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Research Article

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NGAL as a biomarker to predict and diagnose Acute Kidney Injury (AKI)

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ABSTRUCT

Neutrophil gelatinase associated lipocalin (NGAL) (also known as Lipocalin 2 or Lcn2) is a small molecule protein (25 Kd) and belongs to the family of the Lipocalins that transport small hydrophobic materials. Studies have shown the benefit of NGAL in predict and diagnose the acute renal injury after 2-3 hours of its occurrence compared with creatinine who needs 2-3 days to rise. Serum levels of NGAL, creatinine and urea were measured in 44 patients divided into patients group with acute renal injury and patients group whose samples were collected after two hours and 3 days from the occurrence of the acute injury. We find high levels of NGAL in patient groups compared with the control group. We also find high levels of NGAL in patients whose sample was collected after two hours of the acute injury while the values of creatinine and urea is within the normal range. However, the three parameters increased in samples taken after three days. The study found that levels of serum NGAL rise in acute renal injury and thus NGAL level can be used as an early biomarker to predict acute renal injury and diagnose it before the elevation of creatinine and urea levels.

Keywords: acute kidney injury; acute renal failure; biomarkers; lipocalin; neutrophil gelatinase associated lipocalin; creatinine; urea.

INTRODUCTION

Acute kidney injury (AKI) refers to a common syndrome that results from multiple causative factors and occurs in a variety of clinical settings, with varied clinical manifestations, ranging from a minimal elevation in serum creatinine to anuric renal failure. AKI is characterized functionally by a rapid decline in the glomerular filtration rate (GFR), and biochemically by the resultant accumulation of nitrogenous wastes such as blood-urea nitrogen and creatinine.

The term AKI has largely replaced acute renal failure, since the latter designation overemphasizes the failure of kidney function and fails to account for the diverse molecular, biochemical and structural processes that characterize the AKI syndrome (1)

Renal failure causes deficiency in the production of waste containing nitrogen, which is usually measured by urea causing an elevation in the concentration of urea in the serum or the so-called Uraemia. Uraemia classified into:

- Prerenal.
- Renal.
- Postrenal (2).

In recent years, several biomarkers was developed to investigate acute renal failure quickly and specifically, one of which is the Neutrophil gelatinase associated lipocalin(NGAL) which is considered as the most important(3.4).

Human NGAL was originally identified as a novel protein isolated from secondary granules of human neutrophils (5), and was subsequently demonstrated to be a 25 kDa protein covalently bound to neutrophil gelatinase (6). Mature peripheral neutrophils lack NGAL mRNA expression, and NGAL protein is synthesized at the early-myelocyte stage of granulopoies is during formation of secondary granules. NGAL mRNA is normally expressed in a variety of adult human tissues, including bone marrow, uterus, prostate, salivary gland, stomach, colon, trachea, lung, liver and kidney (7). Several of these tissues are prone to exposure to micro-organisms, and constitutively express the NGAL protein at low levels. The promoter region of the *NGAL* gene contains binding sites for a number of transcription factors, including nuclear factor (NF)-κB(7).

It is composed of 8 β-strands that form a β-barrel enclosing a calyx which binds and transports low-molecular-weight substances (8).

Studies in mouse models of renal ischemia reperfusion showed NGAL production to be highly up-regulated at an early stage, and NGAL was detected in the urine within 2 hr following ischemia (9).

The physiological role of NGAL in this setting may be to decrease injury by reducing apoptosis and increasing the normal proliferation of kidney tubule cells. NGAL may also enhance delivery of iron and cause up-regulation of heme oxygenase-1, further protecting kidney tubule cells (9, 10-13). There is the intriguing possibility of using NGAL as a renoprotective agent in acute ischemic renal injury.

EXPERIMENTAL SECTION

Acute renal injury patient group:
The study included 39 patients(12 male+4 females) between the age ranged 33-69.
patients were divided, depending on the cause of acute renal injury, in to the following groups Figure 1:
1)acute renal injury caused by different reasons.
2)acute renal injury caused by heart reasons.
3)acute renal injury caused by dehydration and hypotension.

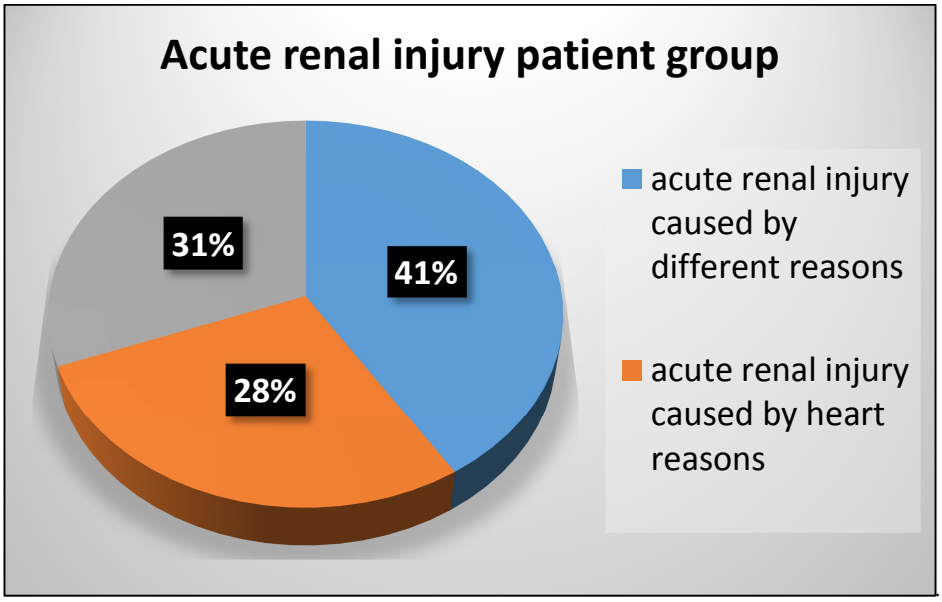


Figure 1: Acute renal injury patient group

acute renal injury caused by different reasons patient group:
This group included 16 patients (12 male +4 female) age ranged from 33-60.

patients were divided, depending on the cause of acute renal injury, in to the following groups Figure 2:
1) acute renal injury due to a given shady substance(2 patients).
2) acute renal injury due to the dissolution of the muscles (3 patients).
3) acute renal injury due to pressure and diabetes (2 patients).
4) acute renal injury due to infection (2 patients).
5) acute renal injury due to medication (2 patients).
6) acute renal injury after surgery (2 patients).
7) Nephrose patient group(1 patient).
8) acute renal injury because of a kidney transplant (1 patient).

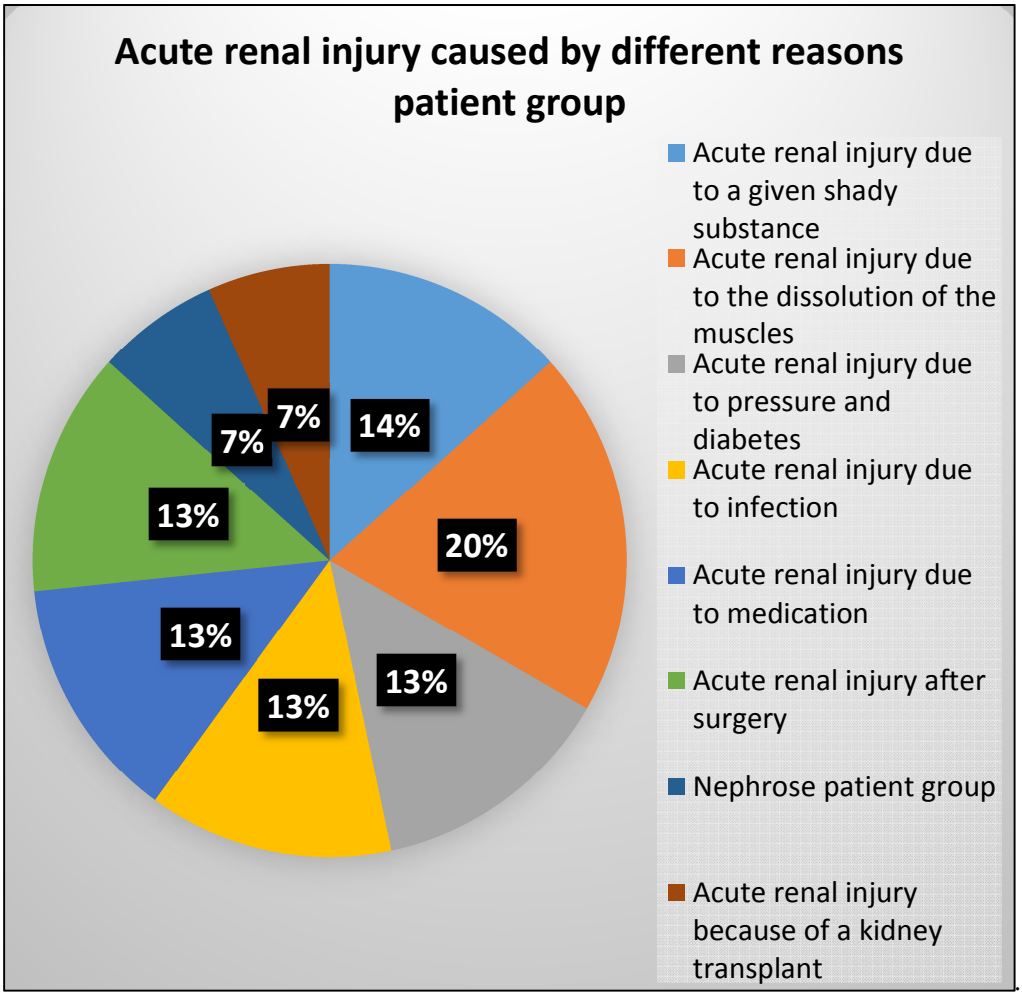


Figure 2: acute renal injury caused by different reasons patient group

acute renal injury caused by heart reasons patient group:
This group included11patients(6 male+5 female) age ranged from 50-69, with an average age of 60.6.
acute renal injury caused by dehydration and hypotension patient group:
This group included12 patients (6 male+6 female)age ranged from 35-66.

patient group who were highly predicted to develop an acute renal injury for any reason:
This group included five male patients aged between 45-55.
I took samples from patients before (2 hours) and after (3 days) the elevation of serum creatinine and urea values.

Control group:
This group included 20 healthy individuals(15 male+ 5 females), who do not suffer from kidney disease, age between23-61years.
the following markers assay were done:
• NGAL: using Human NGAL ELISA kit manufactured by SunRed company CAT. NO. 201-12-1720.
•creatinine: using kit manufactured by Spin react company.
•urea: using kit manufactured by Spin react company.
•Sugar: using kit manufactured by Bio System company.

Statistic analysis:
We used the Excel 2007 and SPSS18 programs for statistical study.
values were defined by using the mean ± standard deviation (Standard deviation).

RESULTS AND DISCUSSION

acute renal injury patient group:
Our study showed that the values of creatinine, urea and NGAL increased dramatically in each of the three causes for acute renal injury, especially for NGAL protein, it recorded the highest value in the case of cardiovascular diseases 1609ng/ml then in the case of different reasons 1556ng/ml and finally the case of dehydration and hypotension1443ng/ml and in all three cases was higher than the normal rate of the NGAL protein for the control group of 447.5ng/ml.

The average concentration of creatinine levels were relatively close between the three groups for acute renal injury, it was higher than the normal average 0.83 mg/dL. The average concentration of urea levels were the highest level in the case of acute renal injury caused by different reasons, and in the case of acute renal injury caused by dehydration and hypotension and the lowest was in the case of acute renal injury caused by cardiac causes, but all of them were also higher than normal levels of urea 24.2mg/dL Figure 3.

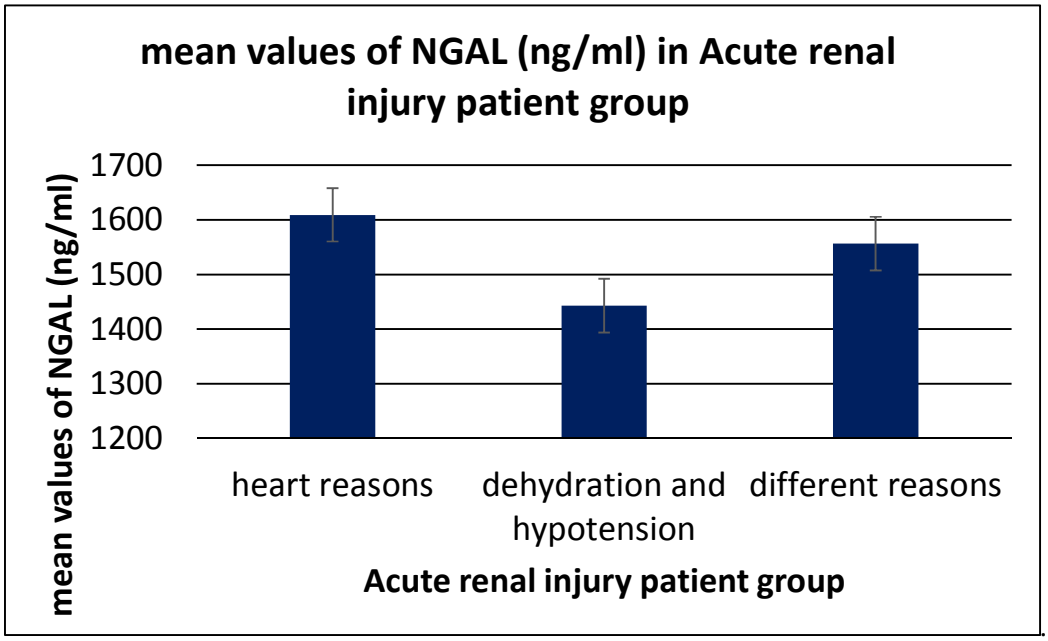


Figure 3: acute renal injury patient group

Based on the above, we can consider that NGAL is strong indicator for acute renal injury, especially among patients with heart disease. This result agreed with the results of Damman study and his colleagues in 2008 (14) and Shrestha study and his colleagues in 2011 (15).

acute renal injury caused by different reasons:

Our study showed that the values of creatinine, urea and NGAL elevated dramatically in all causes of acute renal injury, especially for NGAL which was very highin the case of medication therapy and In the case of shady substance. However, the glucose concentration did not record a significant increase, compared to the natural limits measured from the control group Figure 4.

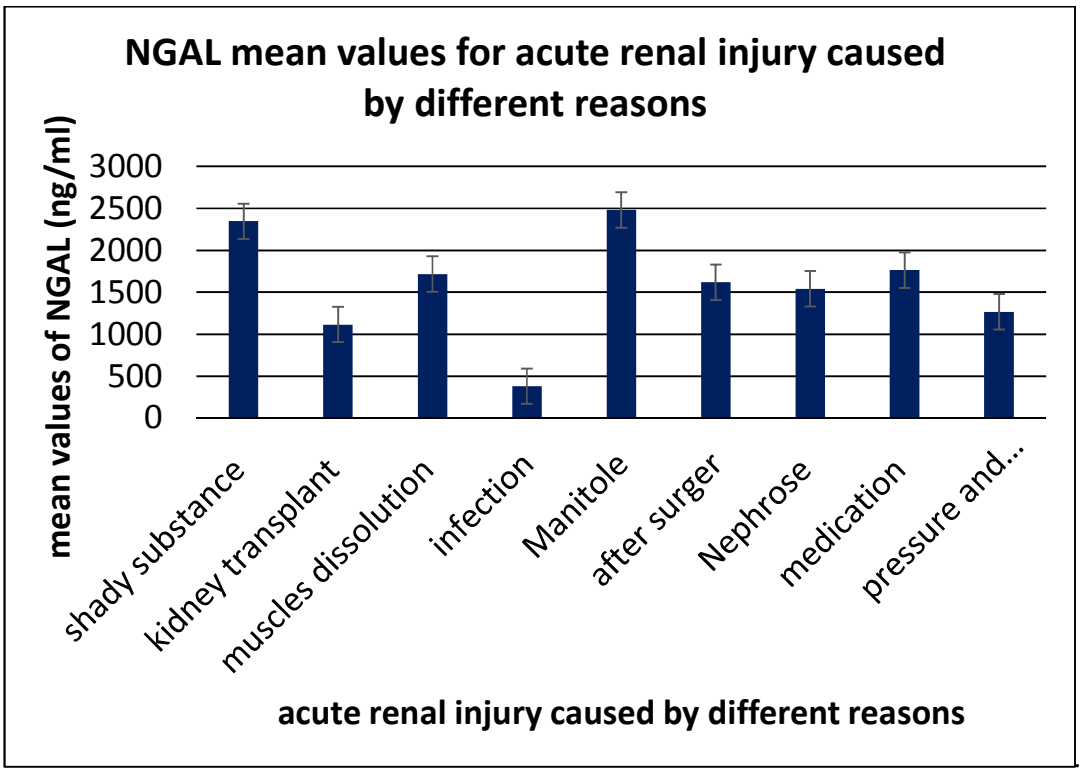
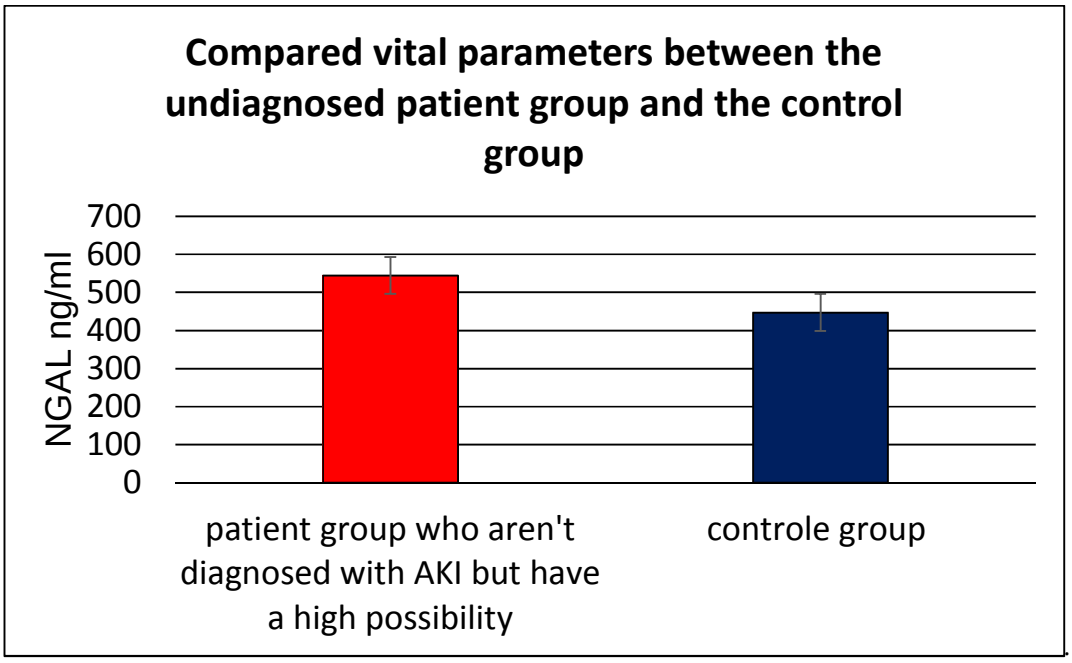


Figure 4: acute renal injury caused by different reasons



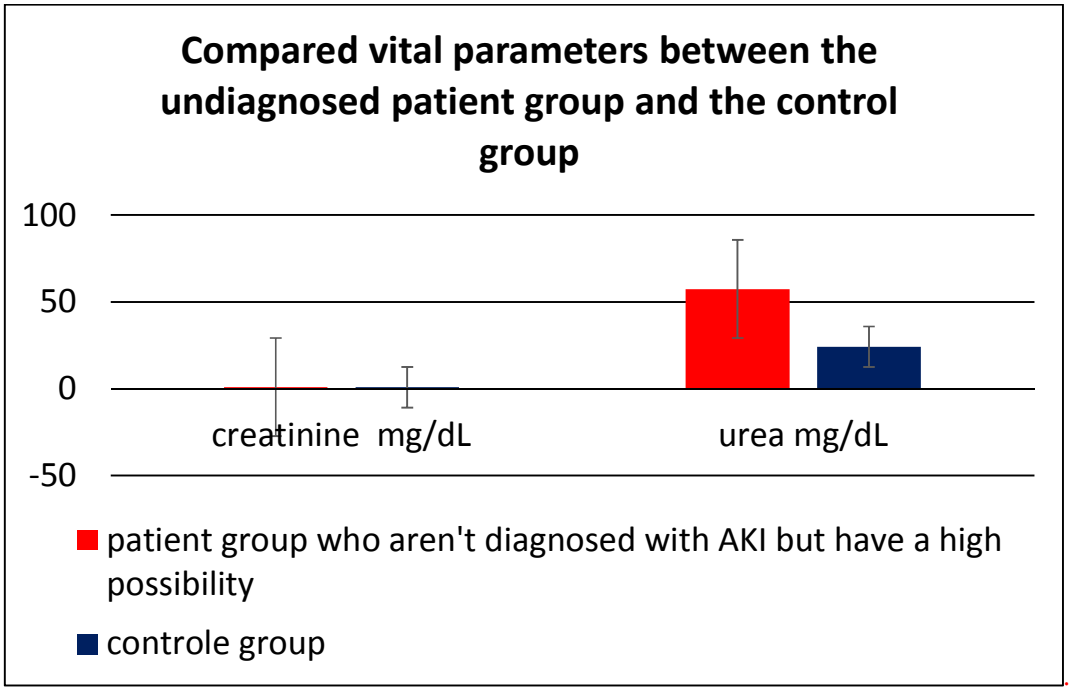


Figure 5: before creatinine and urea levels elevation

The results agreed with the results of Rahman and his colleagues in 2012(16). That can be explained by the fact that drugs and shady substances cause damage for renal cells which cause the increase in the concentration of NGAL.

Patient group who were highly predicted to develop an acute renal injury for any reason:
Before creatinine and urea levels elevation:
Creatinine and urea levels were within normal limits, but we find that the NGAL protein increased significantly compared to the normal value, and that assist its role as a predictive indicator in the event of a high risk of acute renal injury Figure 5, 7.

After creatinine and urea levels elevation:
Creatinine, urea and the NGAL increased dramatically more than normal values demonstrating the incidence of acute renal injury, and this confirms that the high NGAL protein level is an early biomarker to predict acute renal injury Figure 6, 7.

Our study also showed that the levels of NGAL elevated after two hours of the renal injury while creatinine and urea levels remained within normal limits, but it increased after 3 days from the occurrence of the injury and this confirms the importance of the NGAL as an early predicting biomarker for Acute renal injury, and the possibility of prevent a permanent injury in the kidney and these results agreed with Maisel study and his colleagues(17)and Dent study and his colleagues in 2007(18).

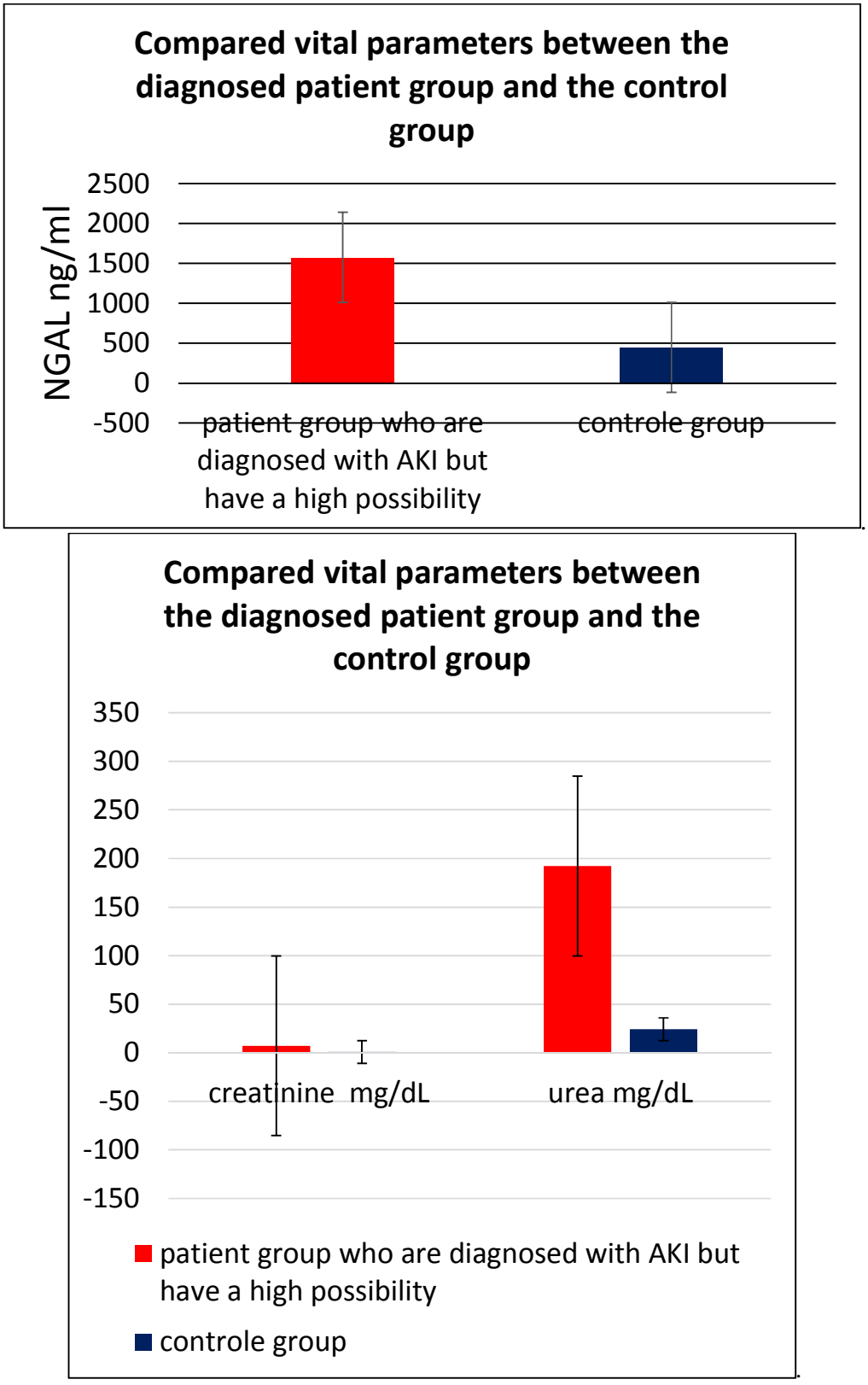


Figure 6: After creatinine and urea levels elevation

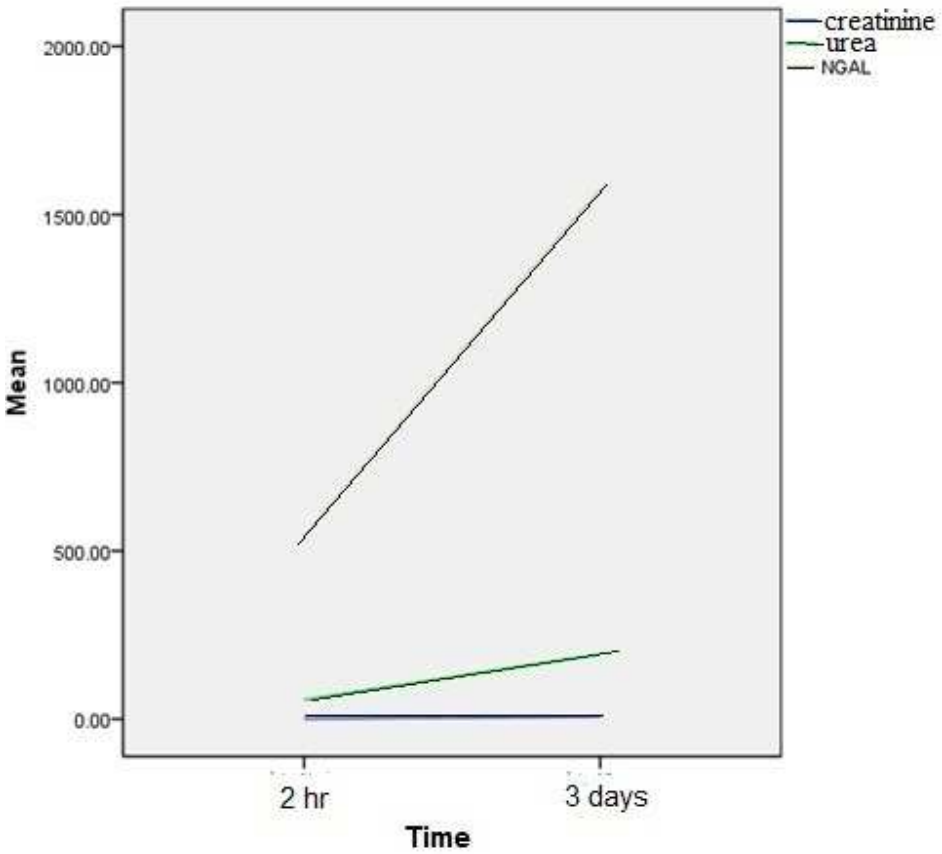


Figure 7: the relationship between the parameters and time

CONCLUSION

We find high levels of NGAL in patient groups compared with the control group. We also find high levels of NGAL in patients whose sample was collected after two hours of the acute injury while the values of creatinine and urea is within the normal range. However, the three parameters increased in samples taken after three days.

We advised to use the NGAL assay in the diagnosis of acute renal injury, and prediction of acute renal injury as its serum levels elevate before creatinine and urea elevation, and thus avoid serious complications that may cause permanent kidney damage and kill the patient.

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