Role of plasma neutrophil gelatinase-associated lipocalin as an emerging biomarker of acute renal failure following kidney transplantation and its correlation with plasma creatinine

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Introduction: Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin protein family, has been advocated as a sensitive, early biomarker for predicting early renal graft after transplantation. The functions of NGAL appears to be expressed in stress conditions and in tissues undergoing involution. It rapidly accumulates in the kidney tubules and urine after nephrotoxic and ischemic insults.

Objectives: This study aimed to examine the prognostic role of NGAL early after renal transplantation.

Patients and Methods: A total of 37 kidney recipients were enrolled from a teaching centre in Tabriz within a 6-month period of time. Plasma NGAL was measured immediately before and at 6 and 12 hours post-transplantation. Changes of serum creatinine were documented daily within the first week post-operation. Acute kidney injury (AKI)/graft rejection during the first week after transplantation was the outcome variable.

Results: There were 22 males (59.5%) and 15 females (40.5%) with the mean age of 34.93 ± 14.97 years (range: 12-59) in the study group. AKI/graft rejection developed in 12 patients (32.4%). The mean post-transplantation plasma NGAL levels and serum creatinine at all time points were significantly higher in patients with AKI/graft rejection. The best prognostic role was found for plasma NGAL at 12 hours (sensitivity = 100%, specificity = 92%; cut-off value = 309 ng/ml), far better than the prognostic accuracy of corresponding serum creatinine (sensitivity = 66.7%, specificity = 61.9%).

Conclusion: Plasma NGAL, particularly 12 hours after transplantation, is a very sensitive and specific biomarker for predicting acute renal injury.

Implication for health policy/practice/research/medical education: Plasma neutrophil gelatinase-associated lipocalin (NGAL), particularly 12 hours after kidney transplantation, is a very sensitive and specific biomarker for predicting acute renal injury.

After providing the required explanations to the patients, the blood samples of patients were obtained before anesthesia and 16 and 12 hours after the surgery. The blood samples were used to determine the plasma NGAL level and serum creatinine level and were sent to the laboratory of the hospital. It is worth mentioning that the serum creatinine level in patients was controlled and examined for at least 7 days.

Considering the persistence of plasma samples at a temperature of -70°C in over one month, in order to increase the precision of the investigation and reduce the number of kits required for calibration of the ELISA device, all of the samples that were sent to the laboratory were centrifuged and the resulting serum sample was stored at a temperature of -70°C after printing codes on the associated microtubes. After completion of sampling all samples were tested simultaneously. All samples were examined in two 3-month periods.

The plasma NGAL level was measured using the Cristal-Day-Biotech kit made in China. The method used for this measurement was the ELISA method (fluorescence de-tected-immunoassay). The 50% (or higher) increase in the maximum serum creatinine level after operation was considered to be an indicator of AKI as compared to the serum creatinine level before operation or the need for dialysis within 1 week after implant. Accordingly, patients were classified into the groups of patients with AKI and patients without AKI. The plasma NGAL level was measured and compared for the two groups at different time periods.

Research variable included age, gender, underlying causes of renal failure, pre-and post-operative plasma NGAL level (6 and 12 hours after surgery), daily serum creatinine level in the first week, ARF, implant rejection in the first week after implant, and mortality within one week after surgery.

**Ethical issues**

The research followed the tenets of the Declaration of Helsinki; all samples were participated after their parent's satisfaction. Participation in this study was voluntary and patients were thus free to withdraw from the study at any time without having any effect on their treatment process. This study was approved by the ethic committee of Zanjan University of Medical Science. The informed consent of all patients was obtained before including them in the study.

**Statistical analysis**

We used descriptive analytical test (Mean±SD), frequency and percentage for presenting descriptive data to compare qualitative data. Chi-square test or Fisher exact test was used. We also evaluated the normal distribution of laboratory data for studying their mean difference and due to their lack of normal distribution we used Mann-Whitney U test. For studying the relationship between plasma NGAL and serum creatinine, Pearson correlation test was used and then we used receiver operating charac-
teristic (ROC) curve for determining the cut off point for NGAL to diagnosing renal failure. In all cases, $P$ value less than 0.05 is considered significant.

**Results**

Of the patients under study, 22 (59.5%) were male and 15 were female (40.5%). The average age of patients at the time of renal implant was $34.93 \pm 14.97$ years (12-59 years).

Underlying causes of renal failure included obstruction (12 cases or 32.4%); hypertension (7 cases or 18.9%); diabetes mellitus (4 cases or 10.8%); infection (3 cases or 8.1%); focal segmental glomerulosclerosis (FSGS) (1 case or 2.7%); and polycystic kidney disease (1 case or 2.7%). None specific causes of renal failure was also observed in 9 patients (24.3%).

The average plasma NGAL level before implant, 6 hours after implant, and 12 hours after implant was $311.14 \pm 102.69$ ng/ml, $317.81 \pm 107.28$ ng/ml, and $312.16 \pm 134.80$ ng/ml, respectively. The plasma NGAL levels measured 6 ($P = 0.70$) and 12 ($P = 0.96$) hours after kidney transplantation were not significant as compared to the average plasma NGAL level before implant.

The average serum creatinine level that was measured daily during the first week after kidney transplantation was $5.33 \pm 2.71$ mg/dl, $2.72 \pm 1.62$ mg/dl, $1.97 \pm 1.24$ mg/dl, $1.83 \pm 1.21$ mg/dl, $1.61 \pm 0.68$ mg/dl, $1.64 \pm 1.15$ mg/dl and $1.77 \pm 1.08$ mg/dl on Saturday, Sunday, Monday, Tuesday, Wednesday, Thursday and Friday, respectively. In the course of the study, ARF, kidney transplant rejection and mortality were seen in 2 (5.4%), 7 (18.9%) and 1 (2.7%) patients, respectively.

The correlation of plasma NGAL levels measured 6 and 12 hour after renal implant with serum creatinine in the first week after surgery is shown in Table 1. In none of the study phases a significant statistical correlation between the variables was observed.

In the ARF group, 9 patients (75%) were male and 3 (25%) were female. In the non-ARF group, 13 patients (52%) were male and 12 (48%) were female ($P = 0.29$).

The average age of patients with ARF and patients without ARF was $39.90 \pm 12.66$ and $32.32 \pm 15.74$ years, respectively ($P = 0.20$).

The changes in serum creatinine level during the first week after implant in the patients with and without ARF are illustrated in Figure 2 ($P = 0.01$). The ROC curve for plasma NGAL level measured 6 and 12 hours after implant is shown in Figure 3 for prediction of ARF.

The area under the curve for NGAL levels measured 6 and 12 hours after implant was equal to 0.68 ($P = 0.08$) and 0.97 ($P < 0.001$), respectively. The NGAL level measured 6 hours after implant (cut off point [COP] > 309 ng/ml)

**Table 1.** Correlation between plasma level of NGAL at 6 and 12 hour after transplantation with plasma creatinine during the first postoperative day week

<table>
<thead>
<tr>
<th></th>
<th>NGAL 6 hours</th>
<th>NGAL 12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>1st Day</td>
<td>0.12</td>
<td>0.49</td>
</tr>
<tr>
<td>2nd Day</td>
<td>-0.05</td>
<td>0.79</td>
</tr>
<tr>
<td>3rd Day</td>
<td>-0.06</td>
<td>0.79</td>
</tr>
<tr>
<td>4th Day</td>
<td>-0.07</td>
<td>0.68</td>
</tr>
<tr>
<td>5th Day</td>
<td>-0.09</td>
<td>0.63</td>
</tr>
<tr>
<td>7th Day</td>
<td>0.09</td>
<td>0.59</td>
</tr>
<tr>
<td>8th Day</td>
<td>-0.01</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Abbreviation: NGAL, neutrophil gelatinase-associated lipocalin.

Figure 1. Changes of plasma NGAL at before and after transplantation between two groups with and without renal failure

Figure 2. Changes of plasma creatinine at before and after transplantation between two groups with and without renal failure
had a sensitivity of 66.7% and specificity of 64% in prediction of ARF. The NGAL level measured 12 hours after implant (COP >317 ng/ml) had a sensitivity of 100% and specificity of 92% in prediction of ARF. The ROC curve for serum creatinine level measured during the first week after implant is depicted in Figure 2 for prediction of ARF. Table 2 presents the area under the curve, sensitivity, specificity and COP of serum creatinine level on a daily basis for one week after the implants.

**Discussion**

ARF or delayed graft function is relatively common among the receivers of graft tissues. So far numerous studies have studied the effectiveness of different markers for prediction of this condition.

NGAL is known as a gene that undergoes rapid changes in the kidney following ischemic injuries. In fact, NGAL is a protein of the lipocalin family which grows through neutrophils and other epithelial cells (including aggregation proximal tubules chains). According to the results of previous research, NGAL is a very useful renal biomarker for the early diagnosis of ARF in children and adults undergoing renal implant and cardiac surgery (20,21).

In this research, it was tried to determine the role of plasma NGAL in prediction of ARF during the first week of surgery. According to the findings of this study, the average plasma NGAL level 6 and 12 hours after implant was significantly higher in the ARF group as compared to the group with normal graft tissue performance.

Results of this study comply with the results of other studies on patients receiving renal implants and patients with other clinical conditions such as patients undergoing cardiac surgery (22) or severely sick patients kept in intensive care unit (ICU) (23).

In the study by Kusaka et al (24) the serum NGAL level helped predict the failure of the graft tissue in a short white after renal implant. Accordingly, in the study by Lebkowska et al (20) the precision of serum NGAL in prediction of post-transplant ARF was studied in 41 patients. The serum NGAL level before and after renal transplant was measured in days 1, 3, 6 and 10.

In this research, a significant decline was observed in serum NGAL level on the first day after implant. In all time periods a significant correlation between serum NGAL and creatinine levels was observed. However, the serum level of NGAL did not decline considerably in patients with delayed graft function. Accordingly, it is recommended to use this serum variable especially as an early indicator (predictor) for prediction of renal graft function.

It is worth mentioning that, unlike the results of the above study, no significant correlation between plasma NGAL level and serum creatinine levels after renal transplantation was observed. This can be ascribed to the early diagnostic capability of NGAL as compared to creatinine.

Malyszko et al (25) studied the relationship between serum creatinine level and estimated glomerular filtration rate in 90 patients who received renal implant. They stated that serum NGAL can be used as an early sensitive marker for prediction of renal graft function.

In the study by Mahdavi-Mazdeh et al (16), serum NGAL level was measured in 52 patients with renal transplant in 0, 2, 4, 12, and 18 hours after transplantation. They detected that serum NGAL level is significantly correlated to serum creatinine level especially 2 hours after implantation. Hence, this parameter can be used as a predictor for prediction of renal graft function.

In another study by Mahdavi-Mazdeh et al (26) the serum NGAL and creatinine levels were measured to predict post-graft renal recovery. It was found out that the serum creatinine and NGAL levels were higher in patients with delayed graft function (6 patients) and slow graft function (9 patients) as compared to patients with immediate graft function. However, the difference was only statistically significant in the case of NGAL. Results of the ROC-based investigations showed that the highest under the curve area belonged to NGAL measured 24 hours after the implantation (0.82).

In the present study, the diagnostic power of serum NGAL level was measured using ROC curves. The area under the

![Figure 3. ROC curve of plasma NGAL at after transplantation to predict of ARF.](http://journalrip.com)

**Table 2. Area under the curve and the prediction of creatinine for ARF during the first postoperative day week**

<table>
<thead>
<tr>
<th>Area under the curve</th>
<th>P</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cut of Point (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Day</td>
<td>0.70</td>
<td>0.09</td>
<td>66.7</td>
<td>61.9</td>
</tr>
<tr>
<td>2nd Day</td>
<td>0.73</td>
<td>0.05</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>3rd Day</td>
<td>0.76</td>
<td>0.03</td>
<td>66.7</td>
<td>76.1</td>
</tr>
<tr>
<td>4th Day</td>
<td>0.79</td>
<td>0.01</td>
<td>77.8</td>
<td>81</td>
</tr>
<tr>
<td>5th Day</td>
<td>0.77</td>
<td>0.02</td>
<td>88.9</td>
<td>81</td>
</tr>
<tr>
<td>7th Day</td>
<td>0.83</td>
<td>0.004</td>
<td>88.9</td>
<td>81</td>
</tr>
<tr>
<td>8th Day</td>
<td>0.82</td>
<td>0.01</td>
<td>77.8</td>
<td>85.7</td>
</tr>
</tbody>
</table>

Abbreviation: ARF, acute renal failure.
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**Authors’ contribution**

All authors contributed equally to the paper.

**Conflicts of interest**

The authors declare that they have no conflicting interest.

**Ethical considerations**

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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**References**


NGAL in acute renal failure after kidney transplantation


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