CHAPTER-3

RESULTS AND DISCUSSION
3. RESULTS & DISCUSSION

3.1 URINARY NGAL, SERUM NGAL AND SERUM CYSTATIN C AS BIOMARKERS FOR ACUTE KIDNEY INJURY

The study was carried out on first group of 100 consecutive patients undergoing coronary angiography procedure. The basal clinical characteristics of first group of 100 patients with normal serum creatinine values undergoing angiography are presented in Table 1.

Table 1: Basal clinical characteristics of patients undergoing angiography.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>51.4 ±13.0</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>138.5±30.0</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81.5±20.0</td>
</tr>
<tr>
<td>Heamoglobin, g/dl</td>
<td>13.7±2.2</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>4.9±2.8</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>1.2±0.18</td>
</tr>
<tr>
<td>eGFR byMDRD equation, ml/min</td>
<td>80.7±26.0</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>170±45</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>45±12</td>
</tr>
<tr>
<td>Fasting Blood Sugar, mg/dl</td>
<td>117±47</td>
</tr>
</tbody>
</table>

The present study revealed a significant increase in serum NGAL, at 2h, 4h and 8 hour after coronary angiography procedure (Table 2). Serum NGAL returned to baseline values at 48 h after the angiographic procedure. Urinary NGAL levels were increased at 4h, 8h and 24h after the procedure. There was a mild increase in serum creatinine at 24h and milder at 48 hours after the angiographic procedure, but there was no significant increase in eGFR during 48 hours of the procedure (Table 2).

3.2 Impact of Diabetes, hypertension and Gender on Serum and urinary NGAL, Urinary IL-18 and Cystatin C.

The serum Cytatin C levels increased significantly at 8h and reaching peak at 24 hours after the procedure. There was a mild increase in urinary IL-18 levels at 24 hours after the angiographic procedure but returned to baseline at 48 hours. It was observed that serum NGAL levels before contrast administration were significantly higher in diabetic patients than in non-diabetics (123.81±81.22 vs 88.11±35.87ng/ml, p<0.05). In addition to that the serum NGAL levels were higher in diabetic patients
comparison to non-diabetics at 2h, 4h, 8h and 24 hours after contrast administration (137.14±87.28 vs 89.17±45.07 ng /ml, p<0.05; 127.26±89.21 vs8.41±55.13ng/ml, p<0.05; 122.34±80.27 vs 81.27±35.01 ng/ml, p<0.05; and 125.42±96.58 vs88.19±40.74ng/ml, p<0.05 respectively). There is no significant difference in urinary NGAL and serum Cystatin C levels in diabetic and non-diabetic patients. The patients with hypertension revealed higher serum NGAL levels at 2h, and 4 hours after contrast administration, than the normal blood pressure patients (124.14±87.28 vs 81.17±35.61ng/ml,p<0.05; and 137.14±97.28 vs 80.17±42.07ng/ml, p<0.05 respectively. Females did not differ significantly with regard to serum creatinine, serum cystatin C, serum and urinary NGAL and urinary IL-18 when compared to males. Females had lower eGFR values by MDRD formula relative to males (60.15±26.75 vs. 85.25±25.15 ml/min, <0.01) before angiography which remained still significantly lower 48 hours after angiographic procedure.

Fig.1. Receiver operative characteristics (ROC) showing correlation among Serum NGAL (2hours), Urinary NGAL (4hours) and Cystatin C (24hours) and Contrast
induced Acute Kidney Injury, defined as a serum creatinine increase >0.5 mg/dl at 48 hours. Using a cutoff value of 106 ng/ml, sensitivity, specificity and area under the receiver operating characteristic (ROC) curve for prediction of Acute Kidney Injury were excellent for serum NGAL at 2 h (96%, 89% and 0.95 respectively). Using a cutoff value of 106 ng/ml, sensitivity specificity and area under the ROC curve for prediction of AKI were excellent for urinary NGAL at 4 hours (95%, 100% and 0.96 respectively). Using a cutoff value of 1.1 mg/l, sensitivity, specificity and area under the receiver operating characteristic (ROC) curve for prediction of Acute Kidney Injury were excellent for cystatin C at 24 hours (83%, 91% and 0.93 respectively).

Fig: 2 Serum NGAL levels are higher in diabetic patients than non-diabetic patients.

As per definition for acute Kidney Injury (>25% of the baseline levels at 48 h after contrast administration), then the prevalence of acute Kidney Injury was 13%. The serum creatinine levels at 48 hours after the contrast administration were significantly higher in the patients with acute Kidney Injury associated with contrast administration then those without acute Kidney Injury (1.40±0.28 vs 1.1±0.31 mg/dl, p<0.01). The serum NGAL levels at 2 hours after the contrast administration were significantly higher in the patients with acute Kidney Injury group associated with contrast administration then those without acute Kidney Injury group (153.76±31.3 vs 98.7±23.7 ng/ml, p<0.01). The urine NGAL levels at 4 hours after the contrast administration were significantly higher in the patients with acute Kidney Injury group associated with contrast administration then those without acute Kidney Injury
group (154.76±29.1 vs 92.5±18.9 ng/ml, p<0.01). The serum cystatin C levels at 24 hours after the contrast administration were significantly higher in the patients with acute Kidney Injury group associated with contrast administration than those without acute Kidney Injury group (2.27±0.6 vs 0.9±0.1 mg/l, p<0.01). The eGFR at 48 h after the contrast administration was significantly decreased in the patients with acute Kidney Injury associated with contrast administration than those without acute Kidney Injury (65.34±17.68 vs 84.07±23.41 ml/min, p<0.05). Cystatin C levels were higher at 8h and 24 hours after the contrast administration in patients with acute Kidney Injury than those without acute Kidney Injury.

![Fig:3 Serum NGAL levels between Hypertensive patients and Non-Hypertensive patients.](image)

When Acute Kidney Injury was defined as an increase in serum creatinine by >50% of the baseline level 48 hours after contrast exposure, the prevalence of AKI was 6% (6 patients). They were all diabetic patients. The cystatin C concentrations were higher 8, 24 and 48 hour after the angiographic procedure in patients with AKI relative to those without AKI.
Table: 2 Changes in serum and urinary NGAL, Cystatin C, IL-18, eGFR and blood pressure (BP) in patients undergoing angiography.

<table>
<thead>
<tr>
<th>Name of the parameter</th>
<th>Before angiography</th>
<th>After 2 hrs</th>
<th>After 4 hrs</th>
<th>After 8 hrs</th>
<th>After 24 hrs</th>
<th>After 48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NGAL, ng/ml</td>
<td>101.3±53.2</td>
<td>116.2±79.2*</td>
<td>130.6±93.6**</td>
<td>118.3±80.7*</td>
<td>104.8±65.4</td>
<td>103.3±59.5</td>
</tr>
<tr>
<td>Urinary NGAL, ng/ml</td>
<td>8.7(0.3-89)</td>
<td>10.8(0.3-174.0)</td>
<td>18.9(0.3-232.0)*</td>
<td>25.5(0.3-240.0)***</td>
<td>20.6(0.5-220.0)*</td>
<td>11.7(0.3-185.0)</td>
</tr>
<tr>
<td>Cystatin C, mg/l</td>
<td>1.2±0.78</td>
<td>1.31±0.72</td>
<td>1.45±0.84</td>
<td>1.47±0.86*</td>
<td>1.65±1.08**</td>
<td>1.42±0.80</td>
</tr>
<tr>
<td>IL-18, urine (pg/ml)</td>
<td>26.0(2-46.0)</td>
<td>26.5(4-58.0)</td>
<td>27.0(4-60.0)</td>
<td>26.0(5-66.0)</td>
<td>28.0(9-79.0)</td>
<td>27.0(5-57.0)</td>
</tr>
<tr>
<td>Serum Creatinine, mg/dl</td>
<td>1.16±0.18</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.14±0.30</td>
<td>1.15±0.31</td>
</tr>
<tr>
<td>eGFR by MDRD equation, ml/min</td>
<td>80.21±27.32</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>78.9±24.34</td>
<td>80.21±26.40</td>
</tr>
<tr>
<td>Cystolic BP, mmHg</td>
<td>139.45±31.15</td>
<td>128.5±32.2</td>
<td>ND</td>
<td>ND</td>
<td>136.55±25.65</td>
<td>137.75±24.25</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81.5±23.1</td>
<td>80.6±21.3</td>
<td>ND</td>
<td>ND</td>
<td>81.7±16.41</td>
<td>81.66±13.55</td>
</tr>
</tbody>
</table>

ND: Not determined. Data given are mean values ± SD or median values (minimum-maximum) *p<0.05 vs baseline; **p<0.001 vs baseline; ***p<0.001 vs baseline.

Fig:4 Serum NGAL levels are higher in diabetic patients than non-diabetic patients.
Using the Youden index, the best cutoff value for both serum and urinary NGAL to predict acute Kidney Injury was: 106 ng/ml, and p<0.5. The cut off value for Cystatin C is 1.1 mg/L with diagnostic sensitivity 83% and specificity 91% and p<0.5.

3.3 KIDNEY INJURY MOLECULE -1 AS A MARKER OF ACUTE KIDNEY INJURY

The study was carried out on second group of 100 consecutive patients undergoing coronary angiography procedure for the evaluation of Kidney Injury Molecule -1. Clinical and biochemical characteristics of all patients with normal serum creatinine values undergoing angiographic procedure are presented in Table 3.

Table 3: Basal clinical characteristics of patients undergoing angiography.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>48.6 ±11.0</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>136.5±29.0</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>80.5±20.0</td>
</tr>
<tr>
<td>Heamoglobin, g/dl</td>
<td>14.5±2.0</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>5.0±2.5</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>1.2±0.17</td>
</tr>
<tr>
<td>eGFR byMDRD equation, ml/min</td>
<td>81.2±22.0</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>168±43</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>44±10</td>
</tr>
<tr>
<td>Fasting Blood Sugar, mg/dl</td>
<td>115±41</td>
</tr>
</tbody>
</table>

We found a significant rise in urinary KIM-1 at 24 h after the angiographic procedure. When acute kidney injury was defined as an increase in serum creatinine of 0.3 mg/dl of the baseline level 48 hours after contrast exposure, the prevalence of Acute Kidney Injury was 12%. The patients with AKI did not significantly differ with regard to age, blood pressure, serum creatinine, serum lipid levels before angiography when compared to patients without AKI. The serum creatinine levels 48 h after the angiography were significantly higher in the patients with AKI than in those without AKI (1.39±0.27 vs 1.12±0.17 mg/dl, p<0.001).
Fig. 5: Receiver operative characteristics (ROC) showing curve against serum creatinine 48 hours vs 24 hours urinary kim-1 and Contrast induced Acute Kidney Injury, defined as a serum creatinine increase by 0.3 mg/dl at 48 hours. Using a cutoff value of 4.5 ng/ml, sensitivity, specificity and area under the receiver operating characteristic (ROC) curve for prediction of Acute Kidney Injury was very good for urinary kim-1 at 24 hours (89%, 81% and 0.95 respectively). Area under curve (AUC) for urine kim-1(24 hrs) to distinguish AKI from non-AKI was 0.95, 95% confidence interval (CI): 4.54 - 5.72 (p < 0.025) and 0.86, 95% CI: 3.93- 4.59 (p = 0.047), respectively.

The eGFR by MDRD formula 48 h after the angiography was significantly lower in the patients with Acute Kidney Injury than in those without Acute Kidney Injury (63.45±18.64 vs 82.95±24.35 ml/min, p<0.045). The AKI was diagnosed in 12 patients at 24 hours following angiographic procedure. No significant differences were noted between patients with and without AKI in respect of age, sex, DM and
Non-DM. (Table No.2). Urinary KIM-1 values were higher at 24h after the angiographic procedure. There was a mild increase in serum creatinine at 24h and milder at 48 hours after the angiographic procedure.

We used Acute Kidney Injury definition as rise in serum creatinine by 0.3 mg/dl over baseline, 48 hours after angiographic procedure. Patients after angiographic procedure are typically discharged within 48 hours sometimes even earlier; therefore, we could miss patients with AKI developing 48 hours after angiography.

Using a cutoff value of 4.5 ng/ml, sensitivity and specificity are very good. Area under curve (AUC) for urine kim-1(24 hrs) to distinguish AKI from non-AKI was 0.95, 95% confidence interval (CI): 4.54 - 5.72 (p < 0.025) and 0.86, 95% CI: 3.93 - 4.59 (p = 0.047), respectively.

Table: 4 Time course changes in serum creatinine and urinary KIM-1, eGFR and blood pressure (BP) in patients undergoing angiography.

<table>
<thead>
<tr>
<th>Name of the parameter</th>
<th>Before angiography</th>
<th>After 2 hrs</th>
<th>After 4 hrs</th>
<th>After 8 hrs</th>
<th>After 24 hrs</th>
<th>After 48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary KIM-1, ng/ml</td>
<td>ND</td>
<td>ND</td>
<td>3.1(2.54-4.16)</td>
<td>4.1(2.66-4.87)</td>
<td>5.3(4.53-5.74)</td>
<td>ND</td>
</tr>
<tr>
<td>Serum Creatinine, mg/dl</td>
<td>1.2±0.17</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.21±0.26</td>
<td>1.32±0.27</td>
</tr>
<tr>
<td>eGFR by MDRD equation, ml/min</td>
<td>81.11±25.30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>77.8±25.32</td>
<td>80.13±24.38</td>
</tr>
<tr>
<td>Cystolic BP, mmHg</td>
<td>135.45±30.5</td>
<td>127.5±30.5</td>
<td>ND</td>
<td>ND</td>
<td>136.45±25.65</td>
<td>135.75±25.25</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81.5±23.5</td>
<td>80.5±21.5</td>
<td>ND</td>
<td>ND</td>
<td>81.5±16.4</td>
<td>81.65±13.55</td>
</tr>
</tbody>
</table>

ND: Not determined. Data given are mean values ± SD or median values (minimum-maximum).

Using a cutoff value of 4.5 ng/ml, sensitivity, specificity and area under the receiver operating characteristic (ROC) curve for prediction of Acute Kidney Injury was very good for urinary kim-1 at 24 hours (89%, 81% and 0.95 respectively). 24 hours after angiography, compared with the non-CIN group, urinary KIM-1 (ng/ml) levels of AKI group increased significantly 5.31; 95% confidence interval, 4.54 - 5.72 vs 4.24 ; 95% confidence interval,3.93 - 4.59, P<0.047, the urinary KIM-1(ng/ml) levels were significantly increased significantly 5.31 ; 95% confidence interval,4.54 - 5.72 vs 3.1; 95% confidence interval, 2.55 - 4.14, P<0.049, from levels of 4 hours to 24 hours after contrast administration in the Acute Kidney Injury group.
3.4 Contrast induced acute kidney injury: Pathophysiological mechanism and renal haemodynamics

Iodinated contrast is a low-osmolar contrast (iodizanol or iopromide) medium (CM), which causes a brief period of vasodilation, followed by sustained intrarenal vasoconstriction with subsequent precipitation of an ischemic injury. The ischemic injury sets off a cascade of events largely driven by oxidative injury causing single cell death of renal tubular epithelium. Significant renal function deterioration and rise of serum creatinine will be apparent when a significant tubular damage has occurred. Usually, CIAKI can be readily diagnosed based on clinical features and renal biopsy is rarely required.

There was an urgent need for a renal biopsy for a patient who was consequently diagnosed as acute kidney injury associated with contrast administration. Upon histological study, it revealed with following. The histology revealed acute tubular necrosis. The renal biopsy will show acute tubular necrosis with dilated tubular lumen with sloughed necrotic material, simplification of tubular epithelium, loss of brush border, and presence of mitosis as a feature of tubular regeneration. In this case calcium phosphate crystals were identified. Light microscopic micrographs demonstrating normal kidney and contrast induced acute kidney injury kidney
Fig:6 Hematoxylin and Eosin stained section demonstrating destroyed tubules with extensive calcium phosphate crystals.

Fig:7 Light microscopic image showing renal cortex within normal limits
3.4.1 Microscopic Description:

The microscopic histology revealed acute tubular necrosis. The renal biopsy will show acute tubular necrosis with dilated tubular lumen with sloughed necrotic material, simplification of tubular epithelium, loss of brush border, and presence of mitosis as a feature of tubular regeneration. In this case calcium phosphate crystals were identified.

Diffuse and focal clear cell transformation with lumenal narrowing of proximal tubules can be seen in the figure no.6. Isometric vacuolization of proximal tubular epithelium noticed the figure no.6 where as such characteristics are absent in normal renal cortex in figure no.7. Fig:6 showing Hematoxylin and Eosin stained section demonstrating destroyed tubules with extensive calcium phosphate crystals. There was a mild cortical interstitial edema with some inflammatory cells in the figure no.6 which is absent in normal kidney figure no.7. Negative immunofluorescence studies were observed with Acute kidney injury associated with ATN due to contrast administration.
3.5 DISCUSSION

The traditional laboratory approach for detection of renal disease does not allow for early detection of acute renal injury. Several medications (trimethoprim, cimetidine, and salicylates) alter the tubular secretion of creatinine, leading to changes in serum creatinine independent of GFR. Damage to renal tubules can be insufficient to result in a change in a parameter of kidney function such as blood urea and serum creatinine. In addition, in cases of more extensive tubular injury, there is a lag in time between the injury and an increase in serum creatinine. Serum creatinine production changes significantly according to the muscle mass of the body and dietary factors. Creatinine is filtered by the glomeruli and also secreted by the renal tubules. This tubular secretion contributes approximately 20% of the total creatinine excretion by the kidney and it can increase as GFR decreases. All of these factors explain why serum creatinine concentration may not be a good parameter for accurate determination of GFR, especially at lower rates.

3.5.1 CYSTATIN C

Cystatin C production in the body is a stable process that is not influenced by renal conditions, increased protein catabolism, or dietetic factors. Moreover, it does not change with age or muscle mass like creatinine does. Its biochemical characteristics allow free filtration in the renal glomerulus and subsequent metabolism and reabsorption by the proximal tubule. For these reasons, serum cystatin C has been suggested to be an ideal endogenous marker of GFR. However, few studies demonstrate that older age is independently associated with higher serum cystatin C levels after adjusting for creatinine clearance. It was suggested that serum cystatin C may have advantages over serum creatinine for estimating the GFR (eGFR), however, with some limitations. Herget Rosenthal et al. 2004, reported that serum cystatin C is a useful detection marker of ARF and might detect Acute Renal Failure (ARF), 1 or 2 days earlier than serum creatinine. Serum cystatin C is an early, predictive biomarker of AKI, which outperforms serum creatinine in the heterogeneous emergency department setting. Serum cystatin C was measured in 25 children in the ICU. The ability of serum
cystatin C to identify a creatinine rate a Schwartz creatinine clearance rate under 80 ml/min/1.73m2 was better than creatinine (area under the ROC curve: 0.85 and 0.79 for cystatin C and 0.63 and 0.62 for creatinine). This study concluded that serum cystatin C was better than serum creatinine to detect AKI in critically ill children. Another study evaluated 85 patients at high risk to developing AKI. Forty-four patients developed AKI and the increase of cystatin C significantly preceded that of creatinine. Specifically, serum cystatin C increased already by more than 50% at 1.5 +/- 0.6 days earlier compared to creatinine. Serum cystatin C demonstrated a high diagnostic value to detect AKI as indicated by area under the curve of the ROC analysis of 0.82 and 0.97 on the two days before the R-Criteria (risk of kidney dysfunction) was fulfilled by creatinine. Plasma and urine cystatin C were prospectively collected from 72 adults undergoing cardiac surgery. AKI was defined as a 25% or greater increase in plasma creatinine or renal replacement therapy within the first 72 hours following surgery. In an another study conducted by J.I.Koyner revealed that the plasma cystatin C and NGAL did not predict the development of AKI within the first 6 h following surgery. However, both urinary cystatin C and NGAL were increased in the 34 patients who later developed AKI. The urinary cystatin C at 6 h after ICU admission was the most useful for predicting AKI.

A recent study compared the sensitivity and rapidity of AKI detection by cystatin C level relative to creatinine level after 150 high risk adult patients in the cardiac surgery. Serum creatinine levels detected more cases of AKI than cystatin C level, 35% developed a > 25% increase in serum creatinine level, whereas only 23% had a >25% increase in cystatin C level. This study concluded that cystatin C level was less sensitive for AKI detection than creatinine level. Another study in 2011 examined presurgical values for cystatin C, creatinine, and creatinine based estimated glomerular filtration rate (eGFR), in 1147 adults undergoing cardiac surgery for high risk AKI. Cystatin C also substantially improved AKI risk classification compared with creatinine. Presurgical cystatin C is better than creatinine or eGFR at prediction risk of AKI after cardiac surgery.

### 3.5.2 Impact of thyroid disease on cystatin C

It has been observed that thyroid dysfunction may alter creatinine, which has been found to be increased in hypothyroidism and decreased in hyperthyroidism.
Fuicker M. et al., in their study concluded that thyroid dysfunction has a major impact on cystatin C levels. Therefore, thyroid function needs to be considered when cystatin C is used as a marker of kidney function. In contrast to creatinine concentrations, cystatin C levels are lower in the hypothyroid and higher in the hyperthyroid state as compared with the euthyroid state. Knight and colleagues found that cystatin C concentrations were significantly associated with increased age, male sex, increased weight, tall individuals, current smoking and higher C-reactive protein (CRP) levels, even after adjustment for creatinine clearance. They concluded that the above characteristics must bias cystatin C concentrations as a measure of GFR. The above studies suggested that the serum concentrations of Cystatin C may increase in settings of increased cell turnover. The use of glucocorticoids has also been associated with higher concentrations of cystatin C.

3.5.3 Impact of dialysis on cystatin C

The process of dialysis leads to a significant fall in mean serum creatinine concentrations. In contrast to this, the serum cystatin C levels were significantly higher in the post-dialysis samples as compared with the pre-dialysis ones. The rise in serum cystatin C following hemodialysis was observed in all the patients taken up for the study. This was in spite of the concomitant fall in serum creatinine, which is an accepted indeed for the adequacy of dialysis. The rise in the serum cystatin C following dialysis could be attributed to several factors such as the nature of the dialyzing membrane and the composition of the dialyzing fluid. When dialysis is carried out using low flux membrane, the pore size is smaller than 1.5 nm which does not permit the removal of low molecular weight proteins such as cystatin C. Another factor to be considered is the electrostatic interaction between micro-protein and other plasma proteins adsorbed onto the dialyzer membranes. Cystatin C is strongly cationic and the charged nature of the molecule might hinder its filtration. The rise in serum cystatin C could also be attributed to the effect of hemoconcentration which occurs during dialysis. The fall in serum creatinine despite such change is because of the magnitude of reduction of this metabolite during dialysis. These studies have concluded that serum cystatin C cannot be used to monitor adequacy of hemodialysis.
3.5.4 Impact of jaundice on cystatin C

A study conducted by Lofberg and Grubb revealed that hyperbilirubinemia is associated with higher cystatin C levels. However, it has been shown that bilirubin concentrations up to 700 μmol/l do not interfere with the immunoturbidimetric assays for cystatin C. Despite normalization of bilirubin levels after the first week of life, higher cystatin C concentrations persisted for several months, further excluding assay interference by hyperbilirubinemia. Therefore, the emerging biomarkers of acute renal failure in combination, similar to troponins, can increase the sensitivity of diagnosis of the renal disease. These include a plasma panel [neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C] and a urinary panel [NGAL, interleukin 18 (IL-18), and kidney injury molecule 1 (KIM)-1] 80.

3.5.5 RIFLE and AKIN Criteria:

In 2002, the Acute Dialysis Quality Initiatives (ADQI) group proposed a standard definition and classification system for the syndrome of acute renal failure through a broad consensus of experts across disciplines and international boundaries. The classification systems coins the acronym RIFLE and has three levels: risk, injury and failure; and two outcomes: persistent acute renal failure (termed loss) and End stage Kidney disease. A unique feature of the RIFLE classification is that it provides retrospectively for three grades of severity of renal dysfunction on the basis of a maximum change in serum creatinine, reflecting changes in GFR or duration and severity of decline in urine output from the baseline. Based on the findings that small alterations of serum creatinine result in adverse outcomes, the Acute Kidney Injury International collaborative Network (AKIN) recently changed the definition of Risk group to include patients with an increase in serum creatinine of 0.3 mg/dl81. The proposed diagnostic and staging criteria for ARF are designed to facilitate acquisition of knowledge and to validate the emerging concepts. Serum creatinine is the most widely used parameter for everyday assessment of glomerular filtration rate (GFR), but it has poor sensitivity and specificity in detecting ARF, because serum creatinine lags behind both renal injury and renal recovery 81. Serum creatinine levels began to rise within 24 hours in 80% of the patients, reaching maximum at 48-72 hours after contrast administration, returning to baseline after 2 weeks82.
3.5.6 Urinary Interleukin-18 (IL-18)

Parikh et al., found that 72 patients with acute tubular necrosis and delayed graft function significantly higher IL-18 levels than other kidney diseases (urinary tract infection, chronic Kidney Injury, nephritic syndrome or prerenal azotemia) 83. Coca et al. reported that IL-18 generally showed a low sensitivity but high specificity respectively for assessing an acute kidney injury diagnosis and risk classification 84. A nested case-control study of 157 patients undergoing elective percutaneous coronary interventions (PCI) did not identify significant differences between urine IL-18 levels in the 9.5% of patients who developed AKI and those who did not 85. Conflicting results were reported in another study that included NGAL in its biomarker analysis. This study of adults undergoing PCI compared 13 patients who developed CIN with 27 controls. ROC curves were created for urinary IL-18 and NGAL measured 24 h and the AUCs were found to be 74.9% and 73.4% respectively 86. IL-18 levels were compared with plasma creatinine levels. A weak positive correlation was found between precontrast creatinine and urine IL-18 level although it was not statistically significant. Furthermore, they also found a weak positive correlation between post contrast 2 h creatinine and urine IL-18 levels, although his was not statistically significant either. A slight increase in plasma creatinine levels at 24 h and 48 h following radiocontrast administration was observed compared with precontrast values, but it was not statistically significant which was regressed to precontrast values at 72h. A statistically significant increase in the levels of spot urine IL-18 levels at 6 and 24 h post contrast was observed, compared with precontrast spot urine IL-18 levels and difference between 6th and 24th hour levels were not statistically significant 87. Delayed Graft Function (DGF) due to tubule cell injury frequently complicates deceased donor kidney transplants. Parikh showed that urinary NGAL and IL-18 represent early biomarkers for DGF. In patients with DGF, peak postoperative serum creatinine requiring dialysis typically occurred 2-4 days after transplant. Urine NGAL and IL-18 were elevated in the first day after transplant in patients with DGF. The receiver operating characteristics curve for prediction of DGF based on urine NGAL or IL-18 at day 0 showed an area under the curve of 0.9 for both markers 88. The same author reported that increased levels of IL-18 in patients with AKI of varying etiology, especially those with delayed renal allograft function and ischemic ATN. In kidney transplant recipients, lower urinary IL-18 levels were
associated with a steeper decline in serum creatinine concentrations postoperative days from 0 to \(^{83}\). Immunohistochemical staining of protocol biopsies showed constitutive IL-18 expression in the epithelium of distal tubules with the induction of immunoreactivity in acute rejection patients were also proximal tubules, infiltrating leukocytes, and endothelium were strongly positive. Furthermore, serum levels of IL-18 were significantly elevated in patients with acute rejection of kidney allograft as compared to patients with uncomplicated outcome of kidney transplantation and subjects with acute tubulointerstitial nephropathy\(^{89}\). In a study of critically ill adult patients with acute respiratory distress syndrome, increased IL-18 was found to be an early marker of AKI and it was an independent predictor of death\(^{90}\). A subsequent study involved 451 patients, 86 of them developed AKI. The area under the receiver operating characteristic curve for urinary IL-18 predicted subsequent AKI within 24 hours was 0.62. It was found that urinary IL-18 remained independently predictive of composite outcome of death or acute dialysis within 28 days of ascertainment (odds ratio, 1.86)\(^{91}\).

The predictive ability of urinary IL-18 has been demonstrated in one hundred thirty seven critically ill children. The peak levels of IL-18 corrected with the severity of AKI. In nonseptic Aki patients, urinary IL-18 rises to a level higher than control levels 2 days prior to a significant rise in creatinine. Urinary IL-18 concentration from the first urine specimen was associated with AKI development within 48 h (odds ratio=3.5) independent of the pediatric risk of mortality. This study concluded that urinary IL-18 rises prior to serum creatinine in nonseptic critically ill children, predicts severity of AKI, and is an independent predictor of mortality\(^{92}\). AKI is also common after cardiac surgery in adults and children. The study tested whether urinary IL-18 is a predictive biomarker for AKI in patients undergoing CPB. Serum creatinine was detected 48-72 h after CPB. In contrast, urine IL-18 increased at 4-6 h after CPB. These study results indicated that urinary IL-18 is an early, predictive biomarker of AKI after CPB\(^{93}\). AKI occurs commonly after pediatric cardiac surgery and associates with poor outcomes. 311 children undergoing surgery for congenital cardiac lesions to evaluate whether early postoperative measures of urine IL-18 urine NGAL or plasma NGAL could identify which patient would develop AKI. 53 of them reached the primary outcome of severe AKI. Urine IL-18 and urine NGAL levels strongly associated with severe AKI. Elevated urine IL-18 and urine NAGAL levels associated
with longer hospital stay, longer intensive care unit stay and duration of mechanical
ventilation. The same author also examined urine IL-18, urine and plasma NAL
markers in adults cardiac surgery. They found that urine IL-18 and plasma NGAL at 6 h were strongly associated with risk of AKI.

Rickli et al. 2004, observed that the rise in cystatin C achieved a maximum 24 h after the application of the contrast agent. Therefore, in our study, we simultaneously assessed NGAL, serum cystatin C, serum creatinine, and eGFR and confirmed their findings. It should be stressed that NGAL correlated with both cystatin C and creatinine. In a recent paper by Mitsnefes et al. both NGAL and cystatin C significantly correlated with the measured GFR (r=0.62, p<0.0001, and r=0.71, p<0.0001) as well as with eGFR (r = 0.66, P<0.0001, and r =0.59, P<0.0001, respectively).

Herget Rosenthal et al reported that serum cystatin C is a useful detection marker for ARF and might detect ARF, 1 or 2 days earlier than serum creatinine (Herget Rosenthal et al, 2004). Rickli et al observed that the rise in Cystatin C achieved a maximum 24h after the application of the contrast agent. Hence in our study we simultaneously analysed serum NGAL, Urinary NGAL, Serum Cystatin C, serum creatinine, eGFR and IL-18 and confirmed that our findings can influence the mode of treatment of AKI.

In our present study, we analyzed urinary IL-18 levels in comparison with serum creatinine levels. There was a mild rise in urinary IL-18 levels at 24 hour after contrast administration, but without significance. In conclusion, Urinary IL-18 might not serve as an early diagnostic tool for detecting Acute Kidney Injury associated with contrast administration.

3.5.7.1 Urinary and serum Neutrophil Gelatinase Associated Lipocalin (NGAL):

Serum NGAL at 2h after contrast administration raised maximally and urinary NGAL raised maximally at 4 hours after the contrast administration. The present study revealed that the serum NGAL, urine NGAL and serum Cystatin C can detect acute Kidney Injury associated with contrast administration earlier than serum creatinine. The area under the curve (AUC) for serum NGAL at 2h was 0.956 and for
Urinary NGAL 0.963. Area under the curve for Cystatin C was 0.939. Using the Youden index, the best cutoff value for both serum and urinary NGAL to predict acute Kidney Injury was: 106 ng/ml. The best cut off value for Cystatin C is 1.1mg/L with diagnostic sensitivity 83% and specificity 91%. Similar but not same values were noted by Hirsch et al in which both urine and plasma NGAL levels served as excellent predictors of contrast induced nephropathy both with sensitivity and specificity of 73% and 100% respectively\(^98\).

Bachorzewska-Gajewska et al. Found NGAL is a sensitive biomarker of AKI after contrast administration for coronary angiography. They found a significant rise in serum NGAL at 2 hour and 4 hour after percutaneous coronary intervention (PCI)\(^99\). A similar study of 25 patients with normal serum creatinine undergoing PCI due to unstable angina revealed a significant rise in serum NGAL after 2 hour and 4 hour. Urinary NGAL and urinary liver fatty acid binding protein (L-FABP) also raised significantly after 4 hour and remained elevated upto 48 hour after PCI.\(^100\) In our study the prevalence of acute Kidney Injury association with contrast administration was 13%. The reported incidence of acute Kidney Injury due to contrast administration varies widely, ranging from 0 to >50%\(^101\). This variation results from whether presence or absence of risk factors (primarily renal insufficiency), amount and type of contrast agent used, the exact radiologic procedure and whether other causes of acute Kidney Injury (ARF) unrelated to contrast media were excluded. The underlying pathophysiology of CIN is acute tubular necrosis, a type of ARF, although the mechanism by which this occurs is not well understood\(^102\).

The two major theories, based largely upon studies performed in experimental animals, are renal vasoconstriction resulting in medullary hypoxemia and direct cytotoxic effects of the contrast agents. Other factors that were postulated include rheological alterations, activation of the tubuloglomerular feedback mechanism, regional hypoxia and production of reactive oxygen species\(^102\). The time of the procedure is very important in determining serum NGAL\(^103\).

Even in the relatively homogenous setting of cardiac surgery in children, as studied by\(^104\) and later by Parikh et al,\(^88\) of the same group, ARF may be caused by several pathophysiological mechanisms such as ischemia, hypotension, hypoperfusion, cytokine release, and atheroemboli. These studies were performed in a
completely different population, i.e., in children with congenital heart disease. The only obvious renal insult in these children would be ischemia-reperfusion injury after CPB.

Parikh et al. compared data of children with over 50% increase in serum creatinine after 48-72 h of ARF, where as we used AKI definition as a rise in serum creatinine by 0.5 mg/dl over baseline 48 hours after the angiographic procedure. Mishra et al and Parikh et al. Did not provide baseline creatinine or eGFR in their study. Differences in urinary NGAL levels between their and our patients may also be attributed to the different mechanisms of injury.

In the post ischemic kidney, NGAL is upregulated in proximal tubules and distal nephron segments. In the former it colocalizes, at least in part, with proliferating epithelial cells. In CIN besides vasoconstriction, tubular damage plays a role; however, this hypothesis is based predominantly on animal data. Nephrotoxic injury after cisplatin administration in mice resulted in a similar pattern of NGAL changes. Therefore, probably the levels of NGAL were in a narrow range and were lower than previously reported.

It should be stressed that the degree of renal failure in our study was minor as compared with findings in prior studies performed in mice and humans. Parikh et al, 2006, in a small group of kidney transplant recipients, reported that urinary NGAL may represent an early predictive biomarker of delayed graft function, an other model of ischemia-reperfusion injury.

In their previous study, the NGAL staining intensity in early protocol biopsies was suggested to be a novel predictive biomarker of acute kidney injury following transplantation. An enhanced urinary NGAL excretion mostly likely resulted from a combination of an early intrinsic tubule cell response to injury and a later component reflecting the inability of the damaged tubular cells to completely reabsorb filtered NGAL. Therefore, in CIN (When the insult is milder and shorter than in CPB) the urinary NGAL excretion is lower. It might be also due to the fact that tubules faster regain the ability to reabsorb filtered NGAL.

In a study done by Wagener et in patients after cardiac surgery, the urinary NGAL levels were higher in 16 subjects developing ARF (i.e., increase in serum
creatinine by more than 50% as compared with perioperative values) when compared to 65 patients without ARF. In their study, even the preoperative values of urinary NGAL were higher in ARF patients when compared to non-ARF patients. Moreover, patients who developed ARF on admission had significantly lower eGFR values (57 vs. 73 ml/min). No data concerning detailed clinical and biochemical characteristics of the patients were available (comorbidities, i.e., congestive heart failure, hypertension, and diabetes, haemoglobin, albumin, blood pressure, medications, etc.). CPB and aortic cross-clamp in the study of Wagener et al. (20) lasted much longer than the PCI.

Patients with ischemic heart disease often exhibit some degree of renal dysfunction due to concomitant diabetes and hypertension, despite normal creatinine values. The finding that diabetics had a significantly higher baseline serum, not urinary NGAL levels may speak in favour of this explanation. A rise in NGAL, despite nonsignificant changes in creatinine, may be due to renal injury and / or an inflammatory component of NGAL.

An earlier NGAL rise in serum than in urine may be due to the fact that NGAL is released into the circulation probably secondary to inflammatory activation of neutrophils initiated by the contrast agent. In addition to this, it was noted that NGAL is increased in atherosclerotic plaques109.

3.5.7.2 Urinary Kidney Injury Molecule -1 (KIM-1)

About a decade ago, KIM-1 was discovered in the search for molecule involved in the pathogenesis of acute kidney injury. Ichimura et al. were the first to describe KIM-1 as a type I membrane glycoprotein, which contain a 6-cystein immunoglobulin-like domain in its extracellular portion, and a Thr/Ser-Pro rich domain characteristic of mucin-like O-glycosylated proteins110. KIM-1 presence in the urine is highly specific for kidney injury. No other organs have been shown to express KIM-1 to a degree that would influence kidney excretion111. In the study of nephrotoxicity, urinary KIM-1 levels increased severely earlier than the increase of blood urea nitrogen and plasma creatinine112. KIM-1 is also a tissue and urinary biomarker for nephrotoxicant-induced kidney injury. Tissue and urinary expressions were measured with different nephrotoxic doses of cisplatin, folic acids, cadmium,
gentamycin, mercury and chromium. In fact the Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) have included KIM-1 in the small list of kidney injury biomarkers that they will now consider in the evaluation of kidney damage as part of their respective drug review processes of new drugs. FDA, European medicines agency to consider additional test results when assessing new drug safety collaborative effort by FDA and EMEA expected to yield additional safety data. Therefore new promising markers were investigated to find the ways to diagnose Acute Kidney Injury at the earliest possible time.

In our present study, the prevalence of AKI due to contrast administration was 12%. The reported incidence of AKI due to contrast induced nephropathy varies widely, ranging from 0 to >50%.

This variability results from whether presence or absence of risk factors (primarily renal sufficiency), the definition of CIN, amount and type of contrast agent administered, the exact radiologic procedure and whether other causes of Acute Kidney Injury (AKI) unrelated to contrast media were excluded. There have been over 16 definitions used for the diagnosis of acute renal failure in previously published studies, with most of them based on serum creatinine values. Recently the Acute Kidney injury Network has supported the use of the term ‘acute kidney injury’ to reflect the broad spectrum of acute kidney disease, including conditions that do not progress to failure.

Receiver operative characteristics (ROC) showed curve against serum creatinine 48 hrs vs 24h urinary kim-1 and Contrast induced Acute Kidney Injury, defined as a serum creatinine increase by 0.3 mg/dl at 24hours. Using a cutoff value of 4.5 ng/ml, sensitivity, specificity and area under the receiver operating characteristic (ROC) curve for prediction of Acute Kidney Injury was very good for urinary kim-1 at 24 hours (89%, 81% and 0.95 respectively).

In a study conducted by WK Han, et al revealed that the Urinary KIM-1 increased at 6-12 h after cardio-pulmonary bypass surgery (CPB) and remained significantly elevated up to 48 hours after CPB. In their study urinary KIM-1 had an AUC-ROC of 0.57 at 2h, 0.83 at 12 h, and 0.78 at 24 h. Nejat M et al found in a study that the Kidney injury molecule-1 (KIM-1) is elevated in pre-renal azotemia to a lesser extent than in more severe AKI (lasting more than 48 hours).
2012). Therefore, the heterogeneity of AKI suggests that more than one marker may be necessary to obtain sufficient sensitivity and specificity for AKI screening. Analysis of multiple biomarkers such as NGAL, Cystatin C with KIM-1 may optimize early detection of AKI associated with contrast administration at the earliest.

Urinary KIM-1 has been found to be an early indicator of AKI that compares favorably to a number of conventional biomarkers and tubular enzymes \(^{117-118}\). KIM-1 is also a tissue and urinary biomarker for nephrotoxicant-induced kidney injury. Tissue and urinary expressions were measured with different nephrotoxic doses of cisplatin, folic acide aside, cadmium, gentamycin, mercury, and chromium \(^{117}\). Marked increases in KIM-1 expression localized to proximal tubule cells were detected. As early as 1 day after cisplantin treatment, positive KIM-1 immunostain, observed in the outer medulla of the kidney, and changes in urinary clusterin indicated the onset of proximal tubular injury in the absence of functional effects. Tissue KIM-1 was the most sensitive biomarker for detection of cisplantin-induced kidney damage\(^{119}\). KIM-1 is a biomarker of AKI in humans. Urine samples were collected from 32 patients with various acute and chronic kidney diseases, as well as from eight normal controls. There was extensive expression of KIM-1 in proximal tubule cells in kidney biopsies from six patients with biopsy confirmed acute tubular necrosis (ATN). Urinary KIM-1 levels were significantly higher in patients with ischemic ATN compared to levels in patients with other forms of acute renal failure or chronic renal disease. Adjusted for age, gender and length of time delay between the initial insult and sampling of the urine, a one unit increase in normalized KIM-1 was associated with a greater than 12 fold (OR 12.4, 95% CI 1.2 to 119) risk for the presence of ATN \(^{118}\).

KIM-1 was also measured in 90 patients undergoing cardiac surgery. Thirty six patients who developed AKI within 72 h after surgery. The AUCs for KIM-1 to predict AKI immediately and 3h after operation were 0.68 and 0.65, 0.61 and 0.63 for NAG, and 0.59 and 0.65 for NGAL, respectively. Combining the three biomarkers enhanced the sensitivity of early detection of postoperative AKI compared with individual biomarkers; the AUC s for the three biomarkers combined were 0.75 and 0.78. This study demonstrated that combining multiple AKI biomarkers improve the overall predictive value \(^{120}\). A similar study described that preoperative KIM-1 and alpha-GST were able to predict the future development of AKI \(^{121}\). In hospital patients
with AKI, urinary levels of KIM-1 that higher levels correlated with a higher odds ratio for dialysis requirement or hospital death. This study demonstrated that urinary biomarker of AKI such as KIM-1 can predict adverse clinical outcomes in patients with AKI. Renal KIM-1 expression is significantly increased in human kidney tissue among patients with wide range of kidney diseases, including various types of glomerulonephritis, chronic allograft nephropathy, acute rejection, immunoglobulin A nephropathy, systemic lupus erythematosus, diabetic nephropathy, hypertension and Wegener’s granulomatosis. Both renal and urinary KIM-1 correlate with kidney damage and negatively with renal function, but not with proteinuria. A recent study has explored urinary KIM-1 correlated with kidney function in kidney allograft recipients. Kidney transplant recipient showed significantly higher KIM-1 values than the healthy volunteers.

This study concluded that even a successful kidney transplantation is associated with kidney injury as reflected by elevated urinary KIM-1. In a similar study explored urinary biomarkers in 63 renal transplant recipients who require graft biopsy because of progressive worsening of kidney function. They reported that the rate of renal function decline significantly correlated with urinary KIM-1 expression after being followed for an average of 39.7 months. In kidney allograft recipients, urinary KIM-1 expression provides prognostic information in relation to the rate of renal function decline.

### 3.5.7.3 Contrast induced acute kidney injury: Pathophysiological mechanism and renal haemodynamics

Iodinated contrast is a low-osmolar contrast (iodizanol or iopromide) medium (CM), which causes a brief period of vasodilation, followed by sustained intrarenal vasoconstriction with subsequent precipitation of an ischemic injury. The ischemic injury sets off a cascade of events largely driven by oxidative injury causing single cell death of renal tubular epithelium. Significant renal function deterioration and rise of serum creatinine will be apparent when a significant tubular damage has occurred. Different mechanisms are involved in causing renal tubular damage including, increased vasoconstrictive forces, decreased local prostaglandin- and nitric oxide (NO)-mediated vasodilatation, direct toxic effect on renal tubular cells by oxygen free radicals, increased oxygen consumption, increased intratubular pressure due to
contrast-induced diuresis, increased urinary viscosity, and tubular obstruction.

To date, our understanding of the underlying pathophysiology remains incomplete.\textsuperscript{126}

The pathophysiology of contrast-induced AKI assumes baseline reduced nephron number with superimposed acute vasoconstriction caused by the release of adenosine, endothelin, and other renal vasoconstrictors triggered by iodinated contrast. After a very brief increase in renal blood flow, via the above mechanism, there is an overall all 50\% sustained reduction in renal blood flow lasting for several hours. There is concentration of iodinated contrast in the renal tubules and collecting ducts, resulting in a persistent nephrogram on fluoroscopy. This stasis of contrast in the kidney allows for direct cellular injury and death to renal tubular cells. The degree of cytotoxicity to renal tubular cells is directly related to the length of exposure those cells have to iodinated contrast, hence, the importance of high urinary flow rates before, during and after contrast procedure. The sustained reduction in renal blood flow to the outer medulla leads to medullary hypoxia, ischemic injury and death of renal tubular cells.\textsuperscript{127}

The present renal biopsy revealed acute tubular necrosis with dilated tubular lumen with sloughed necrotic material, simplification of tubular epithelium, loss of brush border, and presence of mitosis as a feature of tubular regeneration. In this case calcium phosphate crystals were also identified. Fig no.6 demonstrate: Hematoxylin and Eosin stained section demonstrating destroyed tubules with extensive calcium phosphate crystals. And surrounding proximal tubules, some of which show mild dilatation of lumen with flattening of tubular epithelium and sloughed necrotic material, representing acute tubular injury.

The proposed mechanism includes interaction of iodine with amino acids found in cell membrane proteins, damaging them and causing loss of cell membrane proteins. Through similar processes, it may exert its direct toxicity on renal vascular and tubular cells.