Original article:

Assessment of serum neutrophil gelatinase-associated lipocalin in kidney diseases and its relation to age

"Serum neutrophil gelatinase-associated lipocalin in kidney diseases and Aging"
Ghada Abd ELtawab I Radwan1*, Ali Elsaid Yousef2, Mohamed Fathy Farag Bayomy1,3
Zoology Department, Faculty of Science, Menufiya University, Egypt; Internal Medicine, Rheumatology and Immunology Department, Teaching Benha Hospital, Egypt; General Biology Deptartment, Center of Basic Sciences, Misr University for Science and Technology, 6th of October City, Egypt. *Correspondence to: ghadaabdeltawabradwan@gmail.com
DOI: 10.21608/RRGG.2023.233419.1013. Received: 03 August 2023; accepted: 15 September 2023; published 17 October 2023

Abstract

Neutrophil gelatinase associated lipocalin (NGAL) is a 25 kDa protein of the lipocalin family. This article's goal is to investigate the role of serum NGAL in kidney illnesses such as acute kidney injury and chronic renal failure, as well as how aging affects NGAL levels. Participants (n=300) in the present study were classified into 3 groups. **Group I**: Normal healthy control participants (n=100). **Group II**: Acute kidney damage (AKI) patients (n==100). **Group III**: Chronic renal failure (CRF) patients (n=100). Those groups further divided into subgroups based on age and gender. Blood samples were collected from patients for laboratory investigation as blood urea, serum creatinine, sodium, potassium, blood Hb, GFR, pH, PCO2 and serum NGAL. Demonstrate that regardless of gender, the levels of serum NGAL were considerably more significant in patients with CRF than in those with AKI, and that the levels of NGAL are correlated with age. The levels of NGAL in CRF and AKI of males groups were (462 ±31.9, 652 ± 83) and (330 ± 45.30, 534 ± 61.1) ng/ ml respectively and in females groups were (668 ± 68.3, 730 ± 75.5) and (380 ±70.4, 466 ± 48.8) ng/ml respectively. **Conclusions**: Serum NGAL may be a valid marker to differentiate between AKI and CRF patients and its level is influenced by age.

**Keywords**: NGAL; acute kidney injury; chronic renal failure; aging
1. Introduction

Acute kidney injury (AKI) is a common complication in a number of therapeutic settings, such as major operations (Zhou et al., 2016), Emergency department (Nicholas et al., 2008), and Intensive Care Units (ICU) Constantin et al.,2010). Poorer outcomes are frequently experienced after AKI, including lengthened ICU and hospital stays, the requirement for dialysis, a decline in glomerular filtration rate (GFR), the onset of chronic kidney disease (CKD), and an increase in mortality (Fabrizi et al.,2010; Chertow et al., 2005). AKI poses a threat to the elderly. Aged kidneys' decreased GFR and nephron count could make them more susceptible to AKI Stumlechner et al., 2016).

CRF is a condition involving a decrease in the kidney’s ability to filter waste and fluid from the blood. It is chronic, meaning that the condition develops over a long period of time and is not reversible. This condition is also commonly known as CKD. One of Egypt's most significant health issues is CKD. The prevalence of CKD in adult Egyptians is about 13%, which has a substantial impact on morbidity, death, and healthcare expenditures. Compared to previous stages of CKD, patients with stage 3 or stage 4 CKD have a higher rate of cardiovascular events and death (Yamang et al., 2014). End Stage Renal Disease affects approximately 74 per million in Egypt annually, and there are 264 per million who receive dialysis overall (El-Arbagy et al., 2016).

Even when creatinine levels are not yet raised, novel renal biomarkers have the ability to identify modest harm to glomeruli and tubules in response to diverse insults (Devarajan, 2008). NGAL is a protein that is produced by activated neutrophils of proximal tubule, covalently bound to neutrophil gelatinase (Malyszko, 2010). Additional research revealed that neutrophils create the monomeric version of the urine molecule, while kidney tubular epithelial cells produce the dimeric form (Cai et al., 2010). This difference has the potential to improve the specificity of NGAL as a renal biomarker. Although epithelial cells can release NGAL, their expression level is typically low (Cowland et al., 2003). However, it can be significantly increased in pathological circumstances. It can be detected in both urine and serum (Soni et al., 2010). Therefore, the purpose of this research was to examine the usefulness of serum NGAL in identifying AKI from CRF as well as the relationship between age and serum NGAL level under these conditions.

2. Materials and methods
2.1 Study design

There were 300 participants in this study, and they were divided into three groups. Group I: Normal healthy people (n= 100), who served as control group, that are divided according to age and gender. The control group consists of
participants who were not suffering from any illnesses, such as diabetes, active infections, chronic metabolic problems, or cancer. Group II consists of 100 individuals who suffer from AKI according to The Kidney Disease Improving Global Outcomes (KDIGO) criteria and is further separated into males group GPIIa and females group GPIIb, first GPIIa consists of 50 males participants and is then subdivided according to age. Patients in GPIIa1 were between the ages of 25 and 35, and those in GPIIa2 were between the ages of 45 and 60. GPIIb consists of 50 females participants and is then subdivided according to age into GPIIb1 (age range 25-35 years), and GPIIb2 (age range 45-60 years). Patients with AKI who showed distinguishing symptoms including severe metabolic acidosis (pH < 7.1), hyperkalemia (potassium >6.8 mEq/L), or serum creatinine levels above 8 mg/dl were excluded from the AKI group. Patients describing anuria or an abrupt decrease in urine production and individuals with no known CRF are included.

Group III: 100 individuals with chronic renal failure (hemodialyzed patients) are included. Exclusion criteria were: presence malignancy, liver, or infectious diseases. It is further divided into GPIIIa, which has 50 males patients and two subgroups. Patients between the ages of (25-35) and (45-60) are included in GPIIIa1 and GPIIIa2, respectively. GPIIIb: It has 50 females participants and is separated into two subgroups. Patients in GPIIIb1 between the ages of 25 and 35 and those in GPIIIb2 between the ages of 45 and 60 are included. Patients were selected from Internal Medicine and Intensive Care Unit and Artificial Kidney Unit of Benha Teaching Hospital, Egypt. They were selected during the period between June 2019 and June 2020. Written consent was taken from the included individuals, and the study was approved by the local ethical committee of the hospital. All patients and controls were subjected to full history taking and laboratory investigation including assessment of blood urea, serum creatinine, sodium, potassium, blood Hb, GFR, pH, PCO2 and serum NGAL.

AKI was diagnosed by an increase in serum creatinine by 0.3 mg/dl or more over a period of 48h or less (Mehta et al., 2007). CKD defined using Kidney Foundation Disease Outcomes Quality Initiative Criteria as kidney damage or a glomerular filtration rate <60 ml/min/1.73 m² for ≥3 months, with or without kidney damage, which is defined as structural or functional abnormalities with or without decreased glomerular filtration rate, pathological abnormalities, markers of kidney damage, or abnormalities in imaging tests (NKF, 2002).

2.2 Samples collections and storage

Blood samples of AKI patients were collected from ICU, Department of Internal Medicine and blood samples of CRF patients were collected from Artificial Kidney Unit and were
Blood samples were allowed to clot 10-20 minutes at room temperature and centrifuged at 2000-3000 RPM for 20 minutes and sera were collected in Eppendorfs stored at -20°C or -80°C. Repeated freeze-thaw cycles were avoided.

2.3 Methods of estimation

Estimation of serum urea, sodium, potassium, and creatinine using Vitros ECIQ chemical auto analyzer made in USA. Measurment of blood Hb by auto hematology analyzer device Celltac Alpha NIHON KOHDEN made in Japan. Estimation of GFR by using (MDRD) equation. Estimation of $p$H and PCO2 value by taking arterial blood samples in heparinized blood-gas syringes and using GEM Premier 3000 blood gas analyzer for measurement made in Italy. Estimation of serum NGAL was done by using ELISA technique by Tecan sunrise reader made in Austria. NGAL ELISA kit: Cat. No: SEB388HU manufactured by Cloud-Clone Corp. 1304 Iangham Creek Dr, Suite 226, Houston, TX 77084, U.

2.4 Method of estimation of serum NGAL

Serum NGAL was measured by enzyme-linked immunosorbent assay (ELISA) technique. All reagents were prepared before starting the assay procedure. Aliquots of 50ul each of NGAL standards were added to standards wells. 10ul of the testing sample was added to 40ul of sample diluent to testing sample well; blank well left empty. A 100ul of HRP-conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60 minutes at 37°C. Each well was aspirated and washed with wash solution. The process was repeated for five times. (50ul) of chromogen solution A and chromogen solution B were added to each well. Inclusions were gently mixed and incubated for 15 minutes at 37°C and protected from light. 50ul of stop solution was added to each well. Optical density (O.D.) was read at 450 nm using a microtiter plate reader within 15 minutes.

2.5 Statistics

The statistical analysis was performed using Minitab statistics version 17. Values were expressed by mean ± standard deviation. One-way ANOVA and t-test analyses were used to compare between different study groups. $P$-value < 0.05 was considered statistically significant and $P$-value < 0.01 was considered statistically highly significant.

3. Results

Regardless of gender, the observed data demonstrate a considerable increase in serum NGAL levels in the CRF groups more than the AKI groups, in all studied groups. Our results show substantial variation in serum NGAL levels of males patients of AKI group GPIIa¹ and CRF group GPIIIa¹ $P$< 0.01, serum NGAL levels were (330 ± 45.3) ng/ml and (462 ±31.9) ng/ml respectively, the results represented by Table 1 and Figure 1.
The recorded data demonstrate a significant difference in serum NGAL levels in males individuals of GPIIa² and GPIIIa² $P<0.05$, serum NGAL levels were $(534\pm61.1)$ ng/ml and $(652\pm83)$ ng/ml, as shown in Table 2 and Figure 2.

In table 3 the females patients show significant variation in serum NGAL levels of GPIIb¹ and GPIIIb¹ $P<0.01$, The values of serum NGAL were $(380\pm70.4)$ ng/ml , $(668\pm68.3)$ ng/ml and the values represented by figure 3.

A significant elevations in serum NGAL levels are shown in Table 4 for females individuals of GPIIb² and GPIIIb² $P<0.01$, the serum NGAL levels were $(466\pm48.8)$ ng/ml and $(730\pm75.5)$ ng/ml, the data explained by figure 4.

All examined groups' levels of NGAL in AKI and CRF had considerably risen as compared to their control groups, $P<0.01$.

The results of the current study show that serum NGAL levels significantly rise with aging at $P<0.01$ for males groups and $P<0.05$ for females groups.

The mean values of blood Hb level show a high significant difference in all examined groups, $P<0.01$; similarly, the mean values of serum urea and serum creatinine show high significant variation in all studied groups in comparison to their control groups.

In addition, the mean values of $pH$ and GFR in comparison to their control groups both exhibit highly significant differences, $P<0.01$, in all studied groups.

There is a significant decrease in PCO2 levels in the studied groups in compared to controls groups. When compared to the controls, there is an imbalance in the levels of sodium and potassium.

**Table (1) comparison between AKI and CRF of males patients their age (25-35) years**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AKI GPIIa¹</th>
<th>CRF GPIIIa¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>8.4 ± 1.14</td>
<td>83 ± 4.8</td>
<td>88.2 ± 5.81</td>
<td>0.061</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.68 ± 0.13</td>
<td>3.8 ± 0.5</td>
<td>5.12 ± 0.47</td>
<td>0.004</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.56 ± 0.51</td>
<td>9.82 ± 0.88</td>
<td>9.34 ± 0.54</td>
<td>0.238</td>
</tr>
<tr>
<td>PCO₂ mmHg</td>
<td>37.0 ± 1.58</td>
<td>33.2 ± 8.23</td>
<td>32.4 ± 6.43</td>
<td>0.417</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.03</td>
<td>7.26 ±0.09</td>
<td>7.17 ± 0.07</td>
<td>0.298</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>139 ± 1.58</td>
<td>135 ± 5.95</td>
<td>130.2 ± 4.97</td>
<td>0.154</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.66 ± 0.55</td>
<td>4.46 ± 0.89</td>
<td>4.64 ± 0.63</td>
<td>0.424</td>
</tr>
<tr>
<td>GFR, ml/min/1.73 m²</td>
<td>124.2 ± 13</td>
<td>9.6 ± 1.14</td>
<td>7.6 ± 1.81</td>
<td>0.093</td>
</tr>
<tr>
<td>Serum NGAL, ng/ml</td>
<td>42.60 ± 6.58</td>
<td>330 ± 45.30</td>
<td>462 ± 31.9</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation. Significant $P<0.05$, highly significant $P<0.01$. 

35
Table (2) comparison between AKI and CRF of males patients their age (45-60) years

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AKI GPIIa²</th>
<th>CRF GPIIIa²</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>8.6 ± 2.41</td>
<td>81 ± 5.66</td>
<td>85 ± 9.06</td>
<td>0.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.72 ± 0.13</td>
<td>3.14 ± 0.05</td>
<td>5.24 ± 0.55</td>
<td>0.004</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.86 ± 0.57</td>
<td>9.78 ± 0.75</td>
<td>8.82 ± 0.46</td>
<td>0.05</td>
</tr>
<tr>
<td>PCO₂ mmHg</td>
<td>40.43 ± 2.99</td>
<td>32.0 ± 10.41</td>
<td>30.43 ± 5.26</td>
<td>0.364</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.045</td>
<td>7.25 ± 0.03</td>
<td>7.18 ± 0.08</td>
<td>0.399</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>140.86 ± 3.13</td>
<td>135 ± 5.74</td>
<td>133.71 ± 4.5</td>
<td>0.258</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.95 ± 0.36</td>
<td>4.37 ± 0.61</td>
<td>4.47 ± 0.83</td>
<td>0.352</td>
</tr>
<tr>
<td>GFR, ml/min/1.73 m²</td>
<td>129.6 ± 7.67</td>
<td>12.6 ± 1.94</td>
<td>10.4 ± 1.14</td>
<td>0.084</td>
</tr>
<tr>
<td>Serum NGAL, ng/ml</td>
<td>40.40 ± 3.21</td>
<td>534 ± 61.1</td>
<td>652 ± 83</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation. Significant P< 0.05, highly significant P< 0.01

Table (3) comparison between AKI and CRF of females patients their age (25-35) years

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AKI GPIIIb</th>
<th>CRF GPIIIb¹</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>11.2 ± 1.92</td>
<td>93.8 ± 4.49</td>
<td>97.4 ± 6.62</td>
<td>0.168</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.72 ± 0.13</td>
<td>2.82 ± 1.83</td>
<td>4.66 ± 0.34</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.52 ± 0.415</td>
<td>9.7 ± 0.57</td>
<td>9.32 ± 0.63</td>
<td>0.253</td>
</tr>
<tr>
<td>PCO₂ mmHg</td>
<td>38.71 ± 2.29</td>
<td>30.14 ± 1.52</td>
<td>33.29 ± 5.53</td>
<td>0.241</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.04</td>
<td>7.27 ± 0.08</td>
<td>7.2 ± 0.09</td>
<td>0.399</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>140 ± 2.58</td>
<td>134 ± 6.05</td>
<td>131 ± 5.06</td>
<td>0.166</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.38 ± 0.67</td>
<td>4.45 ± 0.89</td>
<td>4.41 ± 0.64</td>
<td>0.506</td>
</tr>
<tr>
<td>GFR, ml/min/1.73 m²</td>
<td>134 ± 9.83</td>
<td>17 ± 1.58</td>
<td>15 ± 2.24</td>
<td>0.012</td>
</tr>
<tr>
<td>Serum NGAL, ng/ml</td>
<td>40.00 ± 4.12</td>
<td>380 ± 70.4</td>
<td>668 ± 68.3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation. significant P< 0.05 highly, significant P< 0.01
Table (4) comparison between AKI and CRF of females patients their age (45-60) years

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>AKI GPIIb²</th>
<th>CRF GPIIIb²</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>11.2 ± 1.92</td>
<td>90.2 ± 5.81</td>
<td>91.2 ± 12.79</td>
<td>0.427</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.72 ± 0.13</td>
<td>2.81 ± 0.3</td>
<td>5.00 ± 0.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.52 ± 0.415</td>
<td>9.16 ± 0.439</td>
<td>9.46 ± 0.44</td>
<td>0.234</td>
</tr>
<tr>
<td>PCO₂ mmHg</td>
<td>38 ± 2.16</td>
<td>30.43 ± 7.89</td>
<td>30.43 ± 5.97</td>
<td>0.507</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.04</td>
<td>7.28 ± 0.08</td>
<td>7.24 ± 0.08</td>
<td>0.533</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>138 ± 3.35</td>
<td>138.29 ± 4.07</td>
<td>138.86 ± 3.58</td>
<td>0.414</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.1 ± 0.21</td>
<td>5.12 ± 0.67</td>
<td>5.05 ± 0.41</td>
<td>0.475</td>
</tr>
<tr>
<td>GFR, ml/min/1.73 m²</td>
<td>134.2 ± 9.83</td>
<td>14 ± 2.74</td>
<td>12.8 ± 1.64</td>
<td>0.011</td>
</tr>
<tr>
<td>Serum NGAL, ng/ml</td>
<td>40.2 ± 4.44</td>
<td>466 ± 48.8</td>
<td>730 ± 75.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± standard deviation. Significant P< 0.05, highly significant P< 0.01.

Fig. 1: The mean values: The upper and lower whiskers are the maximum and minimum values.
Fig. 2: The mean values, The upper and lower whiskers are the maximum and minimum values

Fig. 3: The mean values, The upper and lower whiskers are the maximum and minimum values
4. Discussion

Numerous studies have demonstrated that NGAL is produced in renal tissue as a result of ischemia, nephrotoxic, or septic kidney injury (Macdonlad et al., 2017). Biomarkers in both AKI and CKD look for similar effects of the diseases, decrease in nephron number, vascular insufficiency, and cell cycle disruption (Wasung et al., 2015). The results of the current study demonstrate a substantial rise in serum NGAL levels in the CRF groups more than in the AKI groups, which may be consistent with the findings of Ozkan et al., (2014), who demonstrated that plasma NGAL concentrations were higher in CRF patients than in AKI patients. CRF is defined as a progressive and irreversible loss of renal function brought on by nephron loss over time since it is manifested by steadily declining glomerular filtration values (acobson, 1991). Any drop in GFR would result in a reduction in the renal clearance of NGAL, which would then accumulate in patients with CKD’s systemic circulation. Additionally, given that both acute and long-term haemodialysis therapies are significant stimuli for neutrophil degranulation; this may be a significant additional source of elevated NGAL (Horl, 2002).

There is a significant variation in the concentration of serum NGAL in the AKI groups (GPII) regardless of sex, \( P<0.01 \) when compared with their healthy control groups in our study.
According to Parikh et al.’s study (2011), which demonstrated that the elevation of NGAL is detectable as early as 3 h after renal injury and that it peaks at roughly 6–12 h after injury depending on the severity of the injury? Acute tubular damage and the release of NGAL by neutrophils, macrophages, and other immune cells as an acute phase reactant are the causes of the NGAL rise in acute renal failure (Deut et al., 2007).

Regardless of sex, the observed data in this study demonstrate a high level of considerably elevated serum NGAL levels in the CRF groups (GPIII) $P<0.01$, which may be consistent with the findings of Ezenwaka et al.’s study (Ezenwaka et al., 2016) that CKD patients had significantly elevated levels of both serum and urinary NGAL. Also correlate with Mori and Nakao (2007) who reported that the increase of NGAL in CKD might be the consequence of reduced renal clearance and/or sustained production by "burning" tubular cells reflecting active kidney damage ("forest fire hypothesis").

The results of the current investigation indicate a substantial increase in serum NGAL levels with aging $P<0.01$ in males groups and $P<0.05$ in females groups, which is consistent with the findings of Chawla et al. (2014) renal aging plays a vital part in AKI and CRF, which are established to be related syndromes. It has been shown that both AKI and CRF raise the risk of end-stage renal disease (ESRD) in the elderly, and the prevalence increases even more after age 50. Also Naude et al. (2013) found that plasma NGAL concentrations were significantly associated with age. The observed data in this investigation demonstrate a highly significant difference in GFR levels across all analyzed groups ($P<0.01$), and serum NGAL levels are inversely connected with GFR, which may be consistent with Bolignano et al.’s work. (Bolignano et al., 2009) who proved increased serum and urinary NGAL levels in CKD patients with decreased GFR.

The findings indicate a highly significant variation in blood hemoglobin levels across all groups $P<0.01$, which is consistent with research by Devireddy et al., (2001) who established that NGAL plays a crucial role in anemia by causing apoptosis and inhibiting the development of erythroid progenitor cells in vitro. According to recent reports, NGAL may be suggested as a novel method for evaluating iron therapy for hemodialysis patients (Bolignano et al., 2009a). Hopefully, plasma NGAL will replace serum ferritin as a more helpful test for assessing iron status in CKD patients, especially those who require renal replacement therapy (Tomasz et al., 2001).

Additionally, all analyzed groups' pH levels varied significantly ($P<0.01$), and this may be related to the investigation of Viswanathan et al., (2013) metabolic acidosis usually occurs when GFR falls to 20–30 ml/min.
The levels of PCO2 are low compared to controls groups in the studied groups and several studies showed an association of low CO2 with worsening renal function (Debre et al., 2013).

5. Conclusion

The level of serum NGAL correlates with age and may be a useful marker to distinguish between patients with acute kidney injury and those with chronic renal failure.

6. Limitations:

Single Centre study with a relatively small number of patients’ samples.

7. Abbreviation

NGAL: neutrophil gelatinase associated lipocalin
AKI: acute kidney injury
CKD: chronic kidney disease
ICU: intensive care unit
GFR: glomerular filtration rate
ELISA: enzyme linked immunosorbent assay

8. References

338(8764):419–421.


