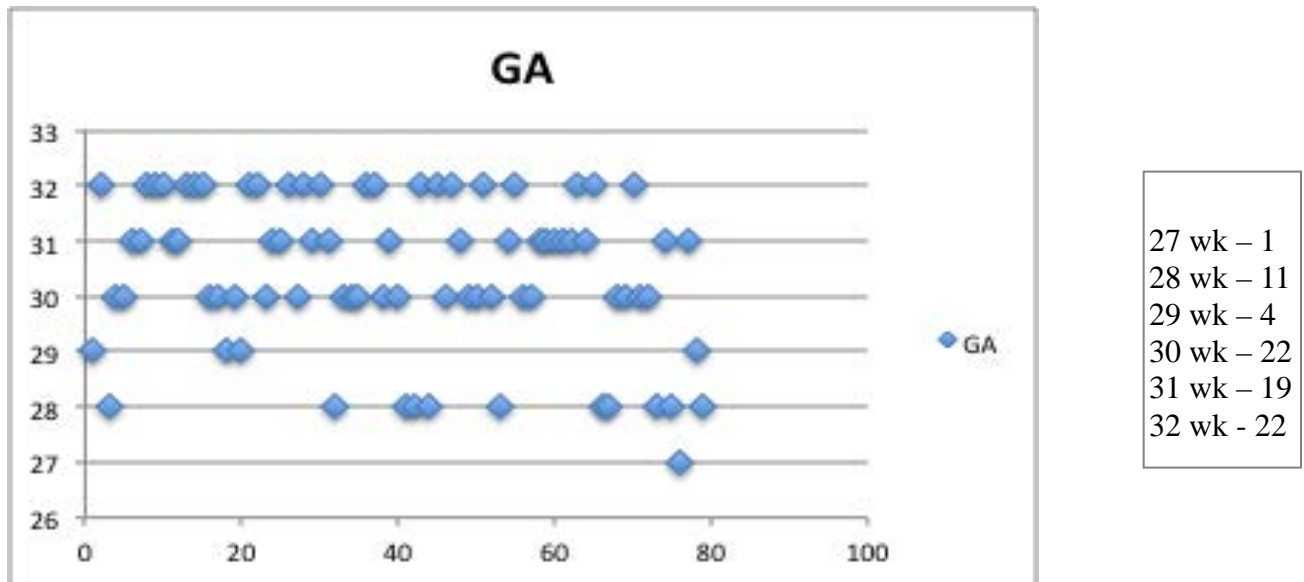28 wk – 11
29 wk – 4
30 wk – 22
31 wk – 19
32 wk - 22

Figure 1: Distribution based on gestational age

There were 12 babies (15.1%) between 27 to 28 weeks, 26 (32.9%) between 29 to 30 weeks and between 31 to 32 weeks there were 41 (51.8%).

BIRTHWEIGHT

Table 2: Birth weight distribution

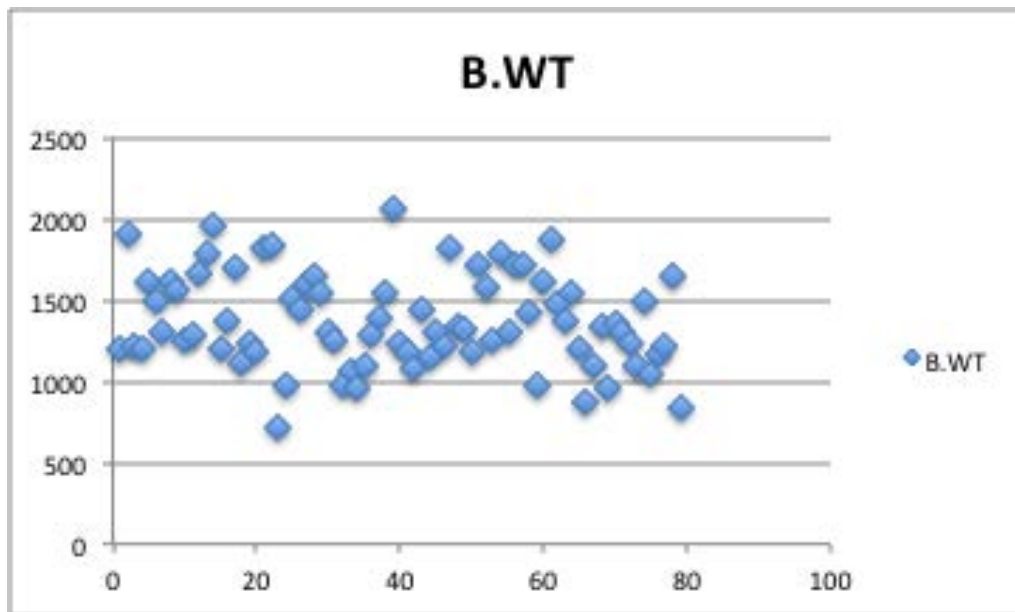| Birth weight (in Gm.)	Number (n = 79)	Percentage (%)
<1000	8	10.1
1001 to 1500	45	56.1
1501 to 2000	25	31.6
> 2000	1	0.2

Figure 2: Birth weight distribution

More than half the babies (56.1%) had birth weight between 1001 to 1500 Gm, while a third

 weighed   between 1500 and 2000 Gm. (31.6%).

GENDER

Table 3: Gender distribution

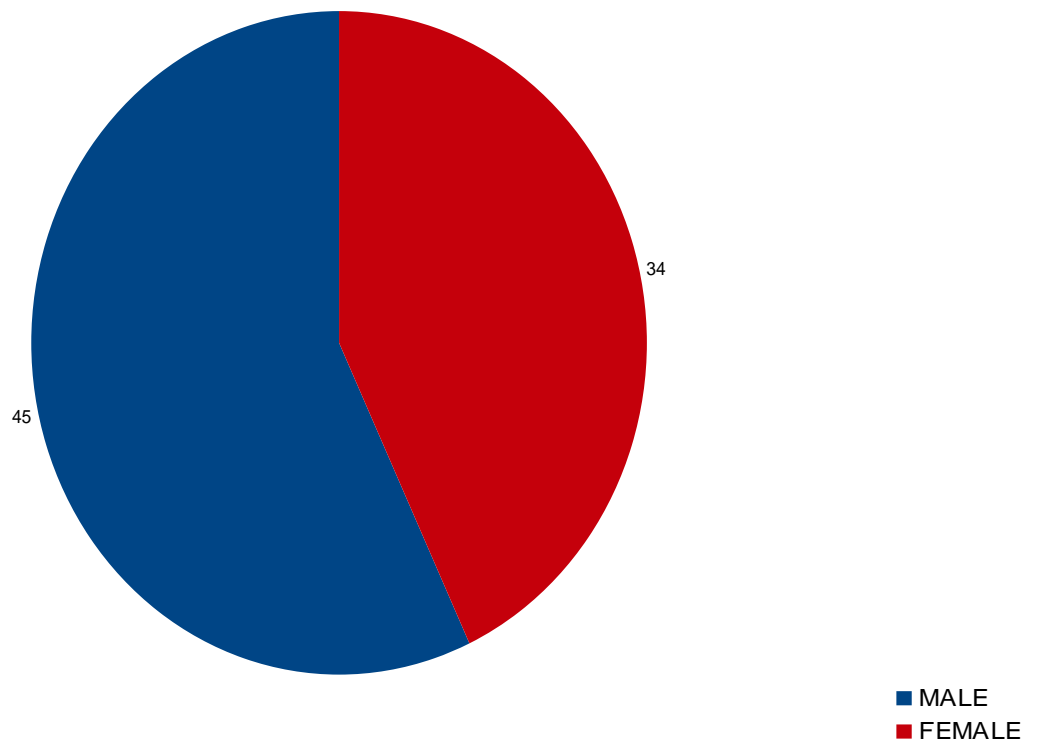| Gender	Total number (n= 79)	Percentage (%)
Male	45	57
Female	34	43

Table 3: Gender distribution

There were 45 males (57%) and 34 females (43%). The male: female ratio was 1.3: 1.

MATERNAL AGE

Table 4: Maternal age distribution

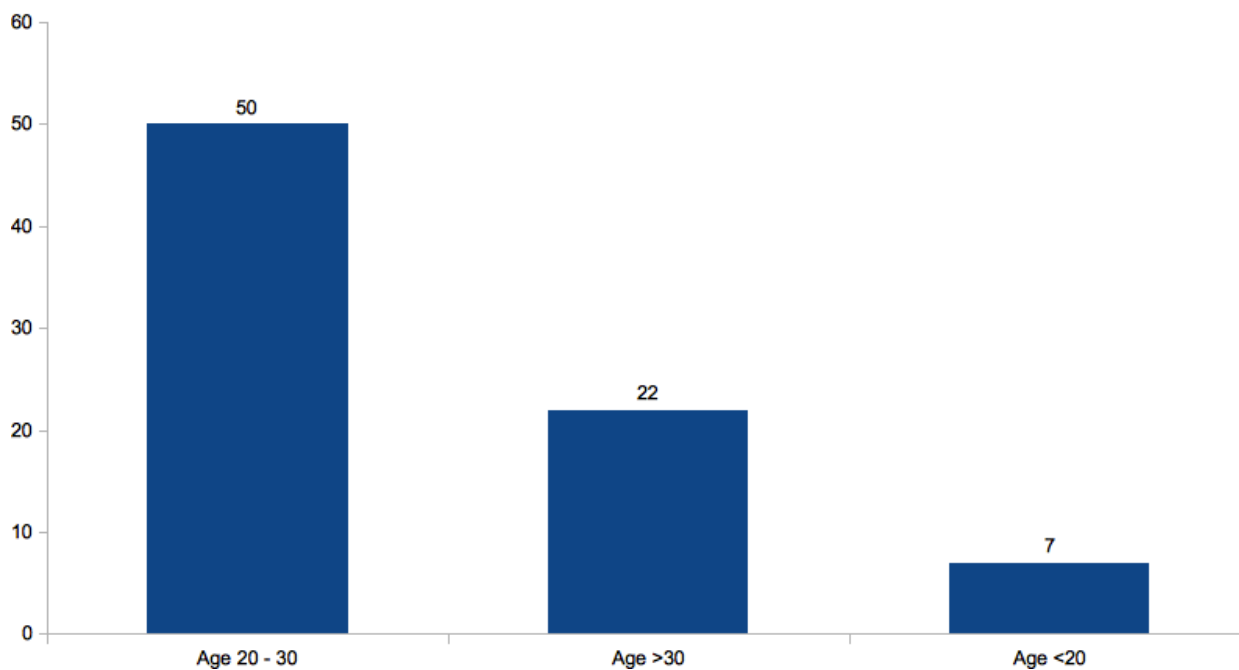| Maternal age	Number (n =79)	Percentage (%)
<20	7	8.8%
20 to 30	50	63.3%
>30	22	27.9%

Figure 4: Maternal age distribution

There were 63.3%% mothers who were between 20 and 30 years , followed by 27.9% more than 30 years and 8.8% less than 20 years.

MATERNAL EDUCATION STATUS

Table 5: Educational status

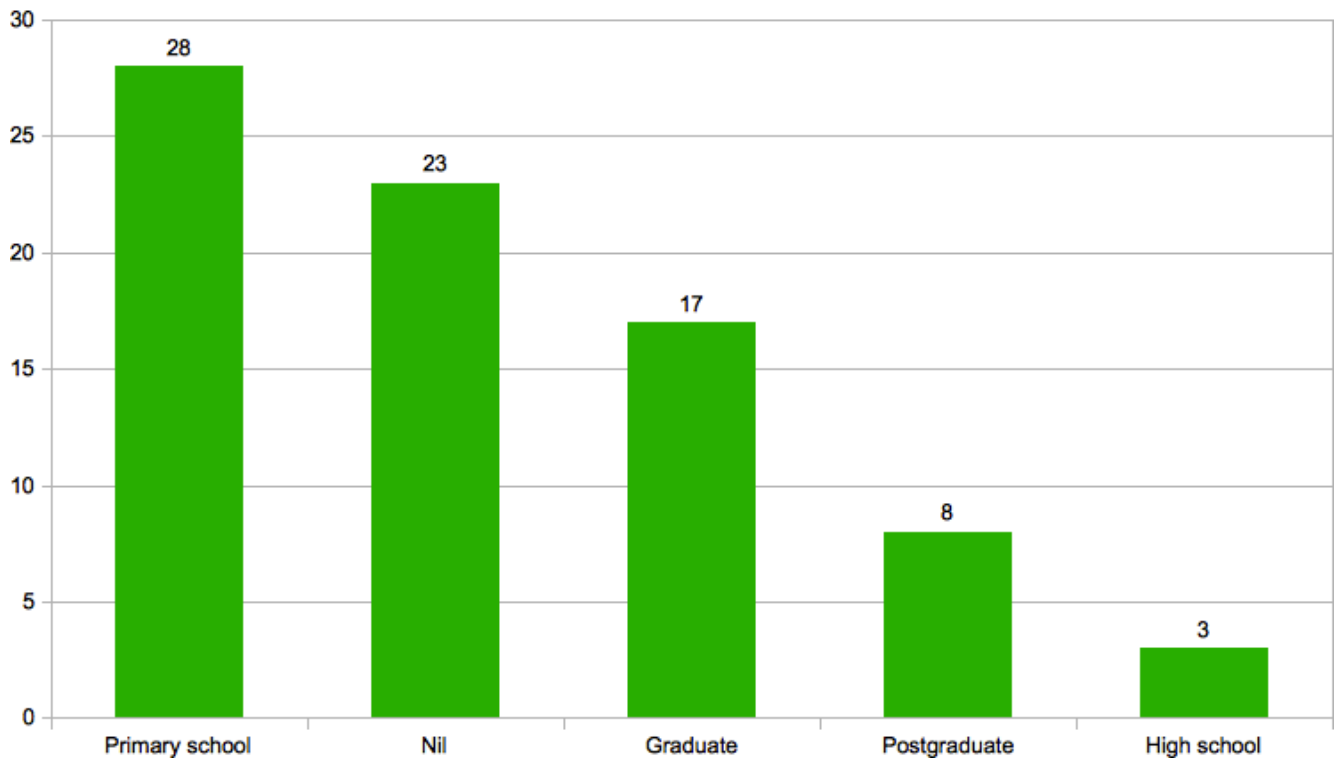| Education	Number (n = 79)	Percentage (%)
Nil	23	29.1
Primary school	28	35.4
High school	3	3.7
Graduate	17	21.5
Post graduate	8	10.1

Figure 5: Maternal educational status

29.1% mothers were uneducated, 35.4% had primary school education.

There were 21.5% graduates and only 10.1% postgraduates

ANTENATAL ULTRASOUND

Table 6: Findings on antenatal USG

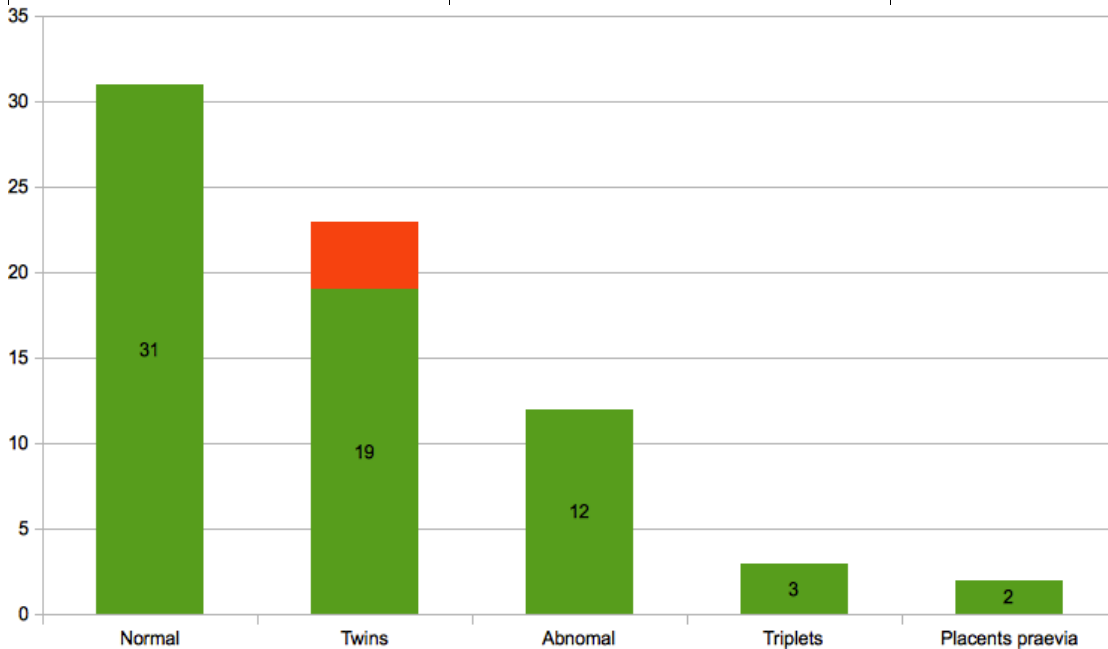| USG findings	Number (n = 69)	Percentage (%)
Normal	31	44.9
Abnormal	12	17.3
Twins	19	27.5
Triplets	3	4.3
Placenta praevia	2	2.8

Figure 6: Ultrasound findings

Of the total, 69/79 mothers had antenatal scans done. Normal scans were noted in 44.9% while 17.3% showed anomalies.

There were 10 sets of twins in the study- of which one of the twins was excluded from the study in view of major congenital anomaly.

Abnormal scans included abnormal Doppler and IUGR, while one baby had fetal ascites.

MODE OF DELIVERY

Table 7: Mode of delivery

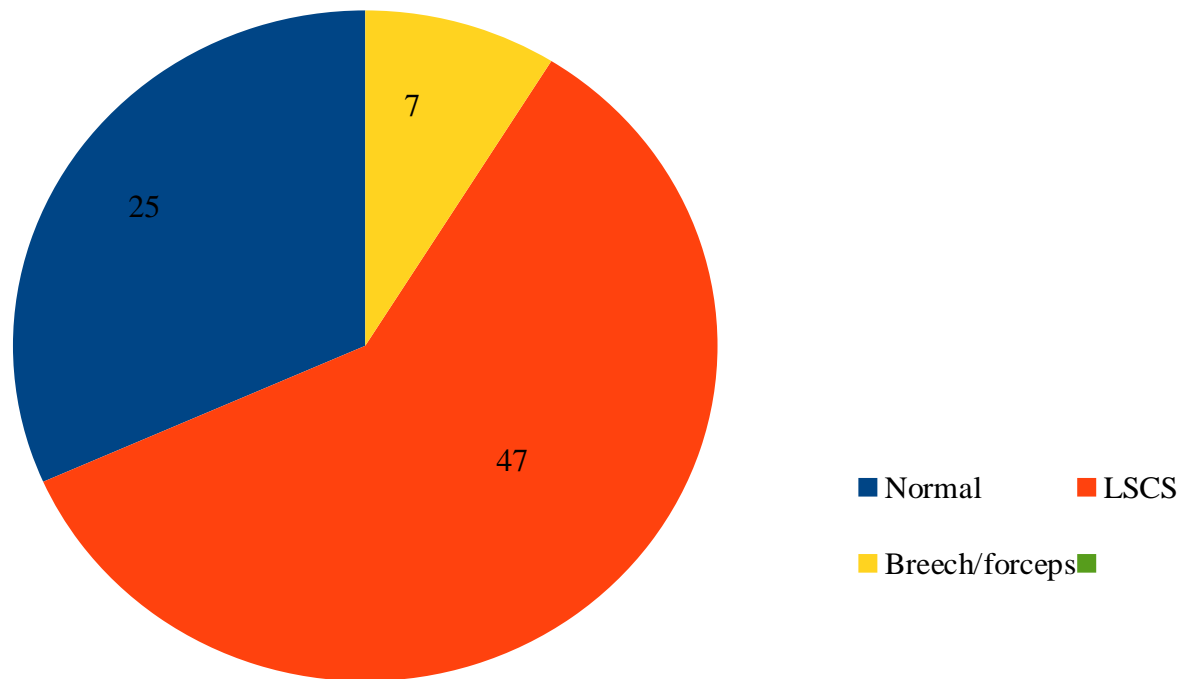| Mode of delivery	Number (n = 79)	Percentage (%)
LSCS	47	59.5
Normal	25	39.6
Breech/instrumental	7	8.8

Figure 7: Mode of delivery

Most common mode of delivery was LSCS in 47 (59.5%) followed by normal delivery in 25 (31.6%)

 and Breech/instrumental in 7 (8.8%)

PARITY OF MOTHER

Table 8: Parity pattern

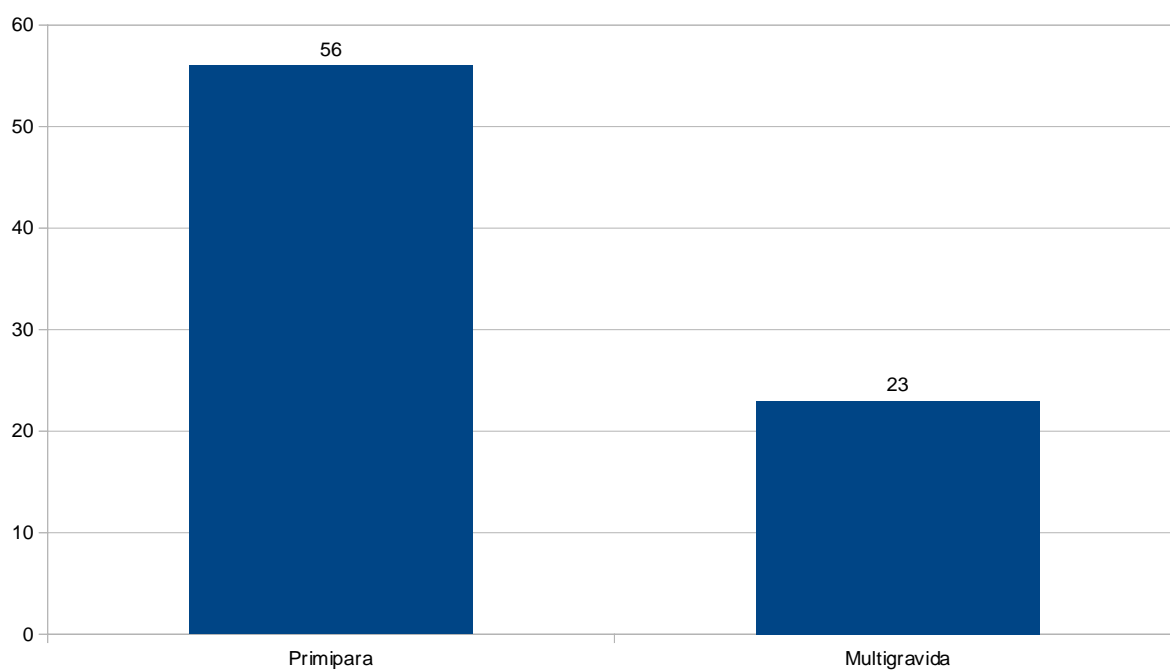| Parity	Number (n = 79)	Percentage (%)
Primipara	56	70.8
Multigravida	23	29.2

Figure 8: Parity

Majority of mothers were primipara - 56 (70.8%); while 23 (29.2%) were multigravida

MATERNAL RISK FACTORS

Table 9: Antenatal and perinatal risk factors (Maternal)

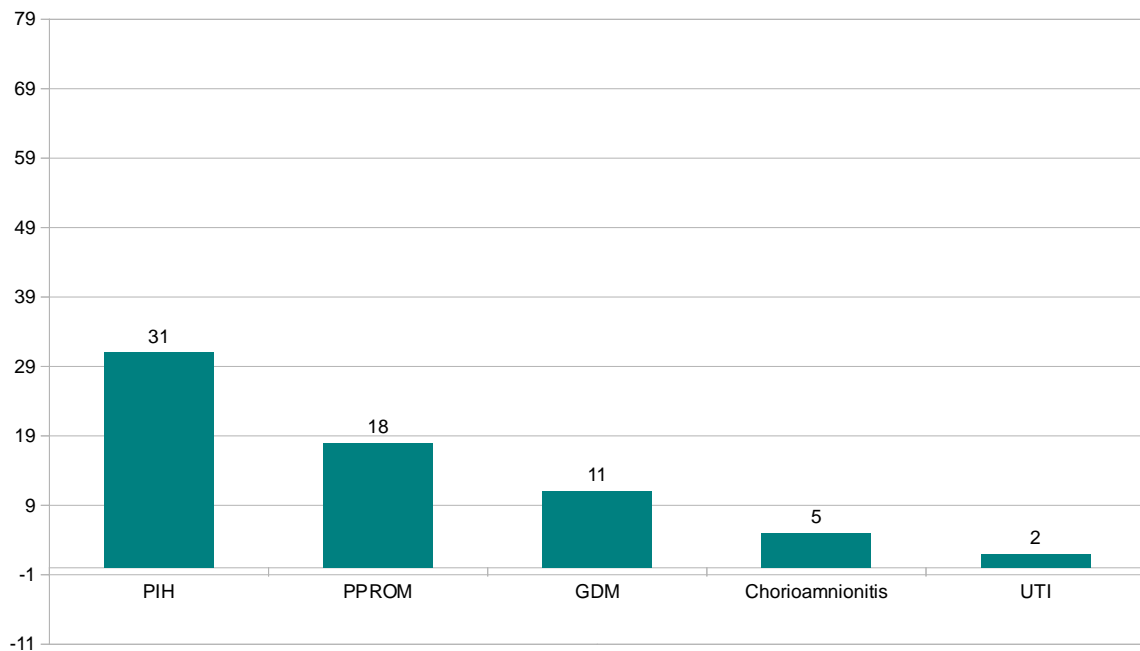| Risk factors	Number (n =79)	Percentage (%)
PIH	31	39.2
PPROM	18	22.7
GDM	11	13.9
Chorioamnionitis	5	6.3
UTI	2	2.5

Figure 9: Maternal risk factors

PIH was the commonest morbidity seen in the Mothers (39.2%) followed by PPROM (22.7%).

Other risk factors included GDM (32.9%), UTI (2.5%) and chorioamnionitis (6.3%).

APGAR SCORE

Table 10: Apgar score

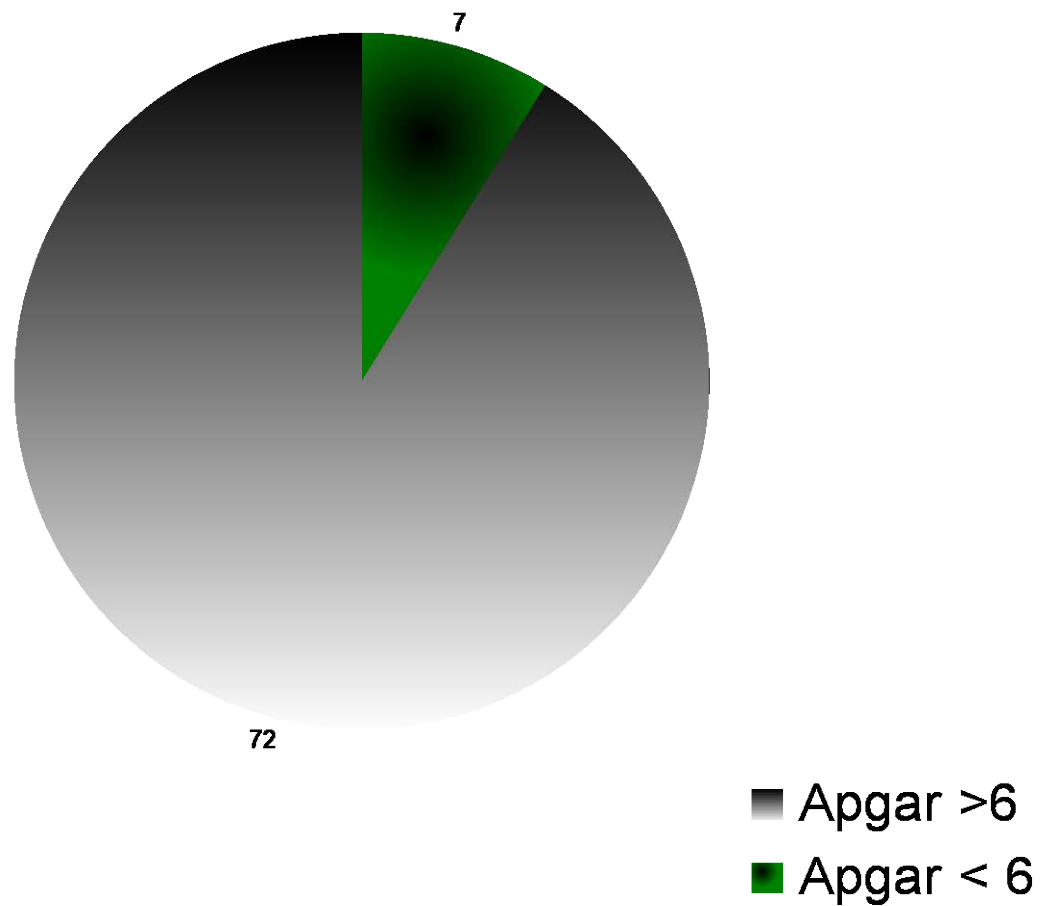| Apgar score at 5 min	Number	Percentage
Apgar >6	72	91.1
Apgar < 6	7	8.9

Figure 10: Apgar at 5 minutes

There were 72 babies (91.1%) with Apgar score more than 6 and 7 with Apgar less than 6 (8.8%)

NEONATAL RISK FACTORS AT BIRTH

Table 11:

Risk factors	Number (n = 79)	Percentage (%)
Resuscitation at birth	17	21.5
Intubation	15	18.9
Symptomatic at birth	55	69.6
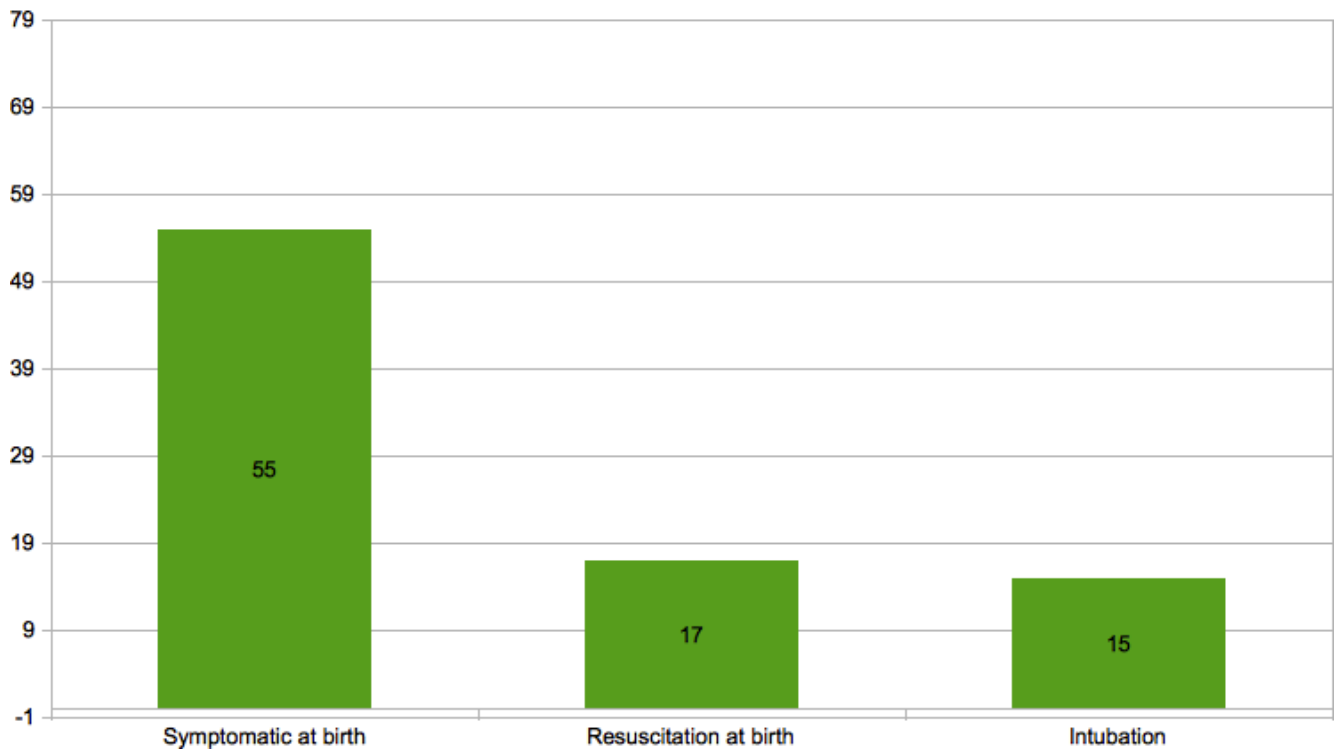
Figure 11: Neonatal risk factors at birth

There were 55 babies (69.6%) who were symptomatic at birth.

Resuscitation was needed in 21.5% and intubation in 18.9%

SYMPTOMS

Table 12:

Symptoms	Number (n= 79)	Percentage (%)
Respiratory distress	52/79	65.8
Poor perfusion	9/79	11.3
Abdominal distension	6/79	7.5
Apnea	5/79	6.3
Temperature instability	4/79	5.0
Shock	2/79	2.5
Oliguria	2/79	2.5
Anuria	1/79	0.2
Seizure	2/79	2.5
Hypoglycemia	1/79	0.2

Figure 12: Symptoms in the neonate
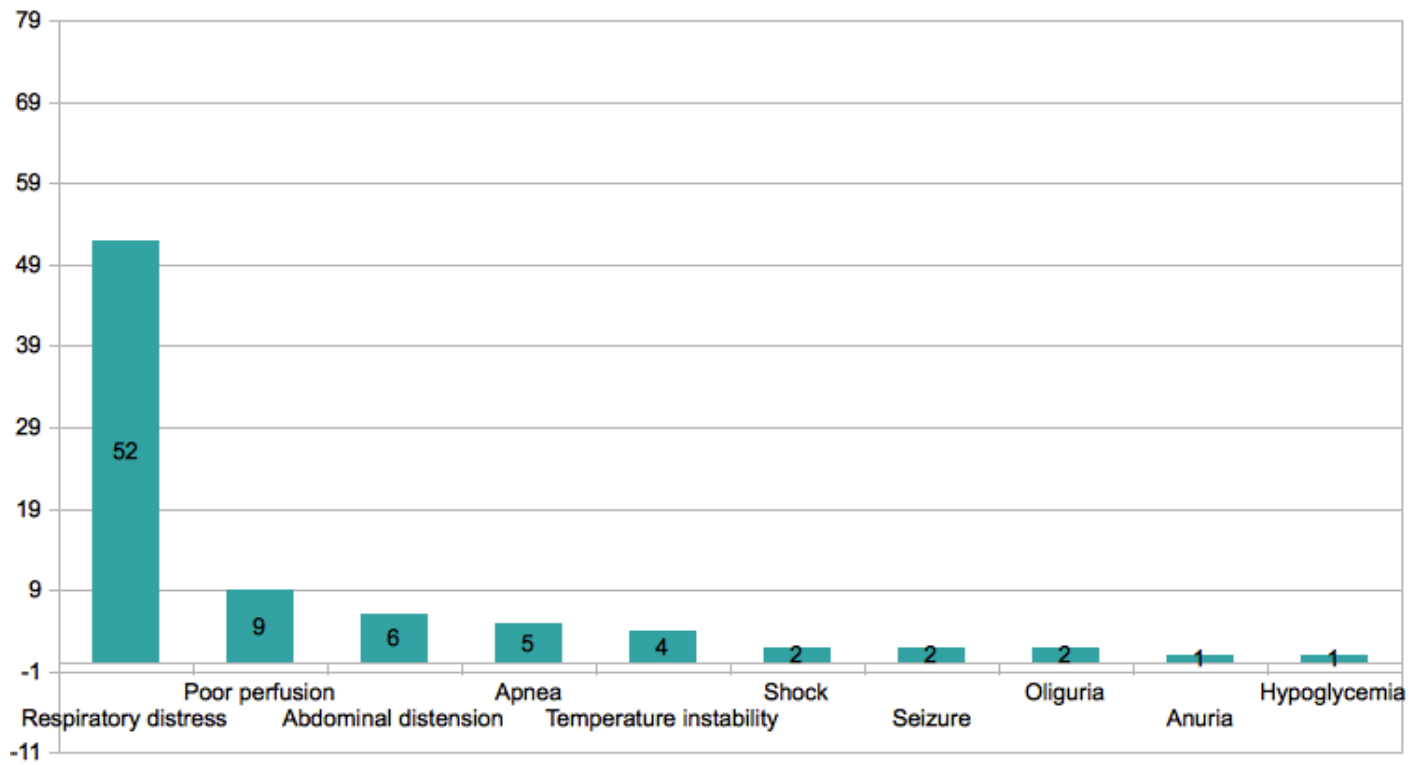
Respiratory distress was the most common symptom (65.8%) followed by poor perfusion (11.3%).

Others included abdominal distension (7.5%), apnea (6.3%) temperature instability, (5%), seizures

(2.5%), hypoglycemia (0.2%) and decreased urine output (2.5%)

CONDITIONS AFFECTING MORBIDITY

Table 13:

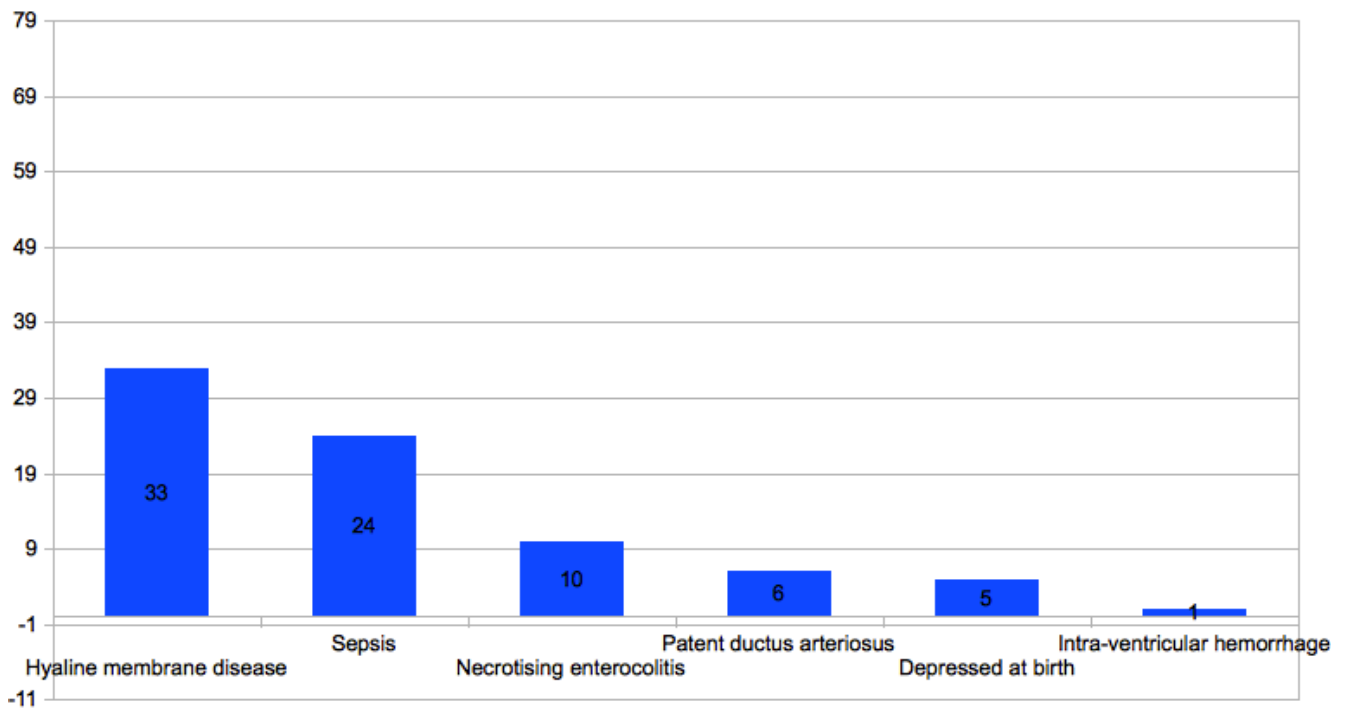| Condition	Number (N = 79)	Percentage (%)
Hyaline membrane disease	33/79	41.7
Sepsis	24/79	30.3
Necrotizing enterocolitis	10/79	12.6
Patent ductus arteriosus	6/79	7.5
Depressed at birth	5/79	6.3
Intra-ventricular hemorrhage	1/79	0.2

Figure 13: Morbidity pattern

Hyaline membrane disease was the commonest condition seen (41.7%) followed by sepsis, which affected one third of them (30.3%).

NEC was seen in 12.5%, PDA in 7.5%, IVH in 0.2% and 6.3% were depressed at birth.

INTERVENTIONS DURING NICU STAY

Table 14:

Intervention	Number (N= 79)	Percentage (%)
IV fluids	79/79	100
Nasogastric feeds	79/79	100
Umbilical venous line	64/79	81
Umbilical arterial line	29/79	45.5
CPAP	36/79	36.7
Mechanical ventilation	9/79	11.3
Percutaneous central line	3/79	3.7
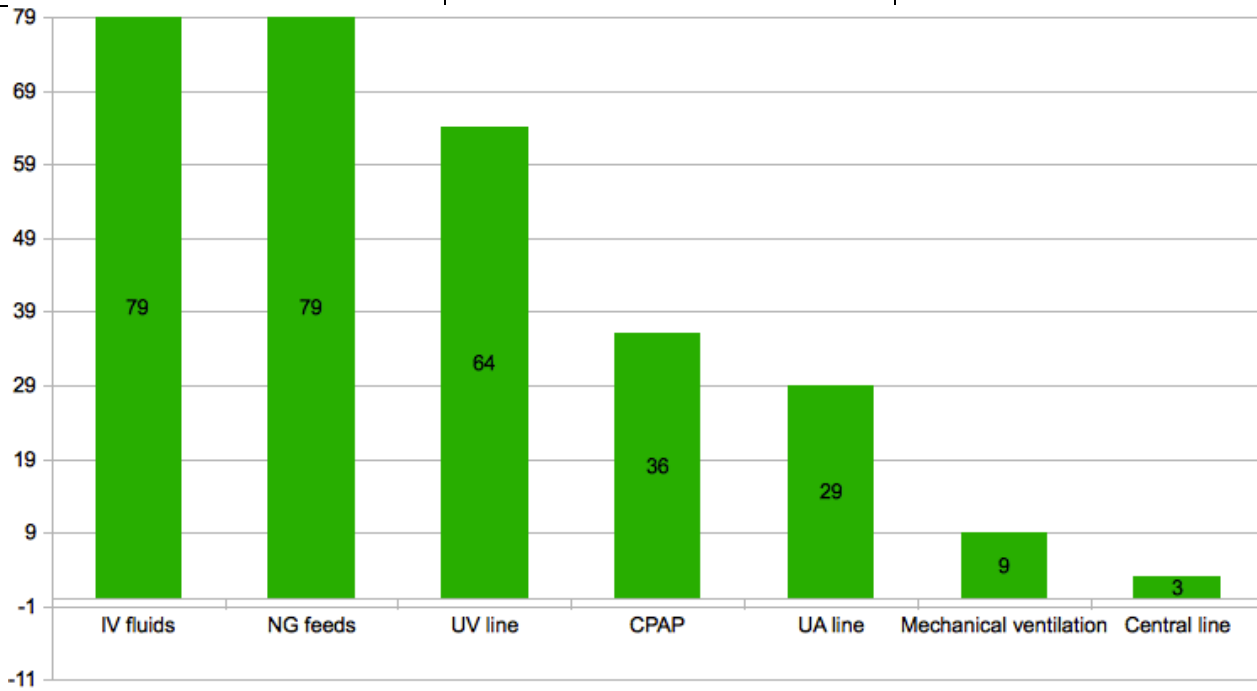
Figure 14: Interventions during neonatal periods

All babies were on IV fluids and Nasogastric feeds.

Majority had umbilical venous lines inserted (81%), umbilical arterial lines in 36.7%.

There were 9 babies (11.3%) requiring mechanical ventilation, 45.5% (36/79) needed CPAP, Inotropic

support in 21.5% and 3.7% needed central venous lines.

NEPHROTOXIC DRUGS USED DURING NICU STAY

Table 15:

Drugs	Number (N =79)	Percentage (%)
Aminoglycosides	71	89.8
Ibuprofen	3	3.7
Indomethacin	3	3.7
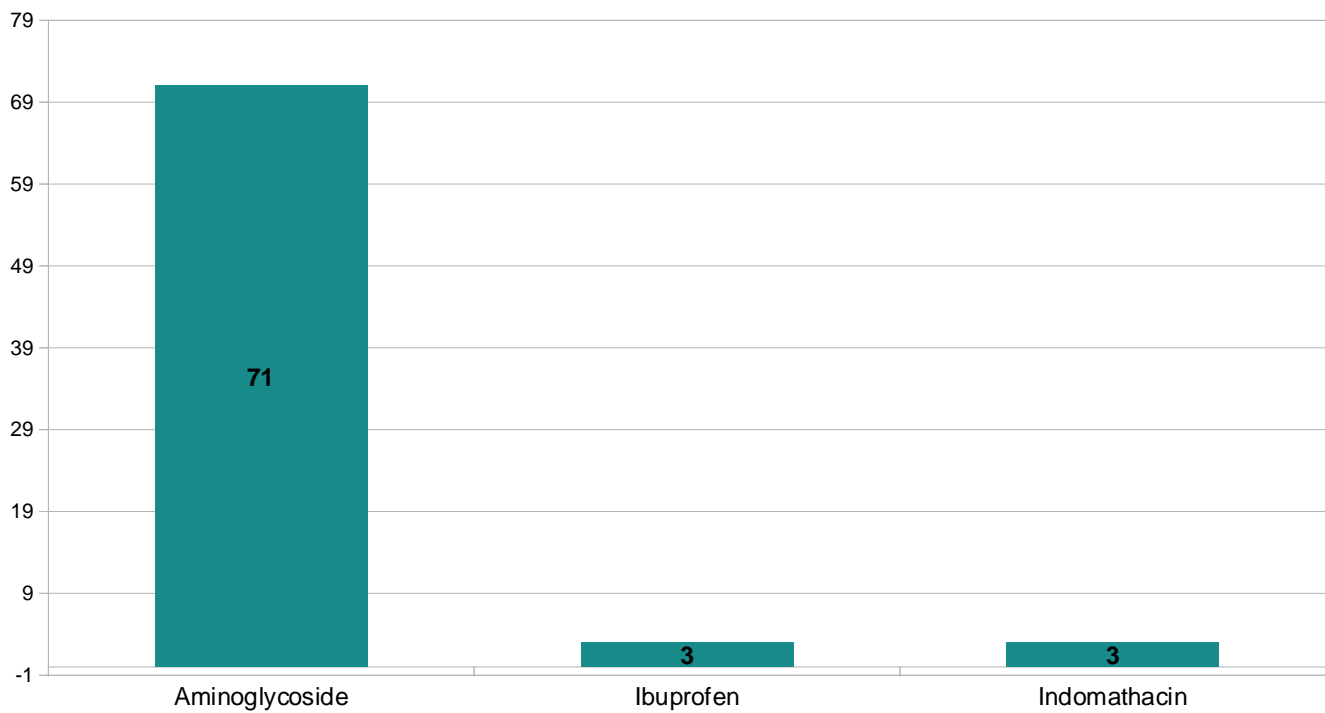
Fig 15: Nephrotoxic drugs used in neonatal period

Aminoglycosides were used in 89.8% of the babies while Ibuprofen and Indomethacin were used in 3.7% each.

II. ASSESSMENT OF RENAL INJURY- LAB EVALUATION

CREATININE LEVELS

Serum creatinine samples were collected weekly starting at Day 3 and then weekly for 4 weeks and analysed.

Table 16: Creatinine trend over 4 weeks

Creatinine(mg/dl)	N	MIN	MAX	MEAN	Std dev
Week 1	78	0.33	1.31	**0.73**	0.21
Week 2	64	0.29	1.22	**0.58**	0.2
Week 3	54	0.1	1.65	**0.44**	0.21
Week 4	46	0.13	1.19	**0.35**	0.19

Figure 16: Trend of creatinine over 4 weeks

The mean value of creatinine decreased from 0.73 mg/dl to 0.35mg/dl over 4 weeks.

COMPARISON OF WEEKLY CREATININE

Table 17: Creatinine levels over the weeks

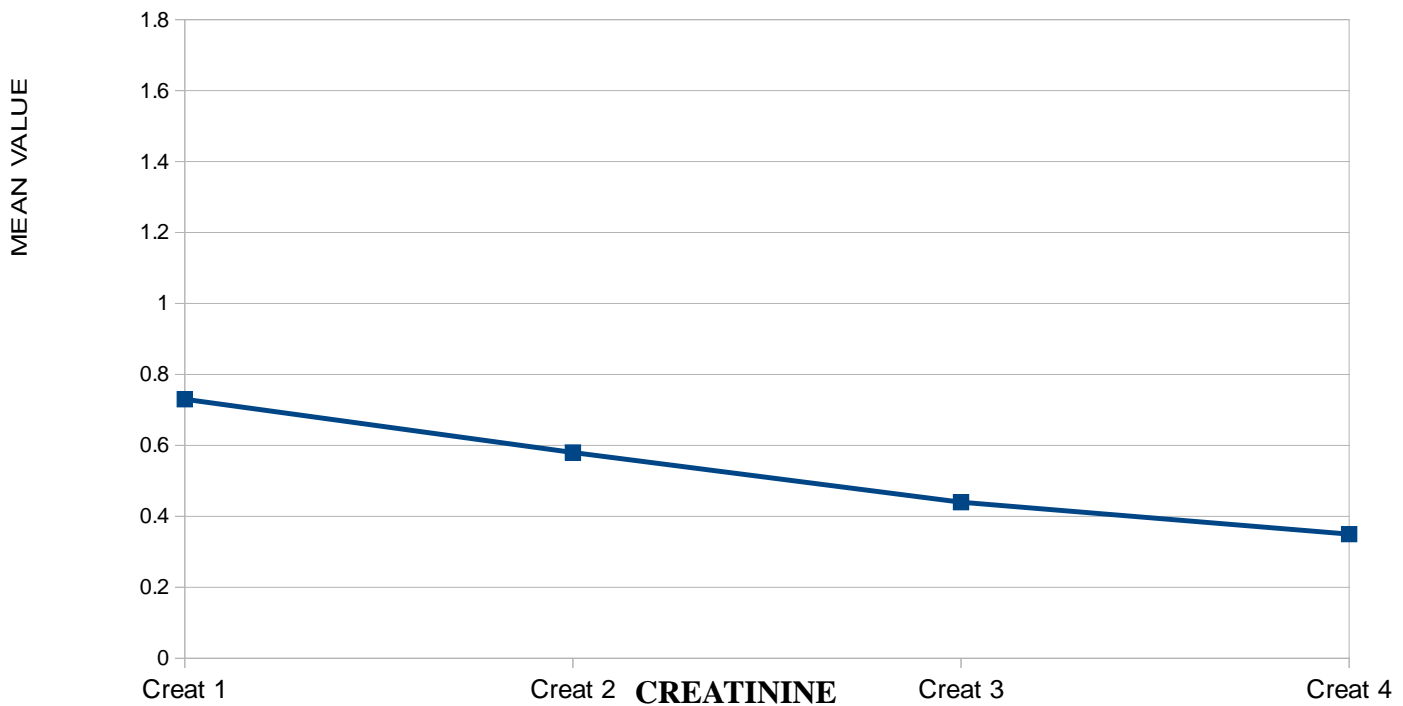| Creatinine level (mg/dl)	Week 1 (n = 79)		Week 2 (n = 64)		Week 3 (n = 54)		Week 4 (n = 46)	
	N	Percentage%	N	Percentage%	N	Percentage %	N	Percentage %
< 0.3	0	0	3	4.7	9	16.7	20	43.5
0.3 to 0.6	21	26.9	36	56.2	40	74.1	22	47.8
0.6 to 0.9	42	53.8	20	31.2	4	7.4	3	6.5
> 0.9	**15**	**19.2**	**5**	**7.8**	**1**	**1.9**	**1**	**2.2**
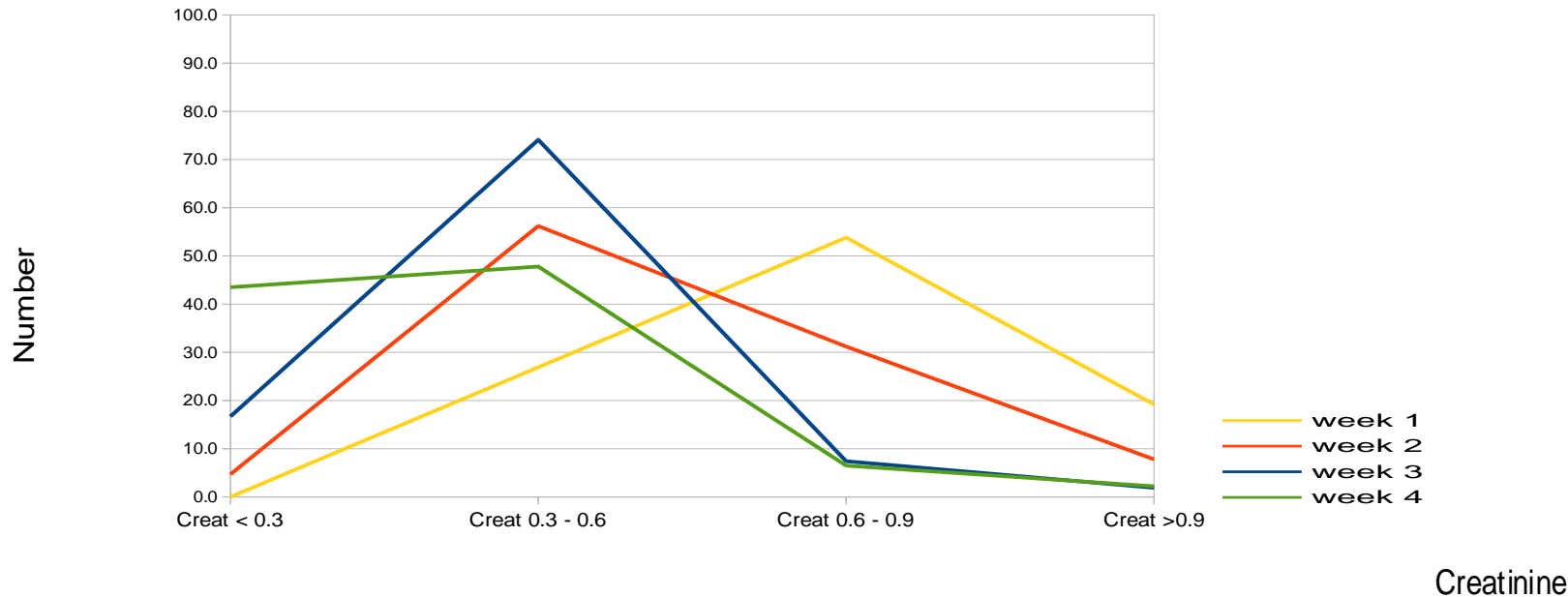
Figure 17: In the <u>first week</u>, nearly half (53.8%) had creatinine in the range of 0.6 to 0.9 and one fifth (19.2) had creatinine levels more than 0.9.

In <u>week 2,</u> highest numbers were in the 0.3 to 0.6 group (56.2%) followed by 0.6 to 0.9 group (31.2%). Only 5 had creatinine value of more than 0.9

By <u>week 3,</u> three fourth had values between 0.3 to 0.6. By <u>week 4</u>, almost 90% had creatinine < 0.6

Thus by week 4, from an initial 19.2 %, only 2.2 % had elevated creatinine values >0.9 mg/dl

CREATININE IN VENTILATED BABIES - (IMV AND CPAP)

Table 18: Creatinine trend in IMV and CPAP groups

CREATININE (mg/dL)	IMV (n= 9)	CPAP (n=36)	Non Ventilation (n= 34)	P value
WEEK 1	0.8	0.7	0.7	0.401
WEEK 2	0.6	0.6	0.6	0.944
WEEK 3	**0.7**	**0.4**	**0.4**	**0.03**
WEEK 4	0.3	0.4	0.3	0.573
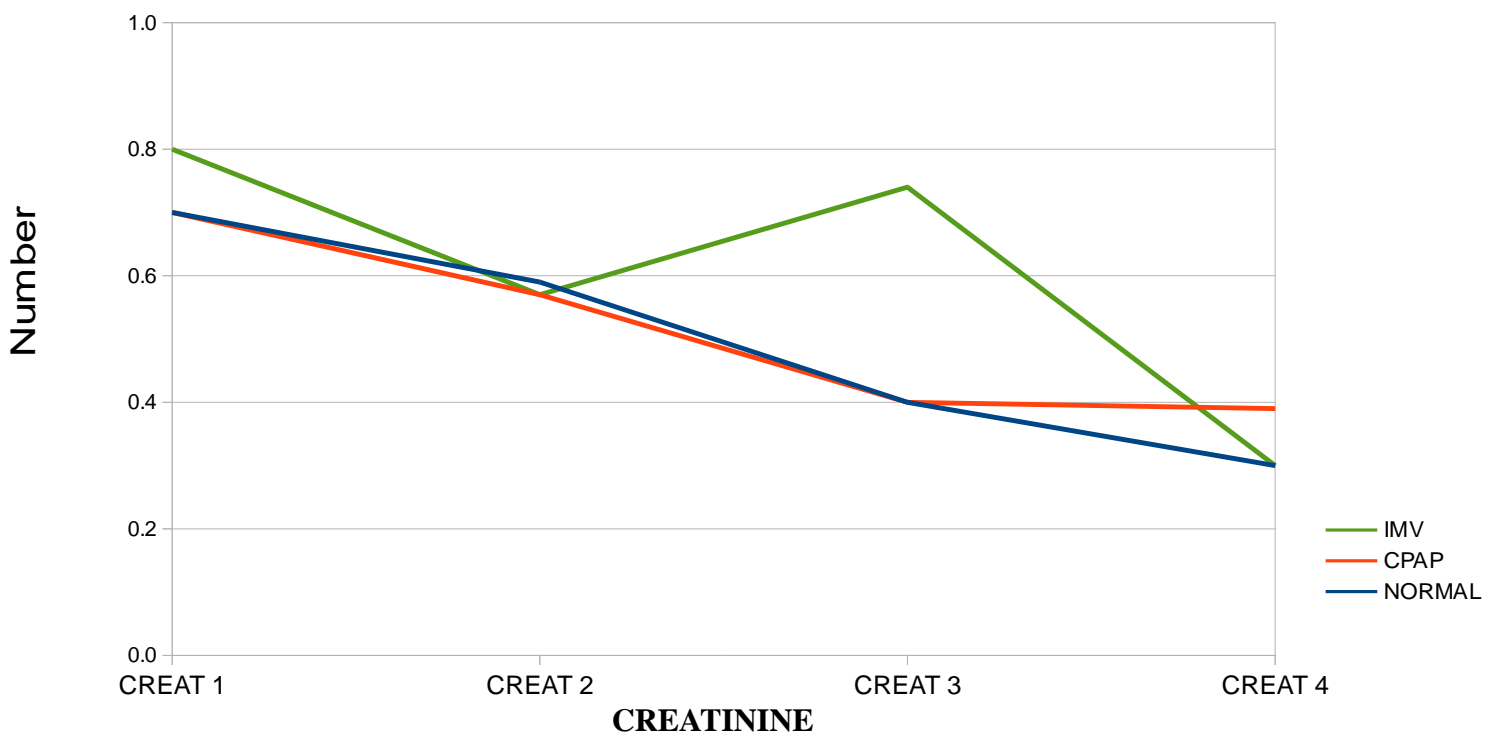
Figure 18: Creatinine levels in IMV and CPAP babies

The Creatinine levels were almost similar in the IMV and CPAP group except in week 3 when the difference was statistically significant (p<0.05)

CREATININE IN ASPHYXIATED BABIES

Table 19:

CREATININE (mg/dl)	ASPHYXIA (n = 6)	NON ASPHYXIA (n=73)	P value
WEEK 1	0.75	0.73	0.778
WEEK 2	0.53	0.59	0.488
WEEK 3	0.43	0.44	0.856
WEEK 4	0.33	0.35	0.784
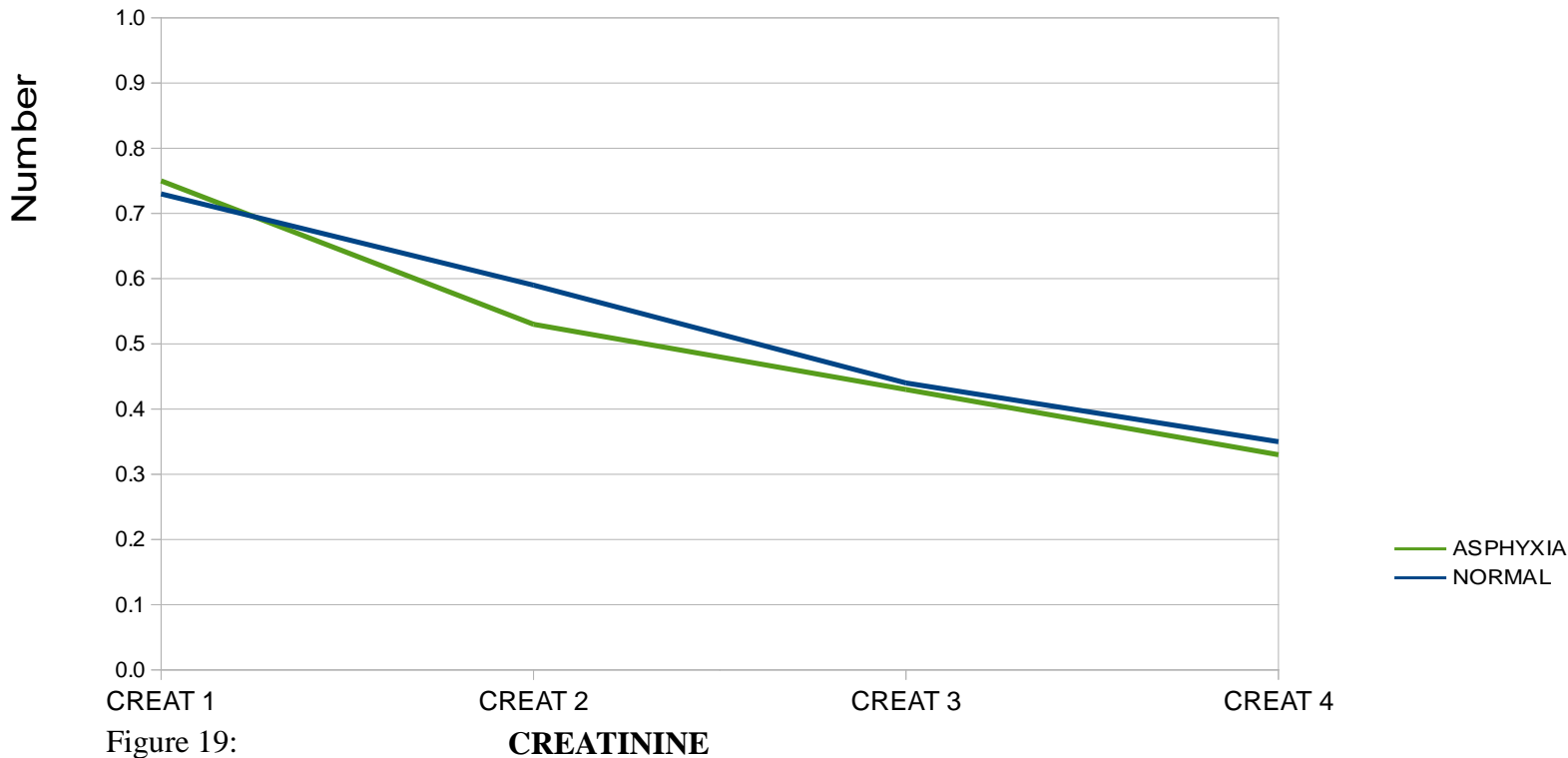
Figure 19: **CREATININE**

The values of Creatinine were almost in the same range in the asphyxiated babies and the non asphyxiated babies.

CREATININE and NSAID DRUGS

Table 20: NSAID drugs and creatinine

CREATININE (mg/dl)	NSAID drugs (n=7)	No NSAID drugs (n=72)	P value
WEEK 1	O.72	0.73	0.884
WEEK 2	0.69	0.57	0.149
WEEK 3	**0.61**	**0.42**	**0.048**
WEEK 4	0.34	0.49	0.102
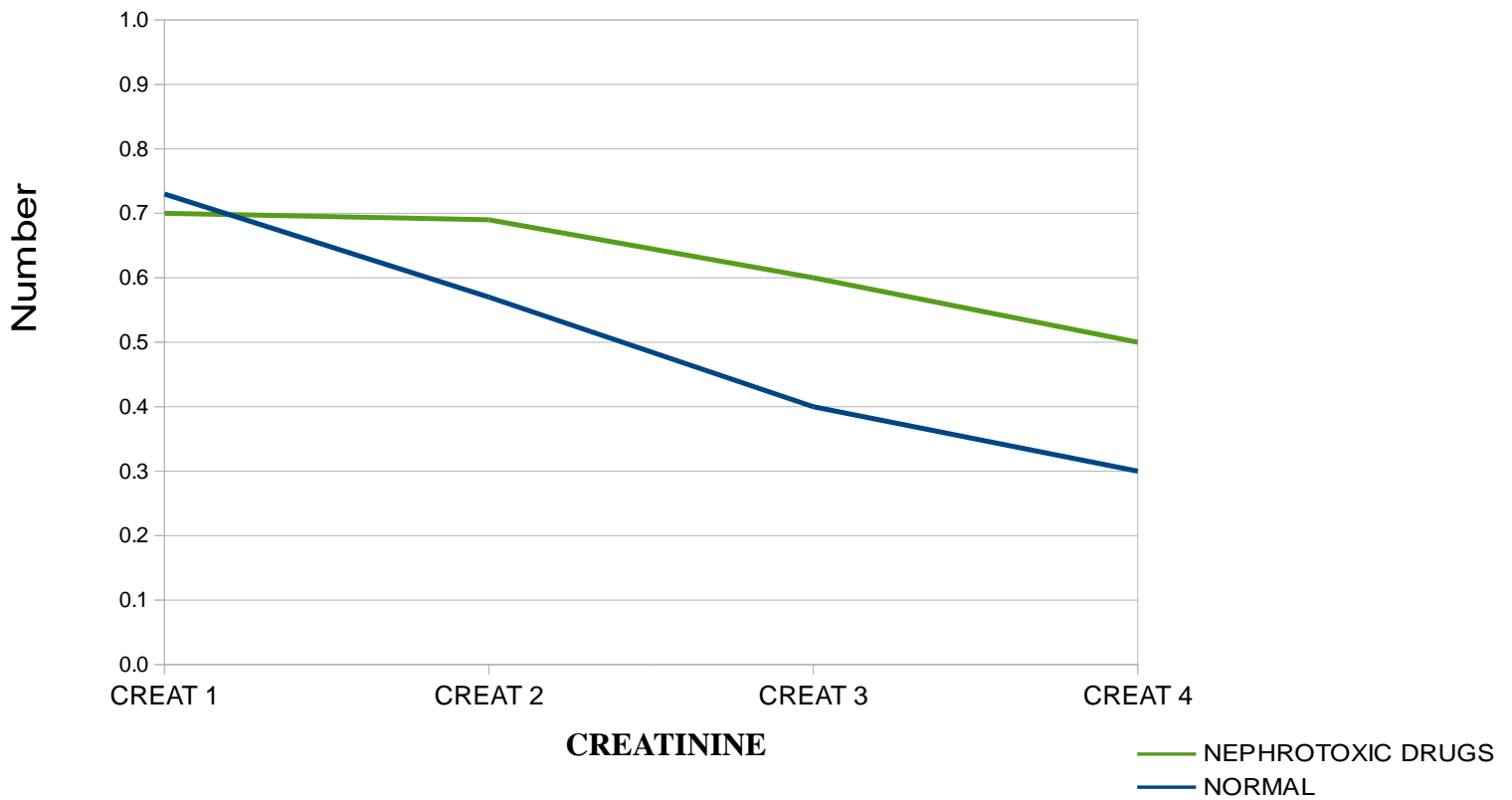
Figure 20: NSAID drugs used were Indomethacin and Ibuprofen.

Weekly creatinine remained high in all weeks but was statistically significant in week 3 (p= 0.048)

CREATININE AND UMBILICAL LINES

Table 21:

CREATININE (mg/dl)	UA + UV lines (n=29)	No lines (n= 50)	P value
WEEK 1	0.78	0.71	0.884
WEEK 2	0.6	0.56	0.149
WEEK 3	**0.51**	**0.41**	**0.048**
WEEK 4	0.39	0.33	0.102
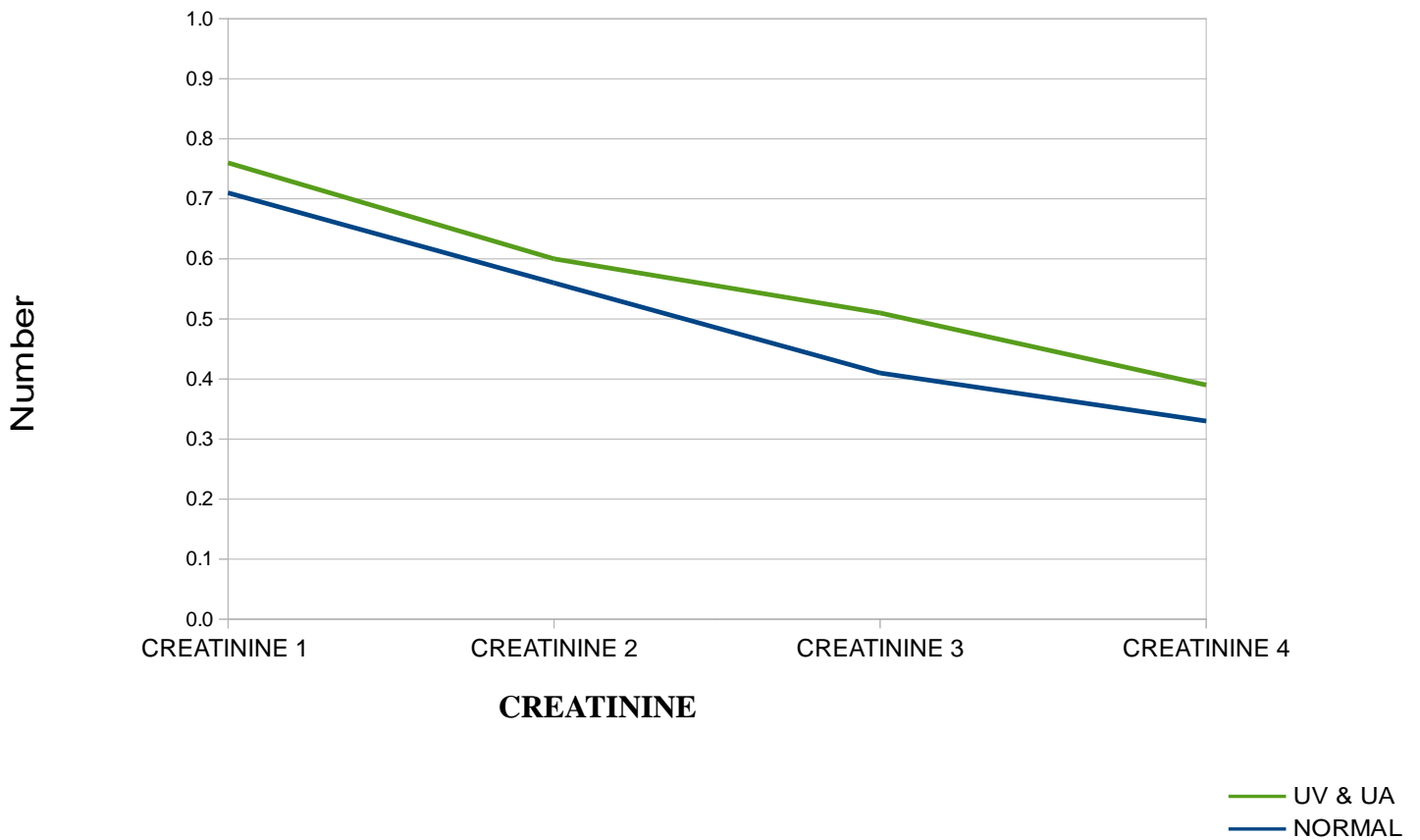
Figure 21:

Creatinine levels were significantly higher in those with umbilical lines in week 3 (p=0.048)

CREATININE AND INOTROPES

Table 22

CREATININE (mg/dL)	Inotropes	No Inotropes	P value
WEEK 1	0.8241	0.7126	0.53
WEEK 2	0.6264	0.5714	0.388
WEEK 3	**0.6429**	**0.4177**	**0.008**
WEEK 4	0.3425	0.3581	0.879

CREATININE

— inotropes
— No inoropes
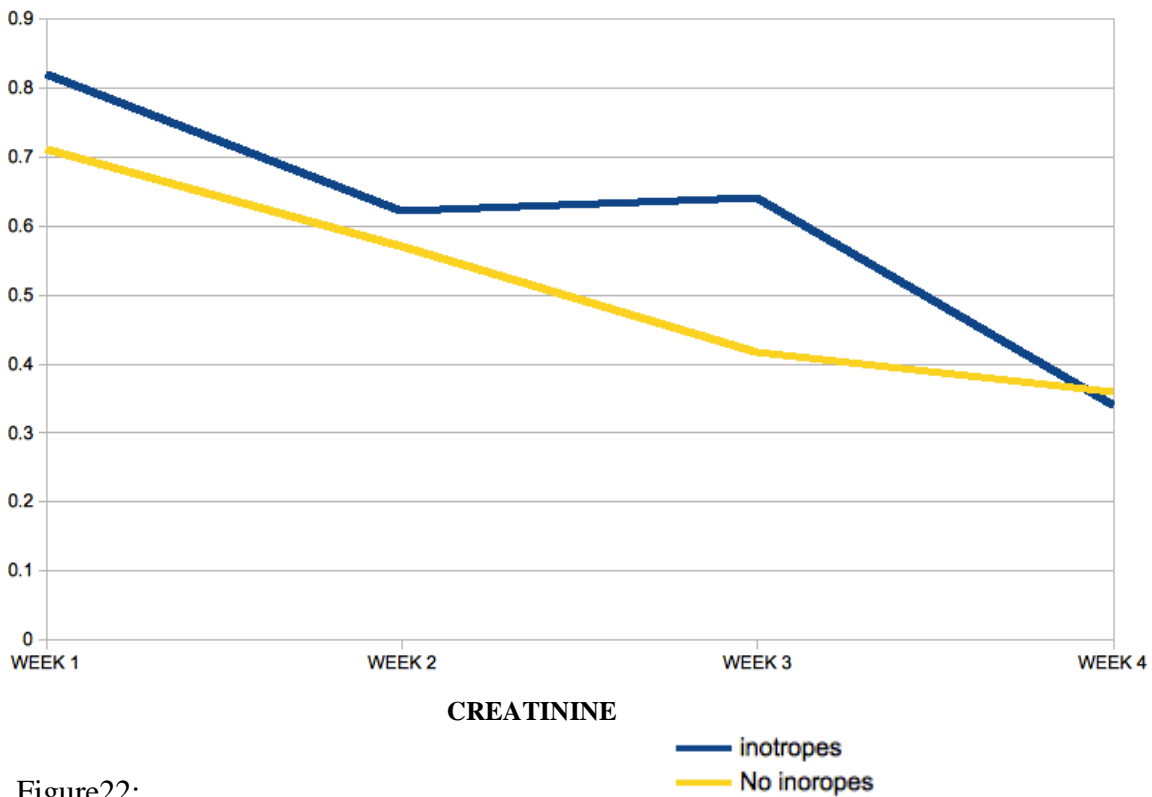
Figure22:

Creatinine values were higher in the inotrope group, but it was significantly higher in week 3 (p<0.05)

III. COMPARISON OF NGAL AND CREATININE

As part of a pilot study Urinary NGAL was studied to assess its usefulness as an early biomarker for AKI in preterm babies. Its ability to predict UTI before a rise in creatinine occurs was studied.

Weekly urine samples from a total of 31/79 babies were processed and the data was analysed.

NGAL values were compared with various risk factors for AKI as well as with concomitant serum creatinine values in the same patients.

URINARY NGAL LEVELS

Urine for NGAL was collected and analysed in 31 babies once a week till discharge (4weeks)

Table 23: Mean values of NGAL

NGAL (ng/ml)	N	MIN	MAX	MEAN	Std dev
Week 1	29	14.38	979.1	**260.51**	264.09
Week 2	31	4.88	850.2	**184.66**	228.12
Week 3	32	0	448.85	**83.68**	93.91
Week 4	17	0	105.08	**36.33**	35.19
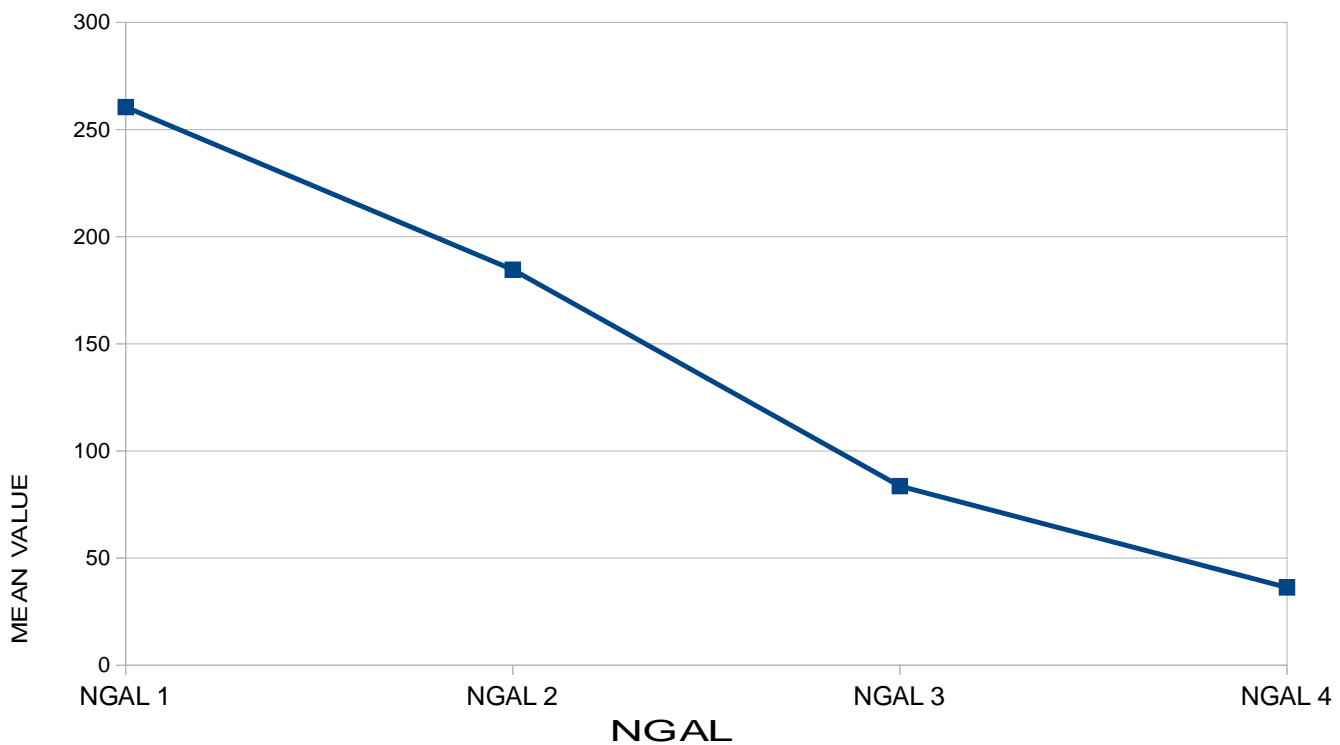
Figure 23: Mean value of NGAL over the weeks

The mean value of NGAL showed a decreasing trend from 260.5 to 36.3 over 4 weeks.

COMPARISON OF WEEKLY NGAL VALUES

Table 24:

NGAL level (ng/ml)	Week 1 (n = 31)		Week 2 (n = 31)		Week 3 (n= 31)		Week 4 (n = 17)	
	N	Percentage %	N	Percentage%	N	Percentage %	N	Percentage %
< 250	18	**58.1**	24	**77.4**	30	**96.4**	17	100
250 – 500	9	**29**	4	**12.9**	1	**3.1**	0	0
> 500	4	**12.9**	3	**9.7**	0	**0**	0	0
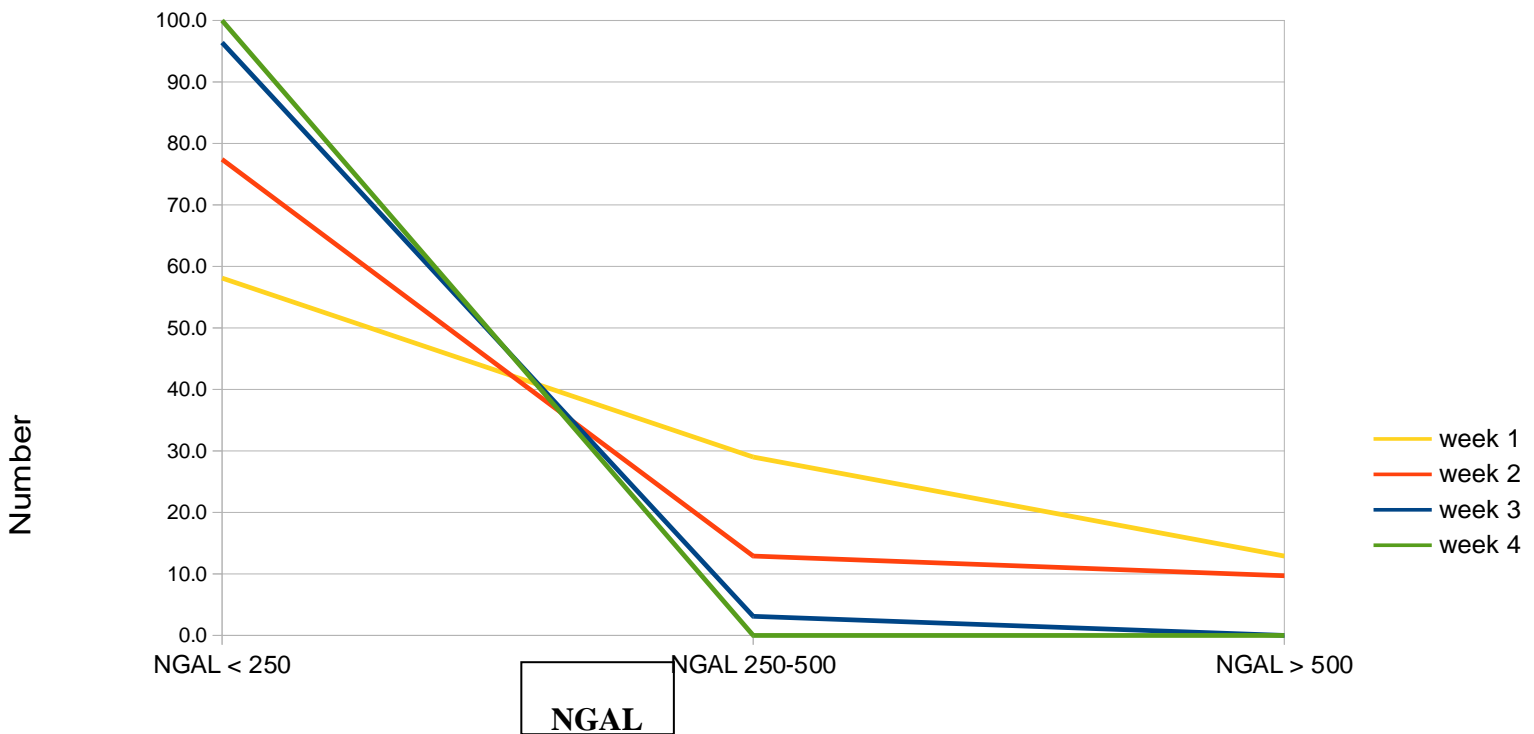
Figure 24:

In the first week, there were 58.1% with NGAL < 250, but 12.9% had values more than 500.

By week 2, nearly 80% had values < 250. By week 3 and 4, almost all (96.4 and 100%) had values < 250.

NGAL VS GESTATIONAL AGE

Table 25:

NGAL (n=31)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Pearson correlation	0.77	0.71	0.135	0..141
P value	0.079	0.704	0.462	**0.015**
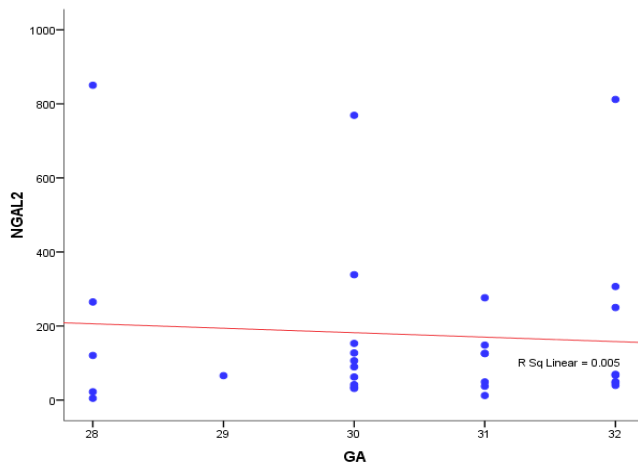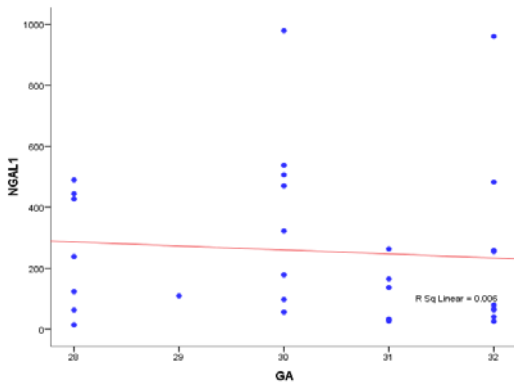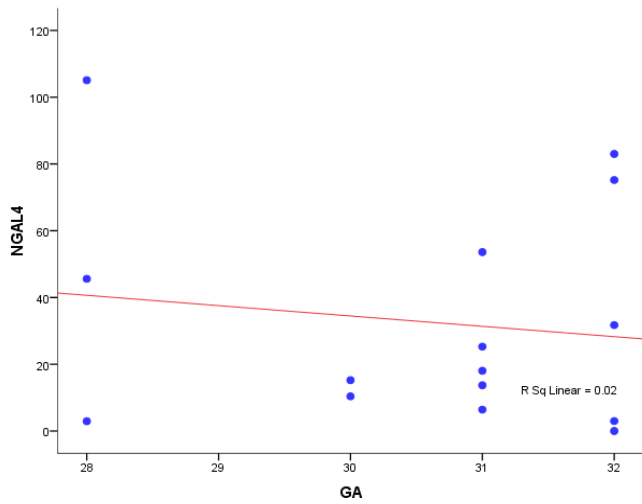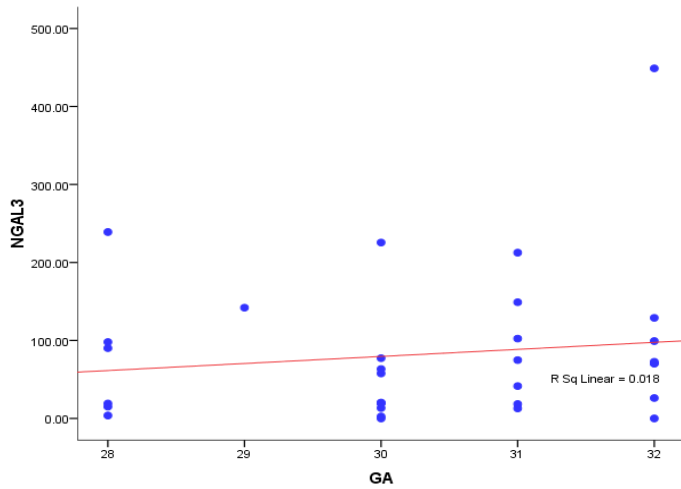
Figure 25:

Weekly NGAL values were plotted against gestational age.

Gestational age and NGAL showed an inverse relationship. However this was not statistically significant. (p=>0.05) except in week 4.

NGAL VERSUS BIRTH-WEIGHT

Table 26:

NGAL (ng/ml)	Week 1	Week 2	Week 3	Week 4
Pearson correlation	-0.112	-0.066	-0.237	-0.446
P value	0.561	0.726	0.192	0.073
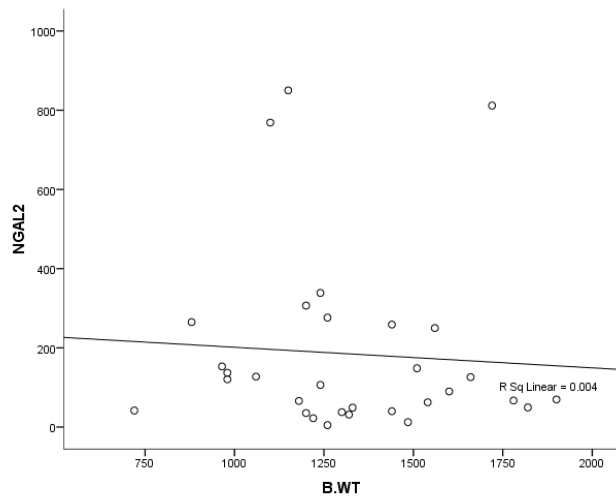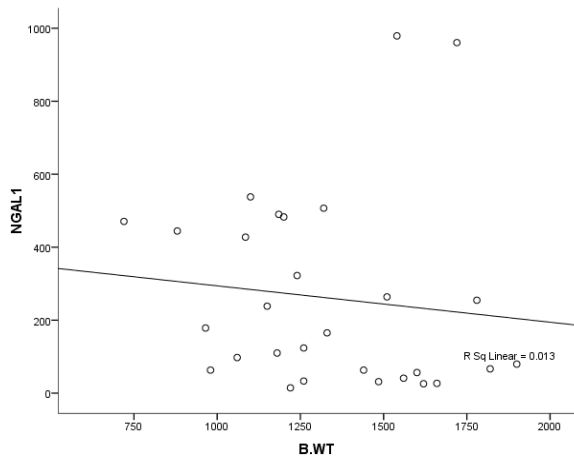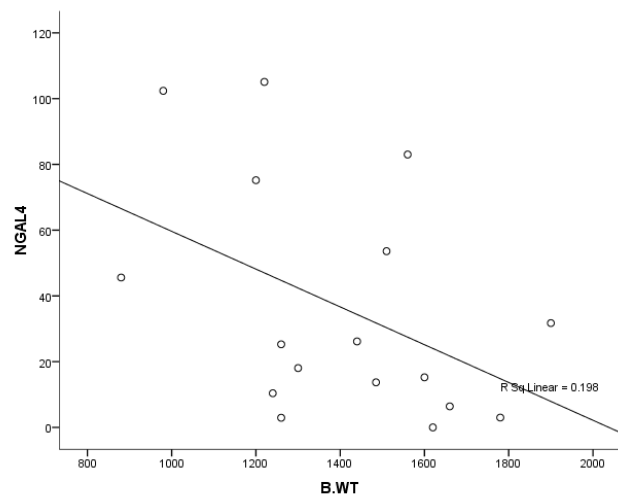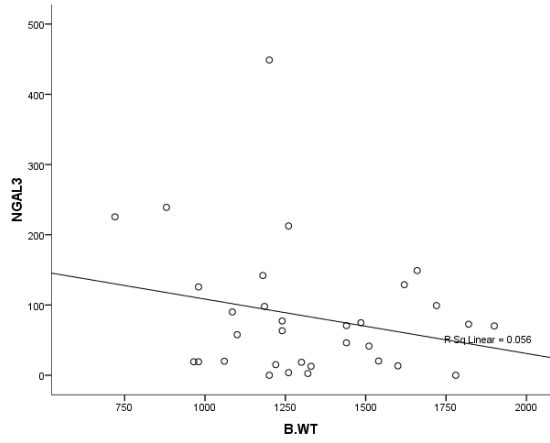
Figure 26:

Weekly NGAL values showed an inverse relation with birth-weight. With increasing birth weight, NGAL values decreased. However this was not statistically significant.

COMPARISON OF NGAL WITH CREATININE

NGAL values for different risk factors were assessed and compared with concomitant creatinine values in the same babies.

NGAL IN VENTILATED BABIES: MECHANICAL VENTILATION AND CPAP

Table 27:

NGAL (ng/ml) n=31	IMV (n= 2)	CPAP (n=13)	No Ventilation (n=16)	P value
WEEK 1	599	230	234	0.147
WEEK 2	**830**	**196**	**93**	**0.000**
WEEK 3	99	83	80	0.982
WEEK 4	0	31	33	0.923
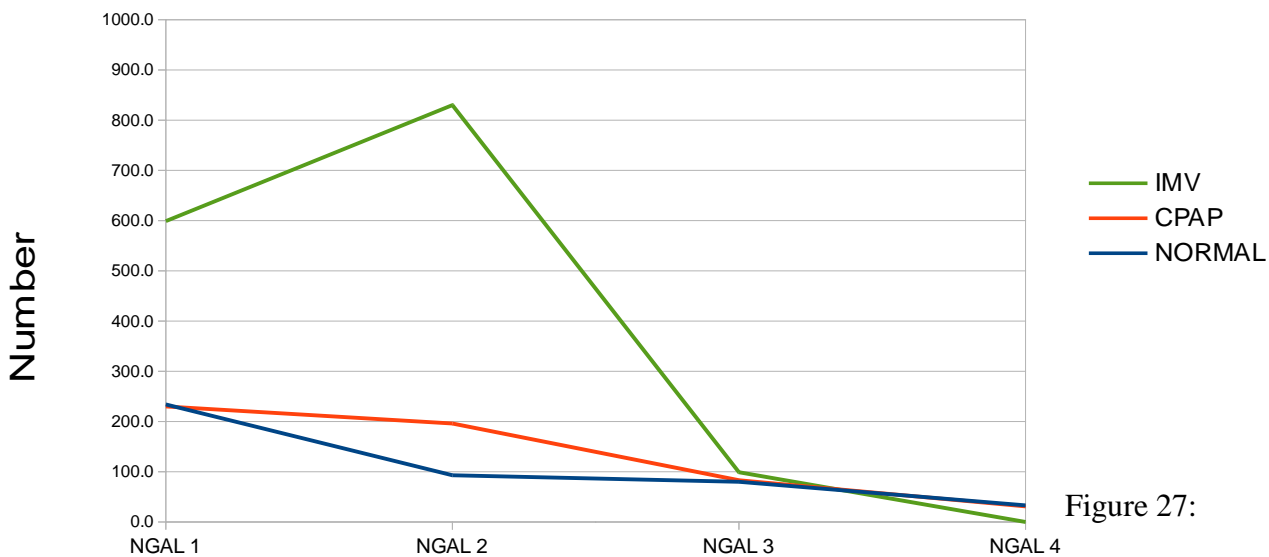
Figure 27: NGAL

NGAL rise was higher in the IMV group compared to the CPAP group in most weeks. This difference was statistically significant (p<0.05) during week 2.

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CREATININE : MECHANICAL VENTILATION AND CPAP

Table 28:

CREATININE (mg/dL) n=31	IMV (n= 2)	CPAP (n=13)	No Ventilation (n=16)	P value
WEEK 1	0.7050	0.7715	0.6488	0.231
WEEK 2	0.6400	0.5331	0.5406	0.719
WEEK 3	**1.2250**	**0.3992**	**0.3838**	**0.000**
WEEK 4	0.37	0.44	0.3021	0.319
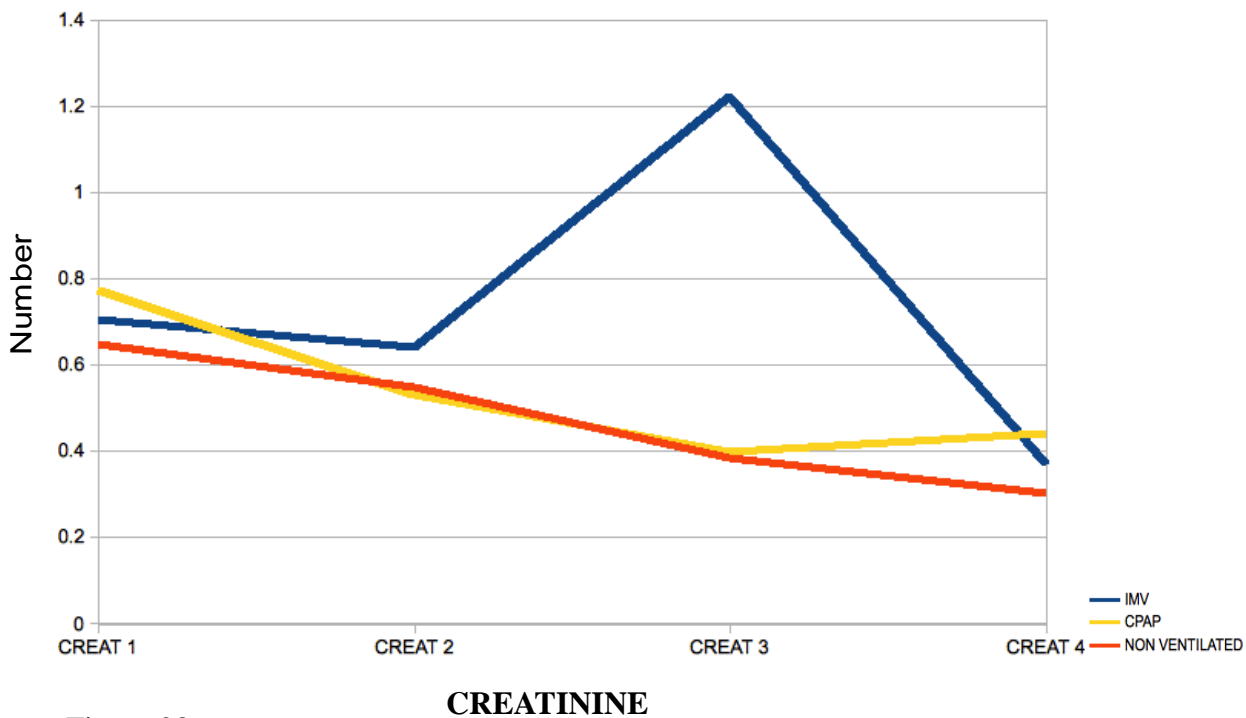
CREATININE

Figure:28

Creatinine rise was higher in the IMV group compared to the CPAP group in most weeks. This difference was statistically significant ($p<0.05$) during week 3.

NGAL IN ASPHYXIATED PRETERM BABIES

Table 29: NGAL in asphyxia

NGAL (ng/ml) n=31	ASPHYXIA n=6	NO ASPHYXIA (n=25)	P value
WEEK 1	59.7	2.8	0.165
WEEK 2	1.5	1.8	0.83
WEEK 3	32	87	0.338
WEEK 4	63	24	0.064
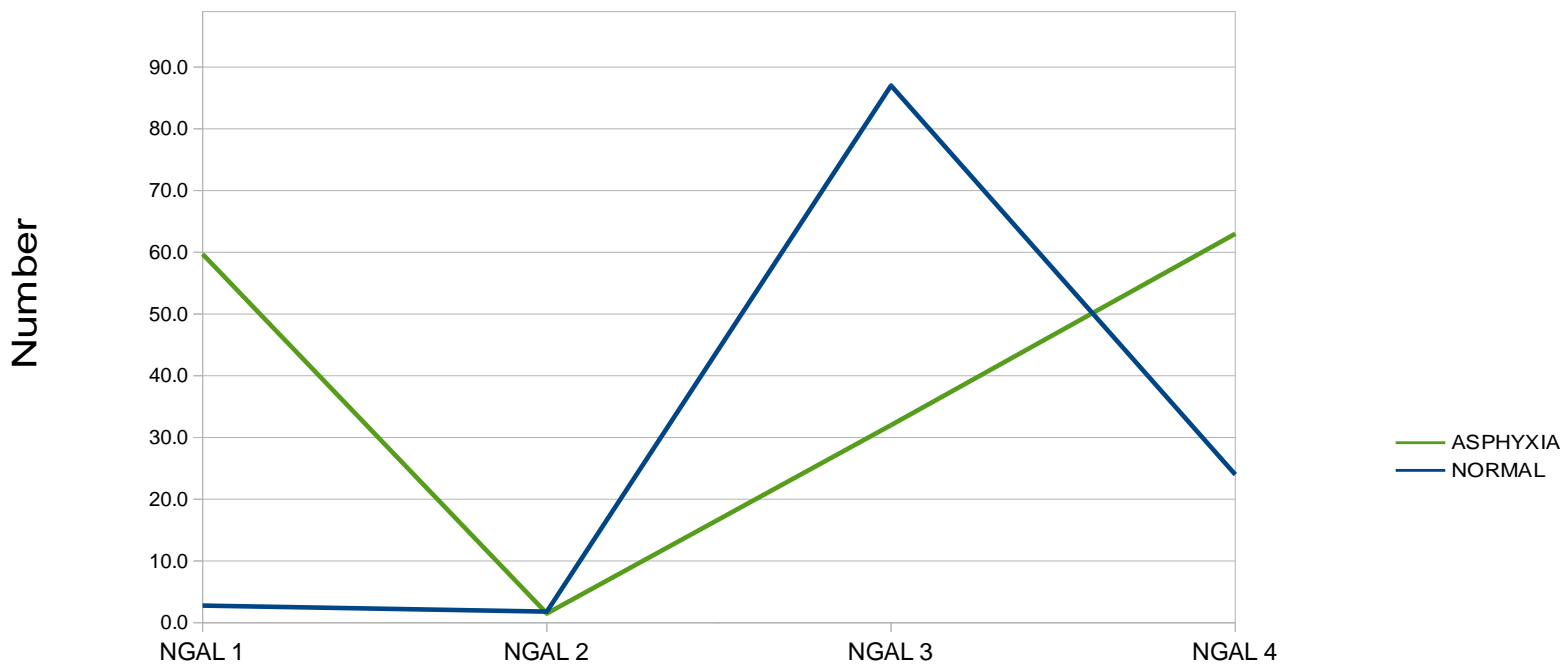
Figure 29:   NGAL

The NGAL values was compared with non asphyxiated babies. NGAL in weeks 1, 3 and 4 were higher but the difference was not statistically significant.

CREATININE IN ASPHYXIATED PRETERM BABIES

Table 30: Creatinine in asphyxia

CREATININE (mg/dL)	ASPHYXIA n=3	NO ASPHYXIA (n=28)	P value
WEEK 1	0.84	0.6879	0.198
WEEK 2	0.55	0.5428	0.938
WEEK 3	0.4267	0.4464	0.904
WEEK 4	0.3275	0.3754	0.711

ASPHYXIA
No iASPHYXIA
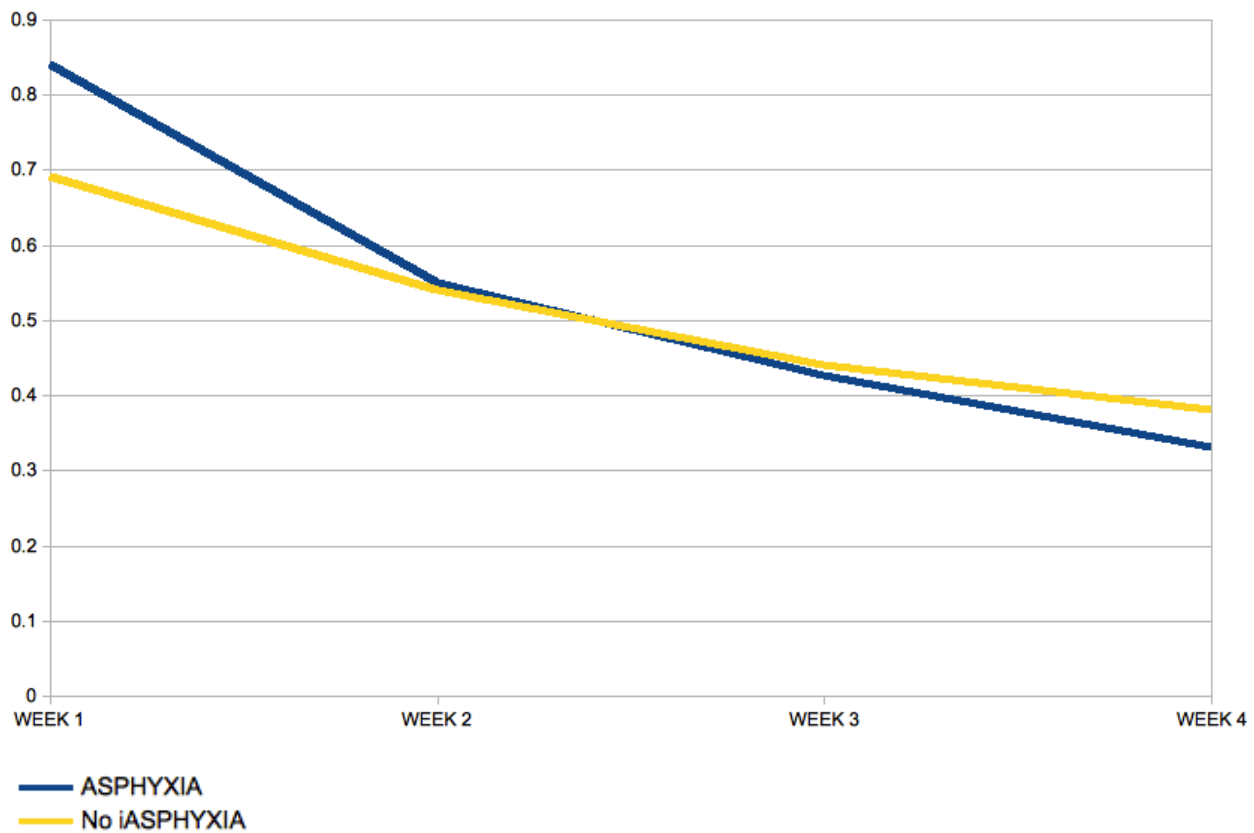
Figure 30: Creatinine in asphyxiated neonates was higher during the first week but this was not significant. ($p > 0.05$)

NGAL AND UMBILICAL LINES

Table 31

NGAL ng/ml	UA + UV (n=11)	No lines (n=20)	P value
WEEK 1	281.82	237.58	0.641
WEEK 2	279.91	127.01	0.212
WEEK 3	98.88	71.94	0.482
WEEK 4	42.12	22.55	0.64
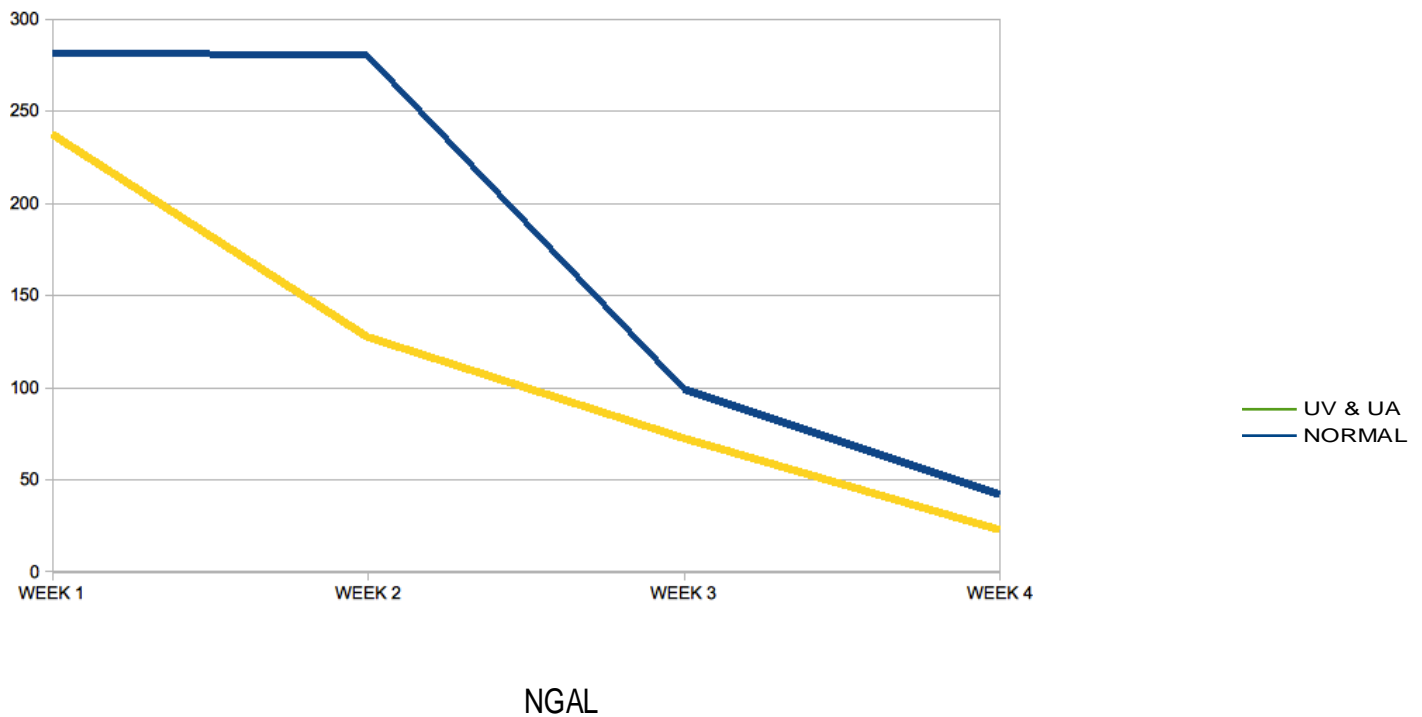
NGAL

Figure 31: NGAL levels in this group were higher in the UA group but the difference was not statistically significant

CREATININE AND UMBILICAL LINES

Table 32

CREATININE (mg/dL)	UA + UV (n=11)	No lines (n=20)	P value
WEEK 1	0.7091	0.6986	0.886
WEEK 2	0.5809	0.5250	0.382
WEEK 3	0.5482	0.3875	0.106
WEEK 4	0.4620	0.3167	0.115
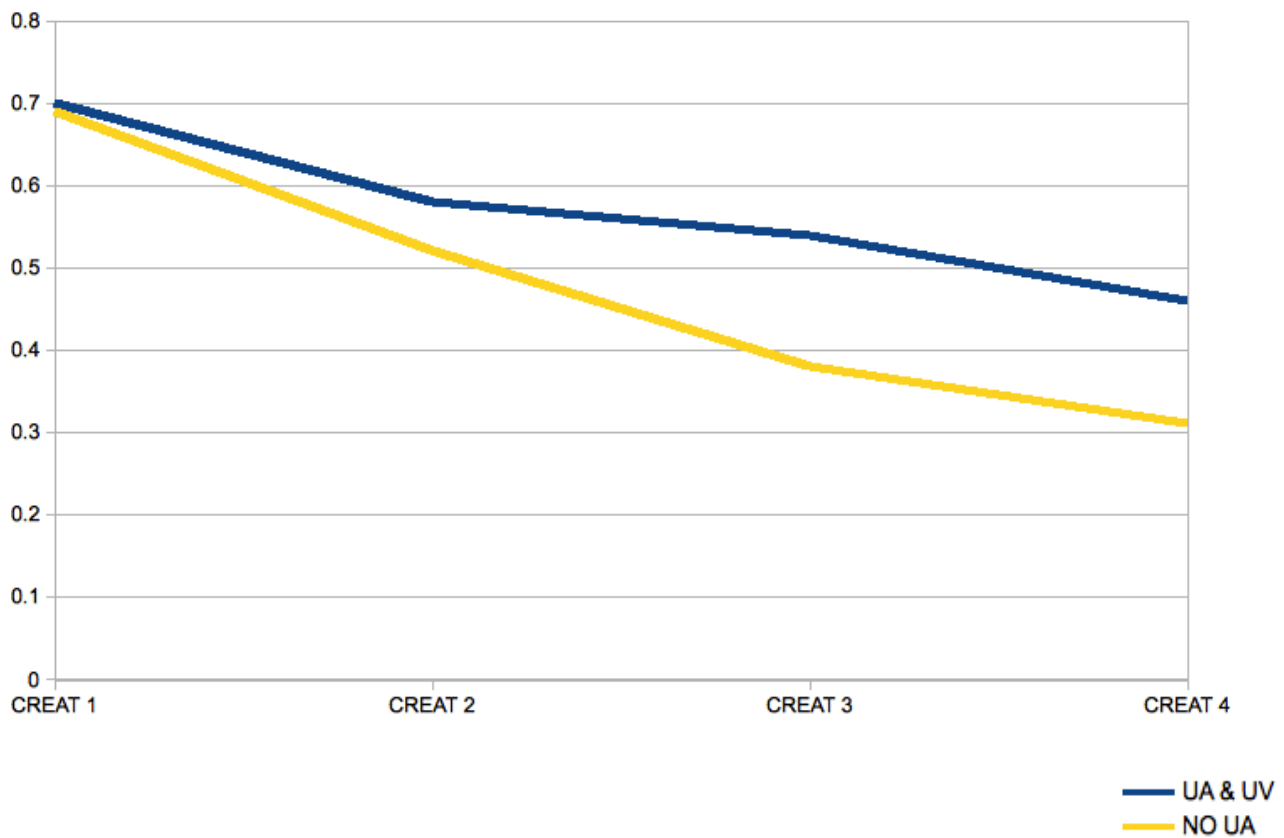
Figure 33 : Creatinine values were higher in those with umbilical arterial and venous lines compared to with no arterial lines. This difference was not statistically significant (p value > 0.05)

NGAL AND NSAIDS

Table 34:

NGAL (ng/ml)	NSAIDS (n=7)	No NSAIDS (n = 72)	P value
WEEK 1	313.89	250	0.69
WEEK 2	375	148.16	0.063
WEEK 3	114.13	79.03	0.547
WEEK 4	45.58	31.68	0.699
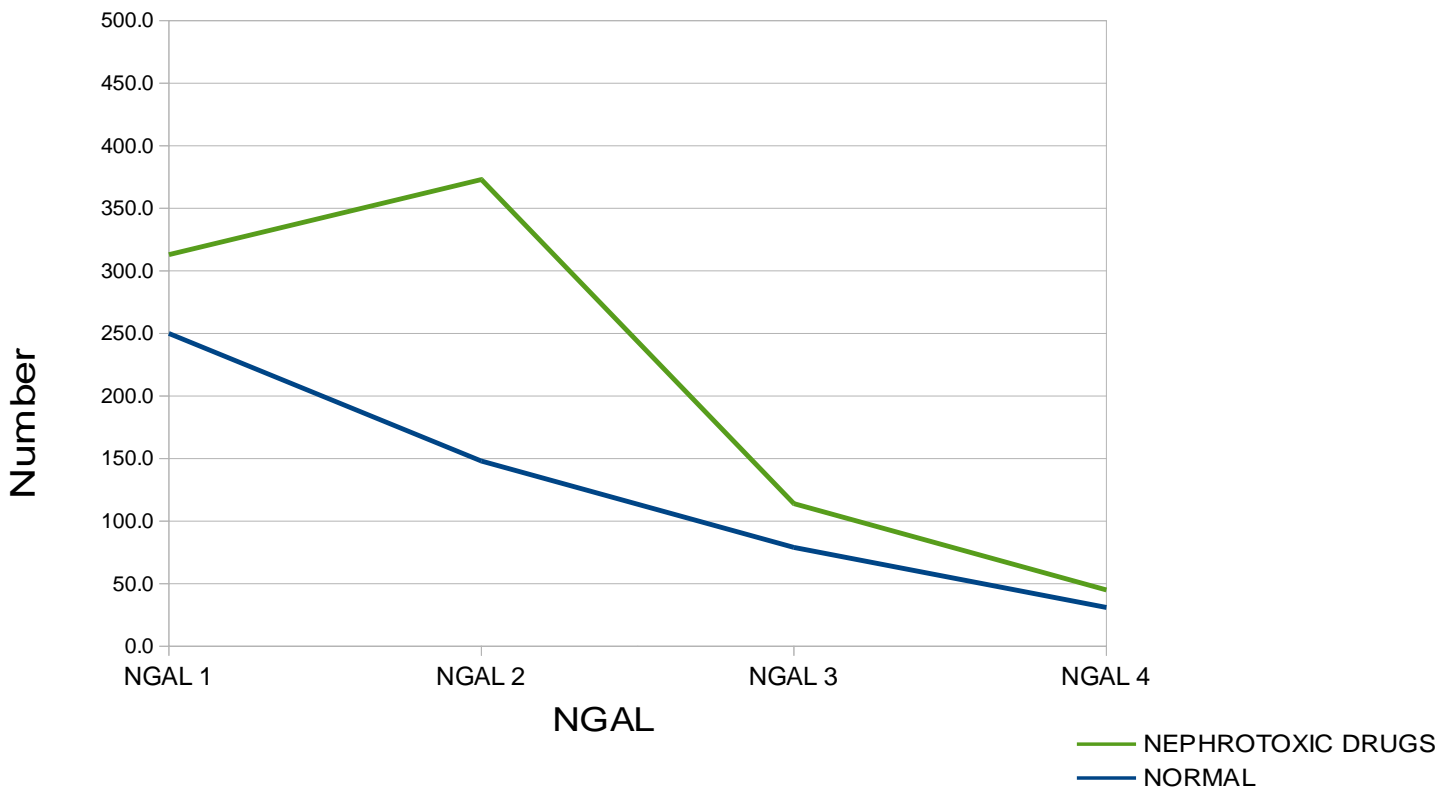
Figure 34:Indomethacin and Ibuprofen were the NSAID - nephrotoxic drugs babies were exposed to.

NGAL values were higher in those receiving NSAIDs but the difference was not significant.

CREATININE AND NSAIDS

Table 35:

CREATININE (mg/dL)	NSAIDS (n=4)	No NSAIDS (n = 27)	P value
WEEK 1	0.65	0.70	0.571
WEEK 2	0.6775	0.5252	0.094
WEEK 3	0.6975	0.4070	0.435
WEEK 4	0.5375	0.3404	0.454
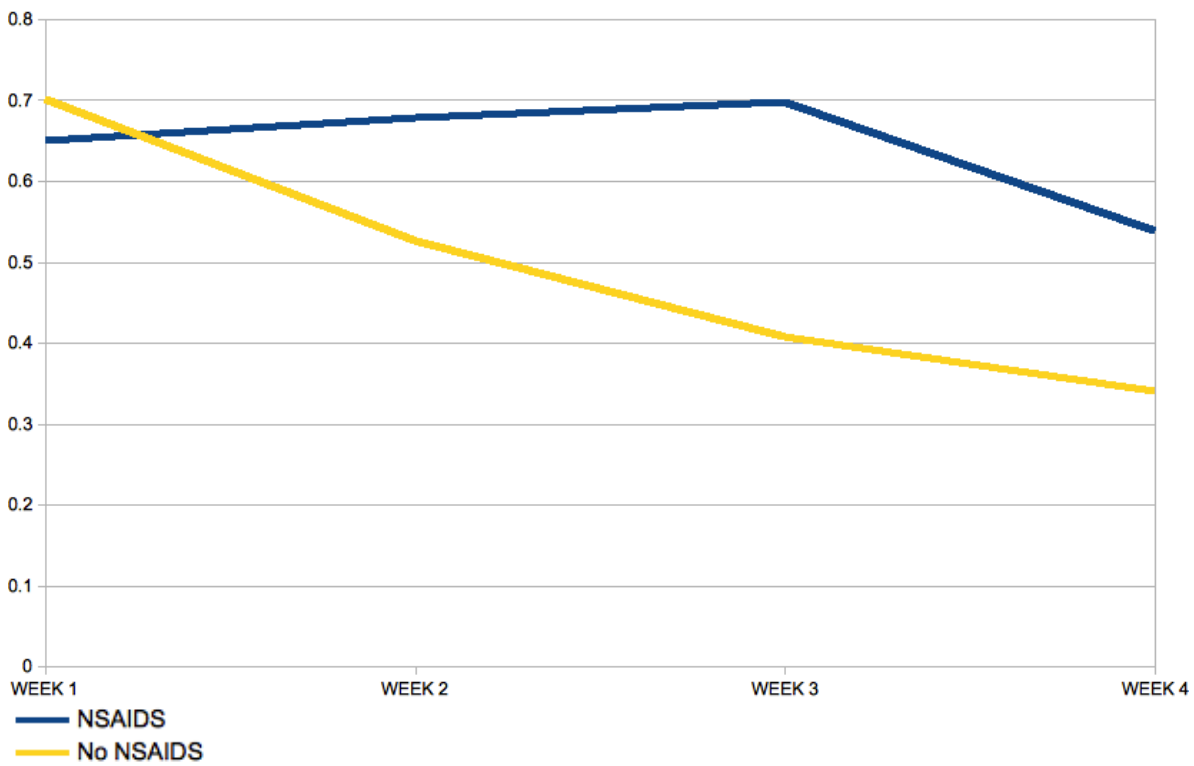
Figure 35: Creatinine was found to persist at a higher value. However on comparing with unexposed babies, there was no significant difference (p>0.05)

NGAL and AMINOGLYCOSIDES

Table 36

NGAL (ng/ml)	Aminoglycosides (n=30)	No aminoglycosides (n=1)	P value
WEEK 1	264	69	0.295
WEEK 2	170	188	0.914
WEEK 3	83	20	0.531
WEEK 4	32	83	0.158
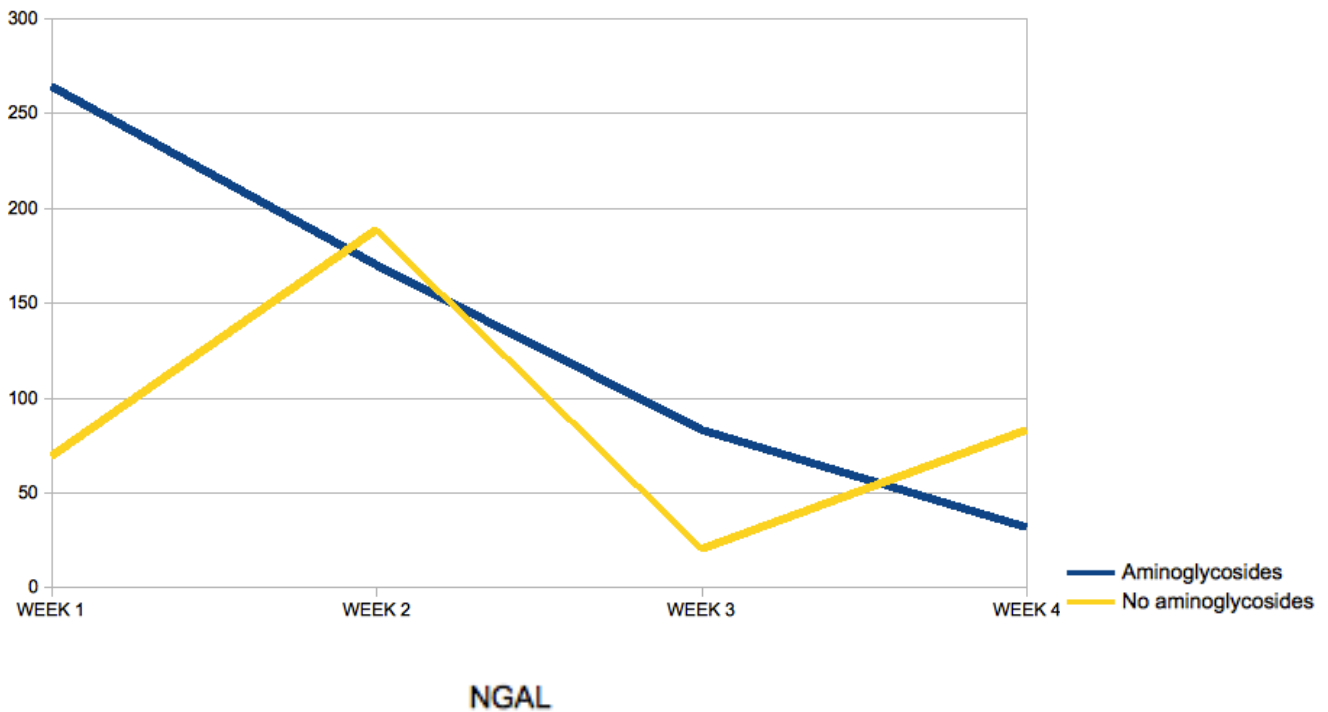
NGAL

Figure 36: Babies were exposed to aminoglycosides in the first week of life. Urinary NGAL showed a decreasing trend over the weeks. Compared to those with no exposure the level of NGAL was not significantly higher (p >0.05)

CREATININE AND AMINOGLYCOSIDES

Table 37:

CREATININE (mg/dL)	Aminoglycosides (n=30)	No aminoglycosides (n=1)	P value
WEEK 1	0.7074	0.54	0.402
WEEK 2	0.5523	0.41	0.259
WEEK 3	0.4393	0.60	0.558
WEEK 4	0.3785	0.24	0.428
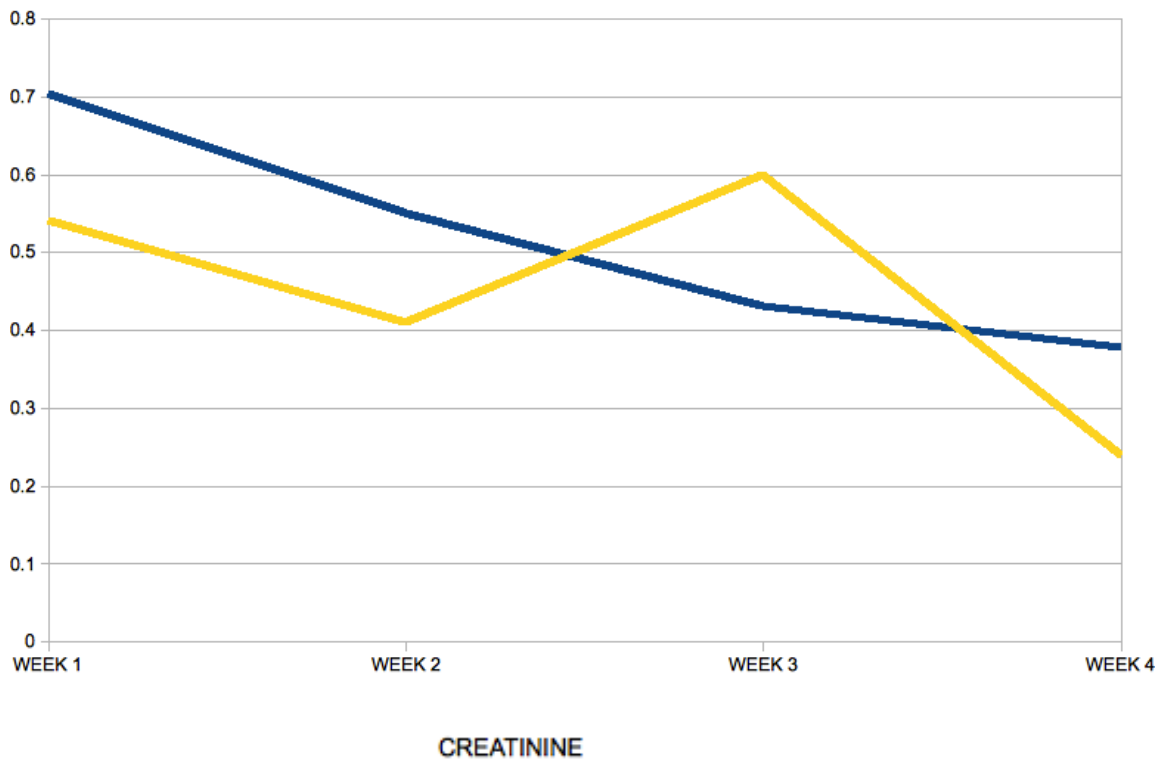
CREATININE

Figure 35: Babies were exposed to aminoglycosides in the first week of life. Serum showed a decreasing trend over the weeks. Compared to those with no exposure the level of creatininee was higher in the first week, however this was not significant (p >0.05)

IV. PREMATURITY AND AKI

A total of 10 babies had AKI (12.6%). This group was studied further.

Four babies with AKI had both creatinine and NGAL done and these values were compared

CREATININE IN THOSE WITH AKI

Table 36

Creatinine (mg/dl)	Week 1	Week 2	Week 3	Week 4
Baby 1	0.7	0.37	0.2	0.8
Baby 2	0.44	0.86	0.26	0.46
Baby 3	0.76	0.71	1.65	0.37
Baby 4	0.71	0.63	0.53	1.19
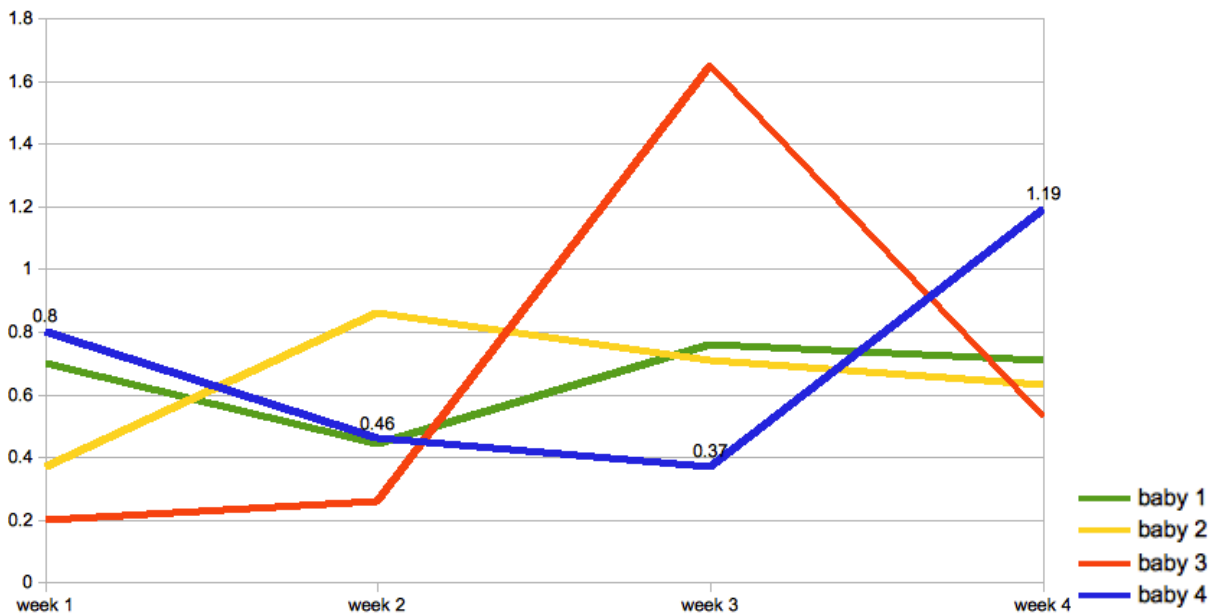
Figure 36

NGAL IN BABIES WITH AKI

Table 37:

NGAL	Week 1	Week 2	Week 3	Week 4
Baby 1	427.5	-	90.1	-
Baby 2	>1000	258.9	46.2	26.7
Baby 3	238	850	>1000	-
Baby 4	444.3	265	239	45.58
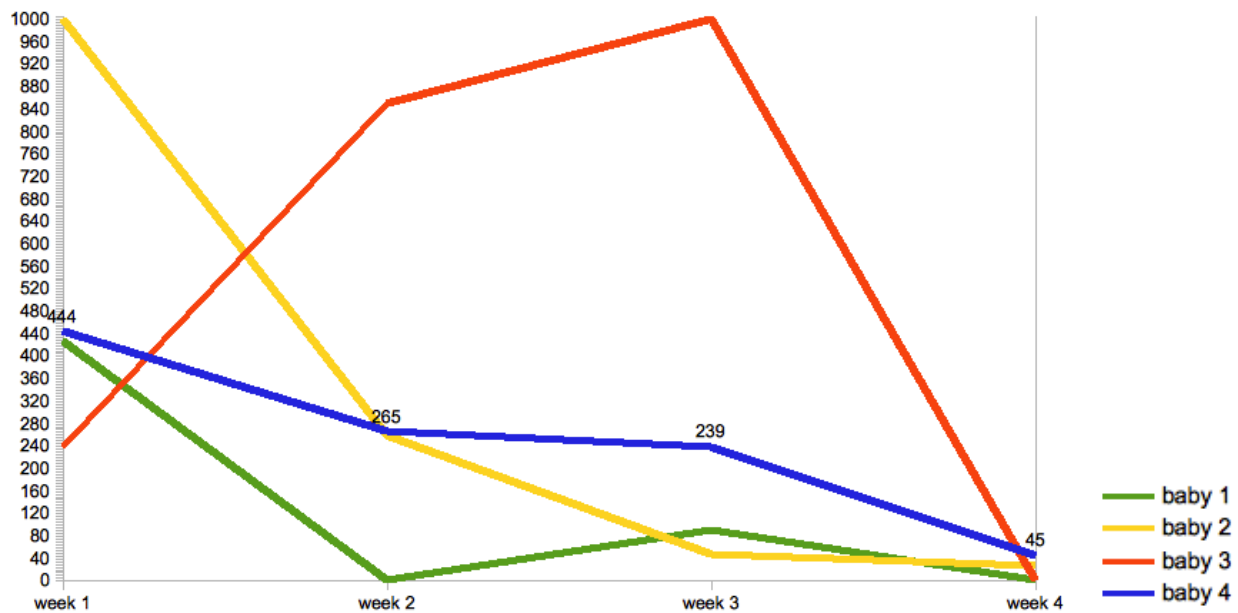
Figure 37

Comparison of creatinine and NGAL in those with AKI showed that the rise of NGAL was earlier than that of creatinine.

PROFILE OF AKI - GESTATIONAL AGE

Table: 38

Gestational age	28 weeks	29 weeks	30 weeks	31 weeks	32 weeks
Number (n=10)	4/10	1/10	1/10	1/10	3/10
Percentage %	40	10	10	10	30
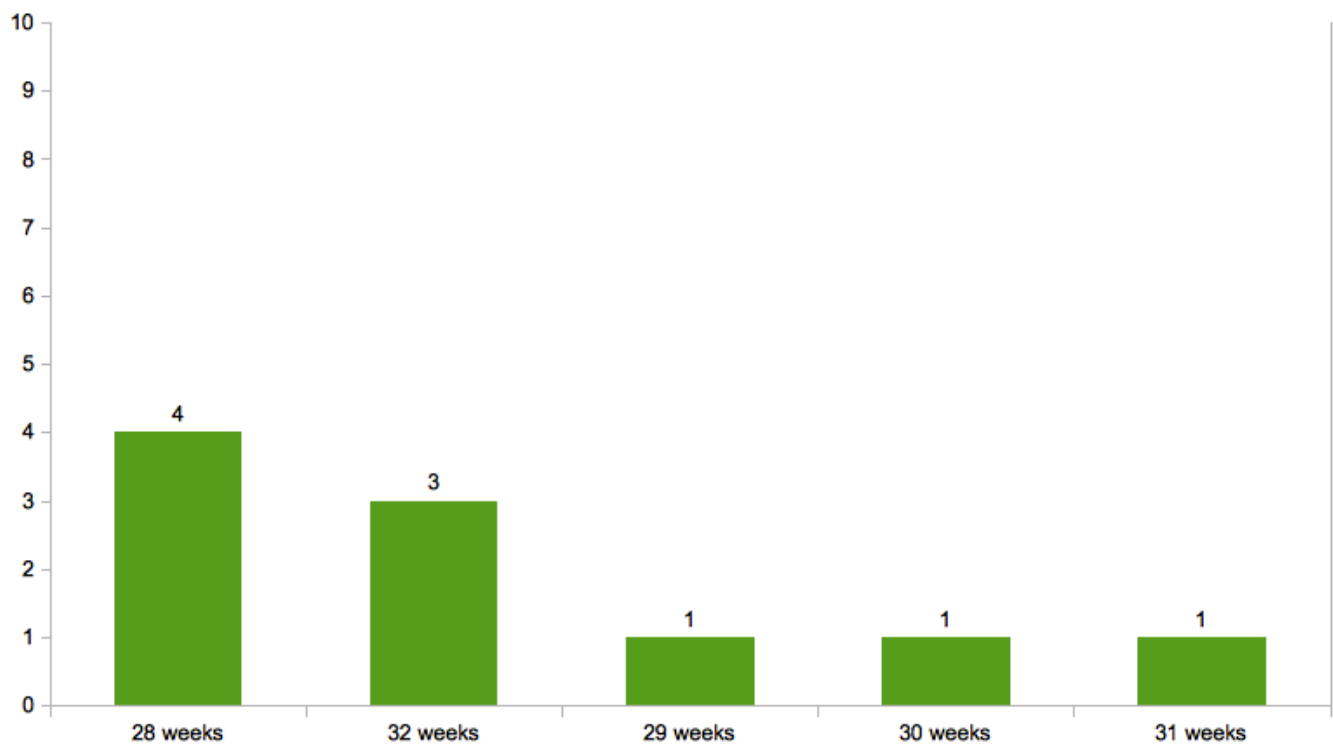
Figure 38:

Of the ten babies with AKI, 40% were born at less than 28 weeks, 30% at 32 weeks and 10% each in 29, 30 and 31 weeks.

BIRTHWEIGHT – AKI

Table 39

Birth-weight	<1000	1001-1100	1101-1200	1201-1300	1301-1400	1401-1500
Number (n=10)	1	2	3	1	1	2
Percentage (%)	10	20	30	10	10	20
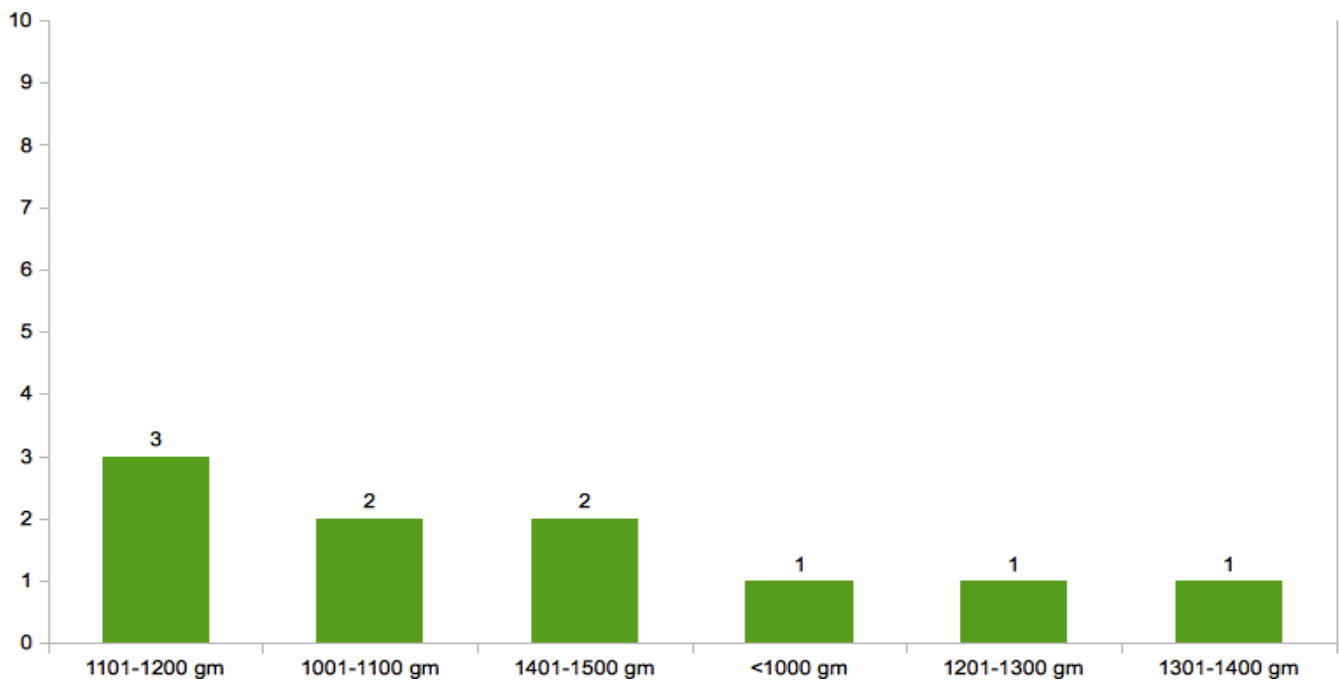
Figure 39

AKI was seen only in babies with birth-weight less than 1500 Gm.

Of the ten, 30% were between 1101 to 1200 Gm, 20% each in 1001 to 1100 Gm and 1401 to 1500 Gm groups, 10% each in < 1000 Gm, 1201 to 1300 Gm and 1301 to 1400 gm.

MATERNAL FACTORS - AKI

Table 40

Factors	Age <20	Age >30	Primigravida	PIH	Abnormal scan
Number (n=10)	1	2	10	3	3
Percentage%	10	20	100	30	30
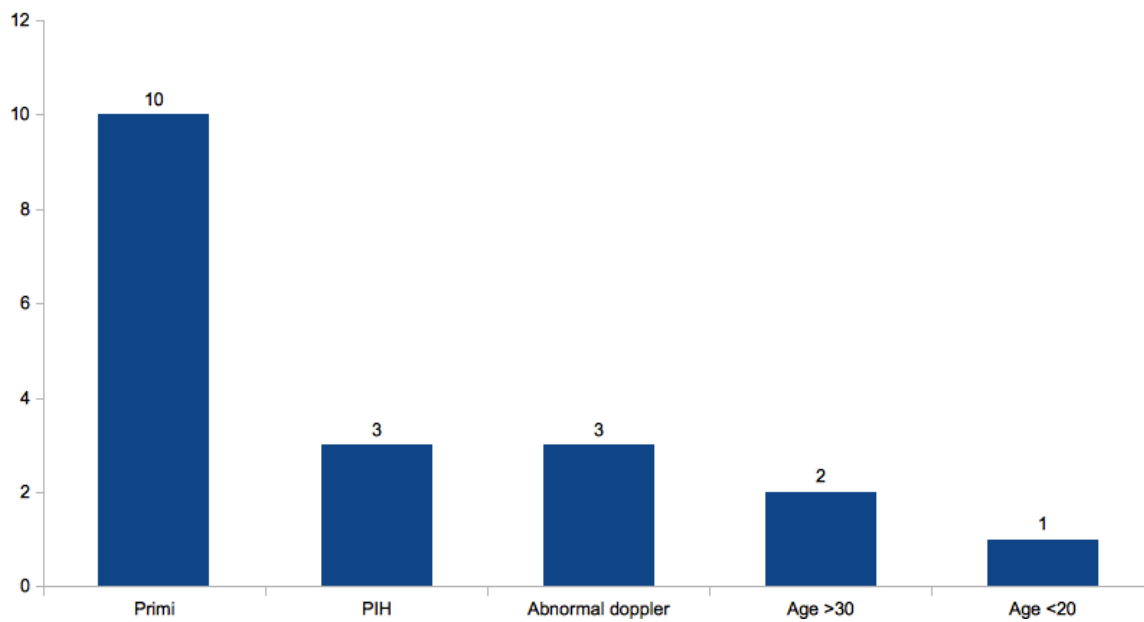
Figure 40

All 10 mothers were primipara, 30% had PIH and abnormal doppler, 2 were more than 30 years (20%)

of age and one teenage mother (10%)

MODE OF DELIVERY AND APGAR AT 5 MINUTES - AKI

Table 41

Mode of delivery	LSCS	Normal	Breech/forceps
Number (n=10)	6	2	2
Percentage%	60	20	20
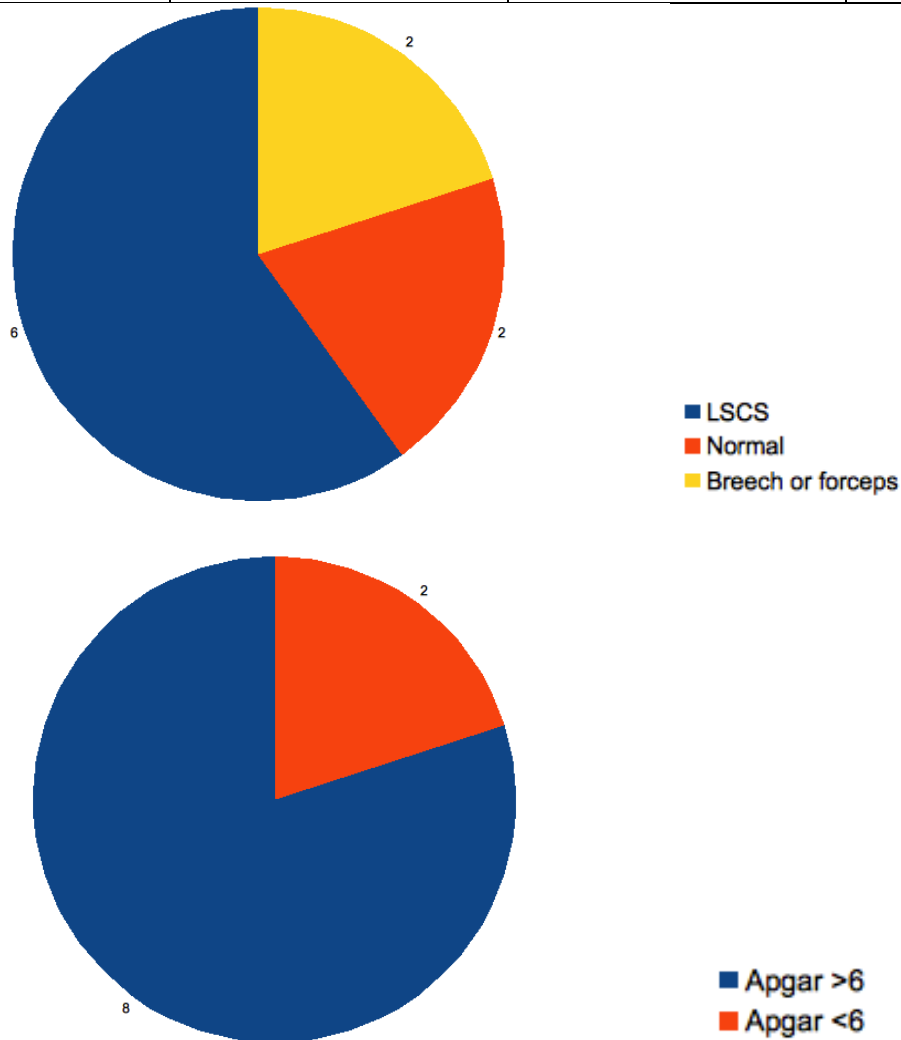
Figure 41: Caesarean section was mode of delivery in 60% and 20% each in normal and breech/low forceps category.

Five minute Apgar was more than 6 in 80% and less than 6 in 20%

RISK FACTORS IN THOSE WITH AKI

Table 42:

	Number (n=10)	Percentage %
Sepsis	10	100
Hyaline membrane disease	3	30
Patent ductus arteriosus	4	40
Nephrotoxic drugs	3	30
Mechanical ventilation	2	20
CPAP	5	50
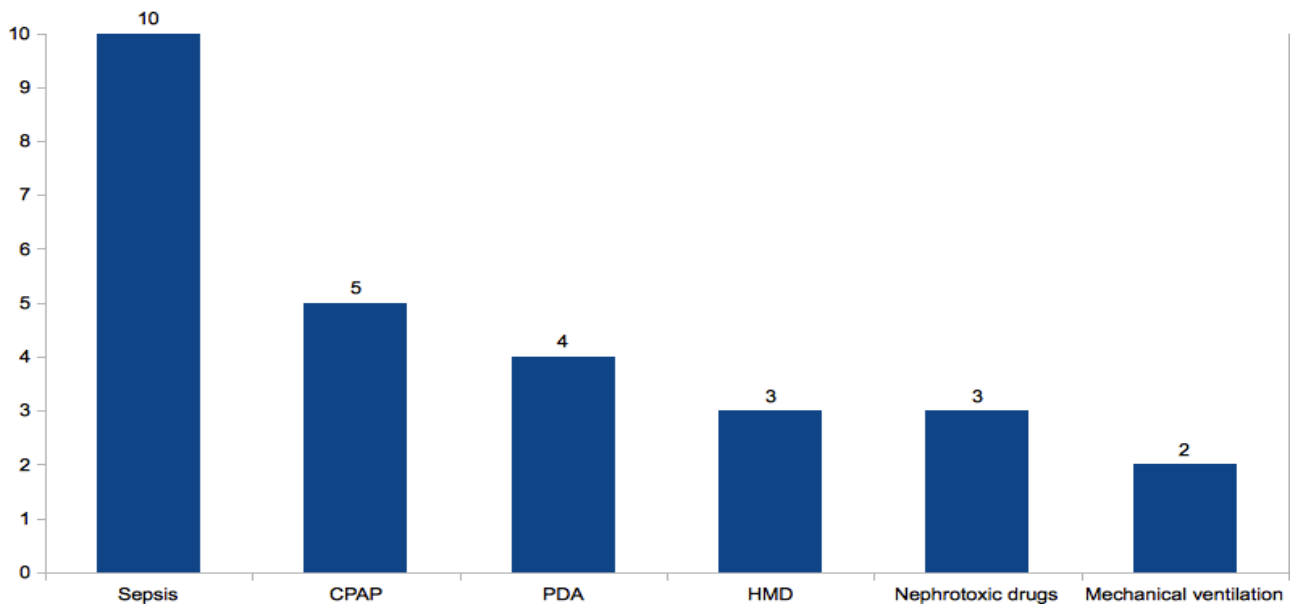
Figure 42: Sepsis was suspected in all, one baby who had culture proven sepsis.

The highest association with AKI was seen in sepsis (100%) followed by CPAP (50%) and PDA (40%)

RELATIVE RISK OF VARIOUS FACTORS IN AKI

TABLE 43:

FACTORS	AKI	NO AKI	Relative risk	95%confidence interval
PIH	3	28	0.664	0.185 to 2.375
Abnormal scan	**3**	**13**	**1.625**	**0.457 to 5.772**
Apgar < 6	**2**	**6**	**2.188**	**0.558 to 8.574**
Asphyxia	0	8	-	
Oligo-anuria	**2**	**0**	**9.652**	**4.995 to 18.547**
Hyaline membrane disease	3	30	0.597	0.167 to 2.141
Patent ductus arteriosus	**4**	**5**	**5.185**	**1.800 to 14.933**
NSAIDS	**3**	**4**	**4.408**	**1.456 to 13.346**
Aminoglycosides	**10**	**69**	**1.164**	**1.059 to 1.279**
Inotropes	**2**	**15**	**1.097**	**0.256 to 4.693**
Mechanical ventilation	**2**	**7**	**1.944**	**0.487 to 7.770**
CPAP	**5**	**31**	**1.194**	**0.375 to 3.802**
Inotropes	2	15	0.912	0.213 to 3.901
Death/DAMA	1	4	1.622	0.253 to 10.387

The risk factors for AKI included Oligoanuria, PDA, Nephrotoxic drugs – NSAIDS and Aminoglycosides, low Apgar, Mechanical Ventilation, CPAP and abnormal Antenatal scan.

FINAL OUTCOME

Table 44: Final outcome

	Number (n = 79)	Percentage
Discharged	74	93.80%
Death	3	3.70%
DAMA	2	2.50%
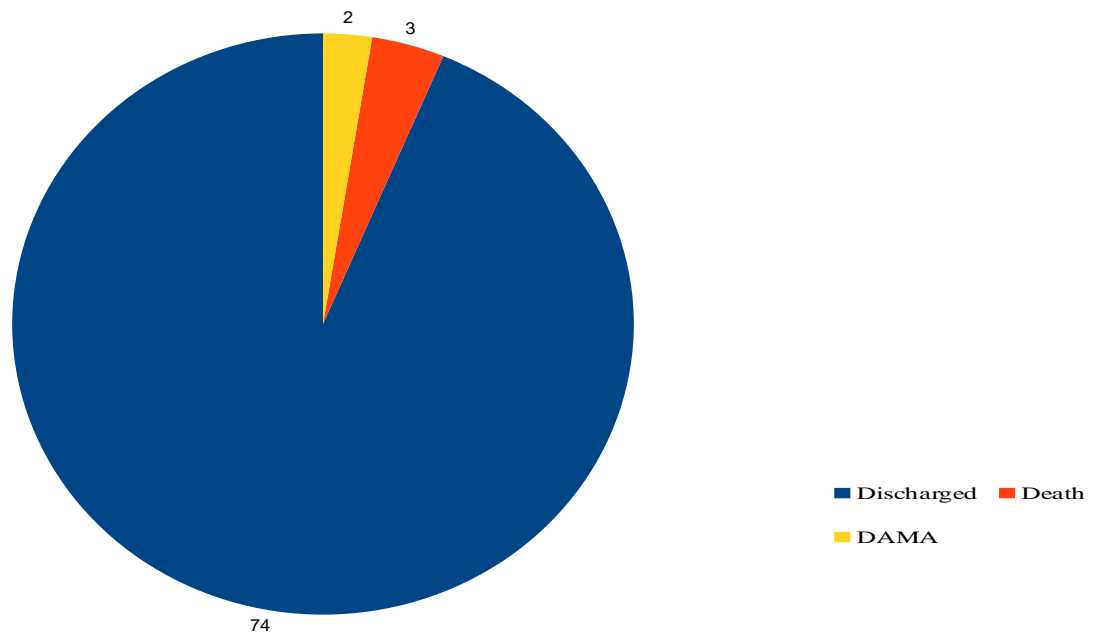
Figure 29: Final outcome

There were 3 deaths (3.7%) and 2 were discharged against medical advice (2.5%).

The remaining 93.6% were discharged well.

DISCUSSION

DISCUSSION

Preterm babies are a vulnerable population and are exposed to various risk factors; predisposing them to multi-organ injury. Due to the myriad of complications preterms are exposed to in the early weeks and multiple interventions with prolonged hospitalization, these babies are predisposed to acute kidney injury and its long term consequences. Factors predisposing to acute kidney injury are usually multifactorial, the most common among these being perinatal asphyxia and sepsis. Both these factors are common in preterm babies.

This study was conducted in Neonatal intensive care unit as collaboration between Departments of Neonatology and Pediatrics unit II during the period - May2014 and August 2014. During the study period, babies born at less than 32 weeks of gestational age were recruited for the study. Those babies with abnormal antenatal renal scans, major systemic congenital anomalies and chromosomal anomalies were excluded.

During the study period, there were 4823 live births. Average live births per month were 1200. Numbers of preterm deliveries were 789 (less than 37 weeks) (16.36%). There were 80 deliveries < 32+6 weeks gestation (10.14%).There were 79 babies that fulfilled the inclusion criteria of the study and were recruited. On an average, there were 23 babies born at less than 32 weeks of gestational age for every 1000 live births.

There were 57% males and 34% females (M:F ratio: 1.3:1). Devarajan et al in their study on urinary NGAL in preterm babies also reported similar sex distribution of 60% males and 40% females (46).

Parity of mothers delivering preterm babies was assessed. It was noted that prematurity of < 32+6 weeks gestation was higher amongst the primipara (70.8%) than multipara (29.2%). An Indian study by Begum etal reported that amongst risk factors associated with preterm labour, primiparity was one

of the many predisposing factors. Other factors included elderly gravida, multi-fetal pregnancy, poor socio-economic status, lower educational status (10).

 Teenage pregnancy is another known risk factor for prematurity. Nearly 62% were between 20 and 30 years of age followed by those with maternal age > 30 years (25.3%). Those < 20 years of age was only 12.6%. This may be a reflection of a gradually increasing marriage age of Indian women. Education status of the Mothers was variable - from no education (29.1%) to post graduation (10.1%). Majority had attended primary school (35.4%).

Multi-fetal pregnancies included 10 sets of twins and one set triplets. Of the 10 sets of twins in the study, one of the twins was excluded in view of major congenital anomaly.

Of the 79, antenatal ultrasonography was done only for 68 of them. Of the 68 scans, 31 were normal, 22 showing multiple pregnancy and 15 were abnormal. Abnormalities included abnormal Doppler in 7, absent end diastolic flow in 3, IUGR in 1, minimal ascites in 1, placenta previa in 2 and breech in 1. There were 19 scans confirming twin pregnancy (ten sets of twins) and 3 scans with triplets. Two sets of twins had abnormal Doppler on antenatal ultrasonography. There was one set of triplet included in the study. An abnormal antenatal renal scan was an exclusion criterion for the study.

Maternal risk factors included preterm pre-labour rupture of membranes (PPROM), chorioamnionitis; Pregnancy induced hypertension (PIH), Gestational Diabetes mellitus (GDM) and urinary tract infections. In our study, PIH was the most common risk factor accounting for 39.2% followed by GDM accounting for 13.9%. The numbers are almost identical to an American study in South Carolina that looked at association of PIH and hyaline membrane disease. Incidence of PIH was 42.6% and GDM was 12.5% in this study. (11) Drugs used in PIH included Magnesium sulphate, anti-hypertensive drugs like alpha methyldopa, Nifidipine and Labetalol. Maternal risk factors'

predisposing to neonatal sepsis like PPROM and chorioamnionitis was seen in 12.7% and 6.3% respectively.

Institutional protocol advises antenatal steroids if there is onset of preterm labour less than 34 weeks of gestation. All mothers had received at least one dose of steroids in the antepartum period during labour as all of them were less than 32 weeks. Intra-partum antibiotics were given to 25 mothers (31.6%) with PPROM, chorioamnionitis and urinary tract infection. There were 2 (2.5%) with documented urinary tract infections.

Mode of delivery included normal vaginal, Caesarean section, low forceps and breech. Caesarean section was the mode of delivery in more than half of them (59.5%) followed by normal delivery (39.6%) and a small proportion (8%) of breech/low forceps delivery. Indication for Caesarean section included both maternal and neonatal factors – severe pre-eclampsia, failed induction, fetal distress and abnormal doppler. The higher rate of Caesarean section many be due to the fact that that ours is a tertiary care center with more referrals for safer delivery in cases of complicated pregnancies.

Apgar score (Appearance, Pulse, Grimace, Activity and Respiration) is an assessment score of neonates at 1 and 5 minutes of life. The 5-minute Apgar less than 7 is abnormal and 3 or less is suggestive of birth asphyxia. There were 71 babies with 5 minute Apgar more than 6 (91.1%) while 7 (8.9%) had abnormal Apgar less than 6. Cord pH of less than 7.0 or first blood gas with pH of less than 7.2 was taken as probable asphyxia. There were 3 (3.7%) babies who fulfilled the criteria. Stojanovic etal also reported metabolic acidosis to be more pronounced in the AKI babies during first 3 days of life, with lower serum pH. (21)

Neonatal risk factors included those who needed resuscitation or intubation at birth and babies who were symptomatic at birth. There were 55 babies who were symptomatic at birth (69.6%). Of these, 17 (21.5%) needed resuscitation and 15 (18.9%) were intubated. International Liaison committee on

resuscitation (ILCOR) guidelines mentions that atleast 10% of all deliveries need neonatal resuscitation. The resuscitation rates were higher in our study as this included only preterm baby up to 32 weeks of gestation as against the ILCOR guidelines who reported on all deliveries. The 2010 ILCOR guidelines also state that lower the gestational age, birth-weight and Caesarean sections are associated with higher risk of resuscitation at birth. (12)

Resuscitation included bag and mask ventilation, use of neopuff and intubation. The indications for resuscitation included depressed at birth with low Apgar scores, secondary apnea and poor perfusion. Those with tachypnea and grunting were transported with neopuff. Neopuff provides positive inspiratory (PIP) and end expiratory pressure (PEEP) and functions like CPAP. Although 15 needed intubation, only 9 required long term mechanical ventilation.

Symptoms varied in the 55 babies. Respiratory distress was the most common presentation (65.8%) followed by poor perfusion (11.3%). There were 2 babies who presented during the late neonatal period with apnea and seizures (2.5%). Abdominal distension (7.5%), temperature instability – hypothermia and hyperthermia (2.5%) and apnea (6.3%) were the other symptoms. Urine output was monitored using urine collecting bags or weighing cotton pads. Oliguria, defined as urine output less than 0.5 ml/kg/hour, was seen in 2 babies and anuria was later noted in one of these two. Cataldi etal found Hyaline membrane disease as a common cause of pre-renal failure. However it was the severity of the disease, rather than just the presence of the disease that increases incidence of AKI (34).

Interventions during the NICU stay included intravenous lines – both peripheral and central lines, umbilical venous and arterial lines, mechanical ventilation, CPAP, intravenous fluids, nasogastric feeds. All 79 babies were on intravenous fluids, nasogastric feeds and had peripheral intravenous catheters. Umbilical venous lines were inserted in 64 babies (81%) and umbilical arterial lines in 29 babies (36.7%). There were no complications associated with umbilical lines like infection, renal vein

thrombosis, displacement of the lines or line related infections. Central venous line was needed in 3 babies (3.7%) and there were no complications.

Non-invasive ventilation in the form of CPAP was used for 36 babies (45.5%). There were no complications like pneumothorax. However, three of them who failed to improve on CPAP went on to mechanical ventilation. There were a total of 9 babies who needed mechanical ventilation. Five of them were given surfactant therapy. There were no complications like pneumothorax or ventilator associated pneumonia. One of them had culture proven sepsis – Non-fermenting gram negative bacilli requiring prolonged ventilation.

The complications adding to morbidity was multi-systemic. These included Hyaline membrane disease (41.7%), probable sepsis (30.3%), probable necrotizing enterocolitis (12.6%), Patent ductus arteriosus (7.5%). Only one case of grade 3 intra-ventricular hemorrhage was seen who went on to develop hydrocephalus. Of the 33 with hyaline membrane disease, 7 were ventilated by conventional mechanical ventilation and 26 needed CPAP support.

There were 24 babies who were at risk of sepsis. There were only three culture proven sepsis – one with non-fermenting gram negative sepsis, one with gram negative sepsis and the third with Enterococcal sepsis. Broad-spectrum antibiotics (Penicillin/Ampicillin and Gentamicin) started for those who were at risk of sepsis according to WHO criteria. (13) Other antibiotics used included Ciprofloxacin, Amikacin, Cefoperazone sulbactum, Netilmycin, Meropenem, Vancomycin and Colistin. Inotropes were used in 17 babies (21.5%) while blood transfusion was given to 2 babies. There were 2 babies with myocardial dysfunction – one requiring mechanical ventilation while the other was conservatively managed with fluid restriction, diuretics and inotropic support.

Patent ductus arteriosus was diagnosed in 7 babies, 3 were given oral Ibuprofen and 3 were given Indomethacin. There was oliguria in one baby post Indomethacin. This was managed conservatively.

A total of 10 babies were diagnosed with necrotizing enterocolitis – 7 had stage 1 while 3 babies had stage 2 NEC. None of them had bowel perforation or needed surgical intervention. Conservative management with antibiotics, IV fluids, total parenteral nutrition and resting the gut by keeping them nil by mouth. There was no mortality seen in this group.

Three babies died who were ventilated – one had severe hyaline membrane disease and was given surfactant and inotropes but died on day 4 of life. One baby had very high bilirubin due to gram-negative sepsis, developed bilirubin encephalopathy and died despite supportive measures and antibiotics, died of late onset gram negative sepsis. The third died due to probable sepsis on day 9 of life. The mortality rate was 3.7%. Two babies were discharged against medical advice. The corrected survival rate was 93.6% (74 of 79 survived).

DIAGNOSIS OF AKI

Blood and urine samples were collected weekly for the first 4 weeks of life during the stay in NICU to assess the risk of Acute Kidney injury in these babies. The first samples for serum creatinine and urinary NGAL were collected after 72 hours of life. Serum creatinine was processed by standard methods in Clinical Biochemistry department. Urinary NGAL samples were collected and stored at sub-zero temperatures. These were processed by rapid ELISA using KIT 037 by BioPorto diagnostics.

 Serum creatinine was collected and processed for all 79 babies. Urinary NGAL was processed only for 31 babies as a pilot study to look at usefulness of NGAL as a biomarker of AKI in preterm babies.

 Creatinine and NGAL values were looked at and correlated with various risk factors.

The mean value of **Creatinine** values decreased from 0.73mg/dl to 0.35mg/dl during week 1 to 4. In the first week, nearly half (53.8%) had creatinine in the range of 0.6mg/dl to 0.9mg/dl and one fifth (19.2) had creatinine levels more than 0.9mg/dl. In week 2, highest numbers were in the 0.3mg/dl to 0.6mg/dl group (56.2%) followed by 0.6mg/dl to 0.9mg/dl group (31.2%). Only 5 had creatinine value

of more than 0.9mg/dl. By week 3, three fourth had values between 0.3mg/dl to 0.6mg/dl. By week 4, almost 90% had creatinine less than 0.6mg/dl. Thus by week 4, from an initial 19.2 %, only 2.2 % had elevated creatinine values.

The Creatinine levels were compared with various risk factors. It was found to be elevated in those undergoing mechanical ventilation- both IMV and CPAP. It was found to be almost similar in the IMV and CPAP groups except in week 3 when the difference was statistically significant (p<0.05). The values of Creatinine were almost in the same range in the asphyxiated babies and the non asphyxiated babies and there was no statistical difference between these values. As there were only 6 babies who suffered asphyxia, this association needs to be studied further as asphyxia is a known risk factor for AKI and other morbidity.

Use of NSAIDS is also considered a risk factor for AKI, especially in preterm babies as it is used in the management of PDA. Weekly creatinine values remained high in all weeks but was statistically significant only in week 3 (p= 0.048). This may correspond to its usage as PDA is one of the known later complications appearing in week 2 or 3. Creatinine levels were significantly higher in those with umbilical lines in week 3 (p=0.048). Creatinine values were also higher in those receiving inotropes, but it was significantly higher in week 3 (p<0.05). The late rise of serum creatinine in all these cases after the initial fall could represent either a delayed detection of AKI or could represent the occurrence of AKI as a cumulative effect of several co-morbid factors.

NGAL estimation in 31 babies showed a decreasing trend from 260.5ng/ml to 36.3ng/ml during week 1 to 4. In the first week, there were 58.1% with NGAL less than 250ng/ml, but 12.9% had values more than 500ng/ml. By week 2, nearly 80% had values less than 250ng/ml. By week 3 and 4, almost all (96.4 and 100%) had values less than 250ng/ml. The change in creatinine was in the range of decimal point while that of NGAL change was in hundreds. NGAL thus may reflect changes in renal function more accurately than serum creatinine.

NGAL levels was compared with gestational age and birth weight over the 4 weeks. It was found that with increasing gestational age, NGAL showed a decreasing trend. However this was not found to be statistically significant. (p= >0.05) except in week 4. Similarly NGAL values for different birth-weight showed that NGAL values were higher in those with lower in birth-weight. This was not statistically singnificant (p value >0.05). Devarajan etal also reported that NGAL values were inversely proportional to gestational age and birth-weight, but the change was statistically significant (p<0.01). Twenty normal preterm babies were grouped based on birth-weight and urinary NGAL was monitored daily for the first 14 days. However further studies are required to establish normal range of NGAL in this group of patients. (31)

NGAL and creatinine in ventilated babies was compared over the 4 week study period. **NGAL rise was significant in the IMV group in week 2 (p<0.05).** However, in the CPAP group a similar rise was not noted in any of the weeks. When creatinine levels were looked at, the **IMV group showed a statistically significant (p<0.05) increase in Creatinine at week 3.** This could be a reflection of the fact that the NGAL rise preceded the rise in creatinine by a week.

Asphyxia was defined as cord pH less than 7.0 or blood gas pH less than 7.2 during the first 24 hours of life. There were 3 babies with suspected asphyxia. The NGAL values of asphyxiated babies was compared with babies with no asphyxia; and it was found that NGAL in weeks 1, 3 and 4 were higher but this was not statistically significant. Similarly, serum creatinine values were higher in babies with asphyxia in the initial weeks compared to later weeks- but the difference was not statistically significant.

Nephrotoxic drugs included mainly NSAIDS and aminoglycosides. NSAIDS used included Indomethacin and Ibuprofen. Both NGAL values and creatinine persisted at a higher level in these babies compared to babies not exposed to NSAIDS. The level of rise of NGAL was much higher than rise in creatinine but the difference was not statistically significance (p>0.05).

Those with umbilical arterial and venous lines were compared with those with no lines. Both NGAL and Serum creatinine showed a decreasing trend and there was no significant difference between the two.

ACUTE KIDNEY INJURY IN THE STUDY

Acute kidney injury was defined as an absolute value of more than 1.3 mg/dL or a rise of more than 50% of the previous value. Different studies have used different cut-off values of creatinine. Cataldi etal reported AKI in preterm babies in a case control study, where creatinine of more than 1.3 mg/dL was used to define AKI. (34) Mortazavi etal looked at both term and preterm babies, wherein creatinine of more than 1.5 mg/dL was used to define AKI (1). The most recent study from Serbia in 2014 by Stojanovic etal defined AKI as rise in creatinine more than 0.3 mg/dL compared to the previous value. (22)

In our study, **the incidence of AKI was 12.6% (10/79).** Of these, 2 babies had high absolute value of creatinine - more than 1.3 mg/dL. One of these also showed a rise of more than 50% compared to the previous value. There were 8 other babies who showed rise of more than 50% compared to the previous creatinine value.

According to the AKIN criteria (23), 3 babies belonged to stage I while 6 were stage II. One was an absolute value of creatinine > 1.3 mg/dL and therefore not defined by AKIN criteria.

Four of the ten babies had concomitant NGAL levels done. The NGAL levels were high and corresponded to the creatinine levels. In all of them, the NGAL levels were found to rise before the rise creatinine level.

Of the babies with AKI, four were females and six were males. There was a male preponderance in the entire study group and in those with AKI. The male to female ratio was 1.5:1. Mortazavi etal also found a male preponderance but male to female ratio was 2:1. (1)

All ten babies had birth-weight less than 1500 gm. Of these, one was less than 1000 gm, two each weighing between 1001 to 1100 and 1401 to 1500, 3 between 1101 to 1200 and one each between 1201 to 1300 and 1301 to 1400. Cataldi etal also reported a higher incidence rate (79%) in babies less than 1500 Gm. (34) However, this study included all preterm babies less than 37 weeks whereas our inclusion criteria was only up to 32+6 weeks of gestational age. A greater sample size of AKI may help to look at this association better.

Among the ten with AKI, 40% were less than 28 weeks of gestational age. This was a finding also reported by Stojanovic etal and Cataldi etal (22, 34). This may be due to higher incidence of co-morbidities like sepsis, perinatal asphyxia, hyaline membrane disease, patent ductus arteriosus, need for mechanical ventilation and nephrotoxic drugs.

Maternal risk factor like pregnancy-induced hypertension was present in three of them (30%). Abnormal Doppler was seen in 3 of them (30%). Only one baby was depressed at birth (10%). Stojanovic etal also found that maternal complications like PIH, Diabetes, chorioamnionitis and antibiotics were common in preterm babies and could contribute to the risk of renal failure. However in their study these factors were not found affect renal function in the neonate. (22)

Hyaline membrane disease was seen in 4/10 of children with AKI. All of them required non-invasive ventilation in the form of CPAP. Cataldi etal reported a higher incidence of Hyaline membrane disease (89%). This   high incidence is probably because their Neonatal ICU provides care to preterm babies born at less than 25 weeks (34). Our NICU has a protocol to admit babies only 27 weeks and beyond, keeping in mind the problems and morbidity associated with such extreme prematurity.

Cataldi et al also reported that Hyaline membrane disease increases pre-renal failure by affecting renal plasma flow and reducing the glomerular filtration rate

Three babies had ECHO proven hemodynamically significant Patent ductus arteriosus; two of them were treated with IV Indomethacin and one with oral Ibuprofen. One of them developed oliguria following Indomethacin and was managed conservatively with IV fluids. The one following Ibuprofen had elevated creatinine at the end of 4 weeks and was otherwise asymptomatic (normal urine output and gaining weight) while the other two had normal creatinine by the 4th week. Though the number is small, Nephrotoxic drugs like Indomethacin and Ibuprofen were associated with development of AKI in our study. Stojanovic etal (22) and Cataldi etal found similar incidence of PDA (40%) in their studies. Cataldi found that Ibuprofen was one of the factors predisposing to AKI. (34) On follow up, the creatinine normalized in these babies exposed to nephrotoxic drugs. Therefore the renal dysfunction was only transient. This needs further study with a larger cohort.

The risk factors for developing AKI were assessed by comparing the AKI group with the non AKI group. The creatinine and NGAL for these was also compared in the two groups and found that the rise of NGAL was earlier than that of creatinine. We noted that the rise in creatinine by decimal points (0.2 to 0.4 mg/dL) may be overlooked compared to NGAL levels which rises in hundreds. This may make it a more useful as a biomarker of in the early detection of AKI.

Sepsis was a factor common to all ten babies while none with asphyxia were in the AKI group. CPAP (50%), Patent ductus arteriosus(40%), drugs like NSAIDS (30%), hyaline membrane disease (30%), mechanical ventilation (20%) were the other factors.

In our study, the Relative risk of developing AKI was found to be associated with factors like Oligo-anuria, PDA, nephrotoxic drugs, low Apgar, Mechanical Ventilation, CPAP and abnormal Antenatal scan. Mortazavi etal found asphyxia and sepsis to increase the risk of AKI. Cataldi etal reported low Apgar, PDA and use of nephrotoxic drugs to increase AKI incidence. The most recent study by

Stojanovic etal showed low Apgar, metabolic acidosis, NEC, intra-ventricular hemorrhage, use of

Vancomycin and Dopamine as independent factors predisposing to AKI.

SUMMARY

SUMMARY

- Descriptive study looking at incidence of Acute renal failure in preterm babies

- This study was conducted in Neonatal intensive care unit as collaboration between Departments of Neonatology and Pediatrics unit II during the period May 2014 and August 2014.

- During the study period, babies born at less than 32 weeks of gestational age were recruited for the study.

- Those babies with abnormal antenatal renal scans, major systemic congenital anomalies and chromosomal anomalies were excluded.

- Sample size was calculated based on incidence of renal failure. Incidence of renal failure worldwide ranges from 3.4 to 24% as mentioned in an Iranian study. Assuming that incidence of renal failure in our population is 10% with a precision of 6% and confidence interval of 95%, sample size was calculated to be 96.

- There were a total of 4823 live births during this period. Of the total 789 preterm deliveries, 80 were preterm deliveries < 32 weeks gestation (10.14%). Of this group, a total of 79 were recruited in the study. One baby was excluded in view of major congenital anomaly.

- Blood sampling for serum creatinine and urine for NGAL was collected once a week.Serum creatinine was processed in Clinical biochemistry lab of CMC Vellore using standard methods.

- Urine NGAL was processed for 31 babies as part of a pilot study to look if it was useful in detecting acute kidney injury in this population. Urine NGAL was processed using a rapid ELISA kit (kit 037).

- Serum creatinine more than 1.3 mg/dL or more than 50% rise of creatinine compared to previous value was used to define AKI.

- Weekly monitoring of risk factors, interventions, drugs were noted. First sample of Serum creatinine and urine for NGAL were collected after 72 hours of age.

- The data was statistically analysed using SPSS software, version

- A total of 79 babies were recruited. Of these, three died (one within 3 days of life and two were late neonatal deaths). There were 3 deaths (3.7%) and 2 were discharged against medical advice (2.5%). The remaining 93.6% were discharged well.

- There were 12 babies (15.1%) between 27 to 28 weeks, 26 (32.9%) between 29 to 30 weeks and between 31 to 32 weeks there were 41 (51.8%).

- More than half the babies (56.1%) had birth weight between 1001 to 1500 Gm, while a third weighed between 1500 and 2000 Gm. (31.6%).

- There were 45 males (57%) and 34 females (43%). The male: female ratio was 1.3: 1.

- There were 63.3%% mothers who were between 20 and 30 years , followed by 27.9% more than 30 years and 8.8% less than 20 years.

- 29.1% mothers were uneducated, 35.4% had primary school education, 21.5% mothers who were graduates and only 10.1% postgraduates

- Of the total, 69/79 babies had antenatal scans done. Normal scans were noted in 44.9% while 17.3% were abnormal. Remaining confirmed multiple preganacies. Abnormal scans included abnormal Doppler and IUGR, while one baby had fetal ascites.

- Most common mode of delivery was LSCS in 47 (59.5%) followed by normal delivery in 25 (31.6%) and Breech/instrumental in 7 (8.8%)

- Majority were primipara - 56 (70.8%); while 23 (29.2%) were multigravida

- PIH was the commonest morbidity seen in the Mothers (39.2%) followed by PPROM (22.7%). Other risk factors included GDM (32.9%), UTI (2.5%) and chorioamnionitis (6.3%).

- There were 72 babies (91.1%) with Apgar score more than 6 and 7 with Apgar less than 6 (8.8%)

- There were 55 babies (69.6%) who were symptomatic at birth. Resuscitation was needed in 21.5% and intubation in 18.9%

- Respiratory distress was the most common symptom (65.8%) followed by poor perfusion (11.3%). Others included abdominal distension (7.5%), apnea (6.3%) temperature instability, (5%), seizures (2.5%), hypoglycemia (0.2%) and decreased urine output (2.5%)

- Hyaline membrane disease was the commonest condition seen (41.7%) followed by sepsis, which affected one third of them (30.3%). NEC was seen in 12.5%, PDA in 7.5%, IVH in 0.2% and 6.3% were depressed at birth.

- All babies were on IV fluids and Nasogastric feeds. Majority had umbilical venous lines inserted (81%), umbilical arterial lines in 36.7%. There were 9 babies (11.3%) requiring mechanical ventilation, 45.5% needed CPAP, Inotropic support in 21.5% and 3.7% needed central venous lines.

- Aminoglycosides were used in 89.8% of the babies while Ibuprofen and Indomethacin were used in 3.7% each.

- The mean value of weekly creatinine values decreased from 0.73 to 0.35mg/dl from week 1 to week 4.

- In the first week, one fifth (19.2%) had creatinine levels more than 0.9mg/dl but by week 4this had decreased to 2.2%..

- Creatinine values almost similar in the IMV and CPAP groups except in week 3 when the difference was statistically significant (p<0.05).

- The values of Creatinine showed no statistical difference in the asphyxiated and non asphyxiated group.

- Weekly creatinine remained high in all weeks in babies receiving NSAIDS but was statistically

significant in only in week 3 (p= 0.048).

- Creatinine levels were significantly higher in those with umbilical lines in week 3 (p=0.048).

- Creatinine values were higher in those receiving inotropes, but it was significantly higher in week 3 (p<0.05).

- The significant rise of Serum creatinine in the 3rd week could represent either a delayed detection of AKI or could represent the occurrence of AKI as a cumulative effect of several co-morbid factors.

- NGAL values were calculated in 31 babies and compared with the concomitant serum creatinine values in 31 babies.

- The mean value of NGAL over 4 weeks showed a decreasing trend from 260.5 to 36.3ng/ml.

- In the first week, 12.9% had NGALvalues more than 500ng/ml, but by week 4 all values were < 250ng/ml.

- NGAL was inversely proportional to gestational age but not this was not statistically significant. (p=>0.05)

- Weekly NGAL values showed an inverse relationship to birth weight, but this was not statistically significant.

- NGAL rise was statistically significant in the IMV group in week 2 (p<0.05) compared to CPAP. Creatinine rise was statistically significant (p<0.05) in the IMV group compared to the CPAP group but in week 3.

- NGAL in weeks 1, 3 and 4 were higher but not statistically significant in the 3 babies with suspected asphyxia. Serum creatinine also did not show a rise.

- NGAL values were higher than creatinine in those receiving NSAIDS and aminoglycosides.

(Indomethacin, Ibuprofen and Gentamicin) but the difference was not significant. (p >0.05)

- There was no difference in the NGAL and Creatinine levels in those with umbilical arterial lines.

- Comparison of creatinine and NGAL in those with AKI showed that the rise of NGAL was earlier than that of creatinine.

- **The incidence of AKI was 12.6% (10/79).**

- Of the ten babies with AKI, 40% were born at less than 28 weeks, 30% at 32 weeks and 10% each in 29, 30 and 31 weeks.

- AKI was seen only in babies with birth-weight less than 1500 Gm. Of the ten, 30% were between 1101 to 1200 Gm, 20% each in 1001 to 1100 Gm and 1401 to 1500 Gm groups, 10% each in < 1000 Gm, 1201 to 1300 Gm and 1301 to 1400 gm.

- All 10 mothers were primipara, 30% had PIH and abnormal Doppler, 2 were more than 30 years (20%)of age and one teenage mother (10%)

- Caesarean section was mode of delivery in 60% and 20% each in normal and breech/low forceps category. Five minute Apgar was more than 6 in 80% and less than 6 in 20%

- Sepsis was seen all of 10 babies with one having culture proven sepsis. Half (5/10) needed CPAP while 2/10 were ventilated. Hyaline membrane disease was present in 3/10 and PDA in 4/10. NSAIDs were used in 3/10 of those with AKI.

- The relative risk of developing AKI was highest with factors like Oligo-anuria, PDA, nephrotoxic drugs, low Apgar, Mechanical Ventilation, CPAP and abnormal Antenatal scan.

<u>CONCLUSIONS</u>

CONCLUSIONS:

- The incidence of AKI in preterms less than 32 weeks of gestational age was found to be 12.6%.

- There was a higher incidence in babies born at 28 weeks or less babies less than 1500 gm at birth.

- The risk factors for AKI included Oliguria, PDA, Nephrotoxic drugs, low Apgar, Mechanical Ventilation, CPAP and abnormal Antenatal scan.

- Serum creatinine was significantly higher in babies receiving mechanical ventilation, NSAIDS and inotropes.

- The rate of rise of NGAL was earlier and the absolute number was also higher than creatinine (decimal points in creatinine while hundreds in NGAL).

- NGAL rise was seen in AKI group as well as babies with risk factors like NSAIDS, mechanical ventilation and asphyxia. NGAL levels were inversely proportionate to gestational age and birth-weight.

- Analysis of NGAL values in all 79 babies in the study group may help to more clearly establish its usefulness as a biomarker.

- A study with larger sample size is required in preterm babies to establish reference values for NGAL and its role as an early biomarker of AKI. It also needs to be studied in greater detail in term babies also who may exposed to various other morbidities including hypoxic ischemic injury.

LIMITATIONS

RECOMMENDATIONS

LIMITATIONS

- As this was a pilot study only representative urine samples could be analysed for NGAL.

- The follow up at 3 and 6 months could not be completed.

RECOMMENDATIONS:

- Follow up of preterm babies who had AKI or risk factors AKI at 6 months to look at growth, serum creatinine, urinary NGAL and renal size.

- Larger study with bigger sample size to study the risk factors of AKI and prematurity.

- NGAL as a maker of AKI in preterm and term neonates needs to be studied further.

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ANNEXURE

Informed Consent form to participate in a research study
Study Title:
Study Number:
Subject's Initials: _____ Subject's Name: _____
Date of Birth / Age:_____

<div style="border:1px solid black; text-align:center;">

NARF study

</div>

(Subject)
(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [   ]
(ii) I understand that my baby's participation in the study is with my consent and that I am
free to withdraw him/her at any time, without giving any reason, without his/her medical care or legal rights being affected. [ ]
 (iii) I understand that the researcher wants to collect blood and urine for this study. I give consent for the collections of such specimens [   ]
(iv) I understand that, instead of throwing away the remaining specimen, the researcher wants to store it and use it for future research projects. I have no objection to the specimen (including urine, blood, blood cells, plasma and serum, collected for this study be used for other research projects [   ]
(v) I understand that the researcher or anyone authorized by the authorities will not need my permission to look at my child's health records both in respect of the current study and any further research that may be conducted in relation to it, even if I decide to withdraw him/her from the trial. I agree to this access. However, I understand that his/her identity will not be revealed in any information released to third parties or published. [   ]
(vi) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [   ]
(vii) I give consent for my baby to take part in the above study. [   ]

Signature (or Thumb impression) of Legally Acceptable Representative:_____
Date: _____/_____/_____
Signatory's Name: _____

Signature of the Investigator: _____
Date: _____/_____/_____
Study Investigator's Name: _____

Signature of the Witness: _____
Date:_____/_____/_____
Name of the Witness: _____

INFORMATION SHEET

Preterm babies are a vulnerable population and at risk for various complications. Renal injury is one of them. As these babies have immature kidneys, they are more prone to kidney damage. Some of the other problems which occur in preterm babies like delayed cry at birth, need for oxygen, breathing difficulty, feed intolerance, etc may also adversely affect the kidney. Tests available for detecting kidney injury are not as useful as in older children. A new test will be done on your baby's urine to see if it is useful in detecting kidney injury before your baby becomes symptomatic. We also want to find out how common is kidney injury in preterm babies and we are often not sure whether the damage completely resolves or not. We need to monitor your child's renal function over the next few months till we are sure it has resolved.

You are requested to volunteer and enroll your baby for the NARF study.

Once enrolled in the study, we will closely follow your baby's progress in the hospital, involves taking weekly special urine and routine blood tests. About 2 ml blood will be withdrawn per week. Blood collection may lead to minimal discomfort to the baby and is unlikely to lead to any complications.

This study involves follow up of kidney function during the first 4 weeks of life and once your baby gets discharged, he/she will be followed up till 6 months of age. The two follow up visits at 3 months and 6 months will be during routine check up in High risk infant clinic. During these visits, a single blood and urine test for kidney function will be done. At 6 months visits, in addition to these tests, an ultrasound scan is also done.

Preterm babies routinely require blood and urine tests. Along with these tests, we'll do an ultrasound scan. The urine test will be done free of cost. The blood test and ultrasound scan are routinely done tests and the cost will need to be borne by the parents.

The data collected and the bloods/urine tests done may be used for future studies which may help us gain further knowledge of renal failure in children.

At any point, you may withdraw your baby from the study. This will not compromise care during his/her stay in the hospital or in the future. He/she will continue to receive the best standard of care.

The study does not involve free hospital care or free medicines.

Your baby's identity is confidential and at no point will be disclosed.

In case you have any queries, please contact me at 0416-2283348.

Dr Indira Agarwal /Nithya J P

QUESTIONS

1. What is this study about?

There is no information available on how common is kidney failure in preterm babies. It is believed to be under diagnosed as these children are not routinely followed up. Because they are a vulnerable population, this study aims to look at how common is kidney damage in preterm babies. We also hope to identify the risk factors for renal damage in this group so that we can suggest appropriate guidelines for follow up.

2. What kind of test will be done?

Your baby will get the standard care of treatment. This includes routine blood tests done as per protocol and additional special urine test will be collected on a weekly basis and stored for few months. Once analyzed, we will try to assess its usefulness in predicting kidney damage.

3. Can these tests harm my baby?

No. Blood tests may lead to discomfort but no complications involved. The volume of blood collected is also minimal. Urine collection is a non-invasive test and will not cause any difficulty in collection.

4. Are these standard tests ?

Yes, one blood test called creatinine is used as marker for kidney damage in all age groups and is being routinely tested all the time. NGAL tested in the urine, is a new marker, which has been tested in other centers. The new test involves collection of urine to detect the level of NGAL, which is a proven early marker of kidney injury.

5. Do I have to pay for these tests?
   You will have to pay for the routine tests but the special urine test is free of cost

6. Does this involve free treatment during the hospital stay?
   No, only the urine tests done for the study will be free of cost. The rest of the bill be borne by the family

7. How many follow-up visits involved and when?
   Two follow up visits at 3 and 6 months

8. What does the follow up visit involve?
   It involves clinical examination, blood and urine tests in both visits and a ultrasound at 6 months

9. Will my transport charges be covered for? Not applicable

10. Can I withdraw from the study?
    Yes, you can withdraw your baby from the study at anytime without giving a reason.

11. Will withdrawing from the study affect the treatment?
No, your baby will receive optimal standard of care

12. Will my baby's identity be disclosed at any time?
    No,your baby's identity will be kept confidential. At no point will his/her identify be disclosed.

STUDY PROFORMA

Name _____   Hosp.No. ☐☐☐☐☐☐☐

Inborn   Y / N   Sex   male/female/others

Date of Birth ☐☐☐☐☐☐   Time __ __:__ __ (24 hour format)

Address _____

Contact number _____

Delivery: 1. Normal 2. Forceps 3.Suction Cup 4. Breech 5.LSCS

Indication: _____

APGAR 1 min: ____   APGAR 5 min: ____

Preterm   Y / N   Gest.Age: _____weeks

Birth Weight:

MATERNAL HISTORY

Mother Hosp.No. ☐☐☐☐☐☐☐

Mat.Age   _____   Booked   Y / N

Education 1. < class 6 2. Class 6 - 12   3.Graduate 4. P.G. 5. Nil

Obstetric Score _____   Primipara   Y / N

PIH   Y / N   DM   Y / N   UTI   Y / N

Maternal medications:

_____

_____

USG:_____

Peripartum Fever Y / N   If yes, Temperature_____

Chorioamnionitis   Y / N   Foul-smelling liquor   Y / N

MSAF   Y / N

ROM - Spontaneous / ARM   Duration of ROM   _____

Prelabour ROM   Y / N   Latent period _____ hours

Duration of Labour_____ hours

No. of PV's after ROM

Induced   Y / N   Induced with_____

Others

Intrapartum antibiotics?   Y / N

Specify: 1._____

               2._____

               3._____

NEONATAL RISK FACTORS

APGAR 1 min: _____   APGAR 5 min: _____

Preterm   Y / N   Gest.Age: _____ ___

Birth Weight: _____

Cord pH/base excess _____

Blood gas pH/base excess _____

Onset sepsis   EOS / LOS

Symptomatic   Y / N

If yes, Symptomatic at birth   Y / N

        Symptomatic within few hours   Y / N   How many hours?

        Symptomatic after few days   Y / N   How many days?

Clinical features:

Respiratory distress   Y / N   Tachypnoea   Y / N

Grunting   Y / N   Retractions   Y / N

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Poor perfusion	Y / N	Shock	Y / N
Poor feeding	Y / N	Lethargy	Y / N
Oliguria/Anuria	Y / N	Seizures	Y / N
Fever	Y / N	Hypothermia	Y / N
Cellulitis	Y / N	Abcess	Y / N
Abd. distension	Y / N	Vomiting	Y / N
Diarhoea	Y / N	Peripheral Cyanosis	Y / N

Others   ---------------

Oxygen	Y / N	How many hours? _____	
Ventilation IMV	Y / N	CPAP	Y / N

 How many hours?

FFP	Y / N	Inotropes	Y / N
Steroids	Y / N	IV Fluids	Y / N
Whole blood	Y / N	Dialysis	Y / N

 Others   _____

Risk factors for renal failure

Resuscitation at birth	Y / N	Intubation	Y / N
UV line	Y / N	UA line	Y / N
CV Cath.	Y / N	IV fluids	Y / N
NG Tube	Y / N	PDA	Y / N

Drugs   Y / N

Aminoglycosides

NSAIDS

Other antibiotics (specify):

Abx X days	Abx X days	Abx X days
Abx X days	Abx X days	Abx X days

 Other drugs   _____

Blood investigations

Haemoglobin _____   MCV _____   retics _____

Total count _____   Differential counts _____

Platelets _____

Electolytes – Sodium _____   Potassium _____   Bicarbonate _____

Creatinine _____   urea _____

Blood culture _____

CRP _____

CSF analysis   WBC_____   P__L___   RBC_____   Glu_____ protein_____

Urine microscopy _____

Ultrasound abdomen dated_____

ECHO _____

Neurosonogram_____

	Week 1	Week 2	Week 3	Week 4
Day of life				
Creatinine				
Urine output				
Urine microscopy				
Urine NGAL				
Neonatal events				
Management				
Outcome				

OUTCOME   Discharged / Died / DAMA

REVIEW VISITS

	3 months	6 months
Weight gain		
NGAL		
creatinine		
Ultrasound abdomen		
Blood pressure		
Urine microscopy		