Urinary NAG as a Biomarker of AKI in Patients with Hepatorenal Syndrome

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Abstract: Early detection of acute kidney injury in patients with decompensated liver cirrhosis by urinary N-acetyl-b-D glucosaminidase (NAG). Background: Renal failure is a challenging complication of liver cirrhosis primarily related to reduction in systemic vascular resistance due to splanchnic vasodilatation triggered by portal hypertension also in some patients, with cirrhosis, intrinsic renal diseases may be present that are related not to alternations in systemic hemodynamics but rather to etiological factors underlying the liver disease such as glomerulonephritis associated with hepatitis B or hepatitis C infection. Methods: Forty patients with advanced decompensated liver cirrhosis, with different ages and developed acute kidney injury (AKI) due to variable precipitating factors such as tapping of large volume of ascites in 25 patients, spontaneous bacterial peritonitis in 9 patients, haematmesis in 6 patients and ten age and sex matched normal persons were screened urinary NAG level 12 & 48 hours after admission. Results: The study group included 28 males and 12 females (M:F = 2.33:1) and their ages ranged from 42 to 67 (mean ± SD = 59 ± 5.3 years). In these patients the first urine sample which taken 12 hours after admission for assessment of NAG level ranged from 40 to 200 IU/L (mean ± SD = 88.7 ± 39.9) and second urinary NAG sample which taken 48 hours after admission ranged from 41 to 198 IU/L (mean ± SD = 100 ± 44.1).Comparison between laboratory data of study group (N= 40) and control group (N=10) revealed significant differences between 2 groups in laboratory data especially regarding mean urinary NAG levels 12 & 48 hours after admission with statistically significant P value = 0.0001. Comparison between Urinary NAG levels 12 hours (mean ± SD = 88.7 ± 39.9) and 48 hours (mean ± SD = 100 ± 44.1) for patients of study group revealed statistically significant P value = 0.0001. We conclude that Urinary NAG is a reliable biomarker for detection of AKI in patients with chronic liver disease and that it allows early detection of AKI before serum urea and creatinine.

Key words: Urinary NAG (N-acetyl-b-D glucosaminidase) - Hepatorenal - Acute Kidney Injury (AKI).

INTRODUCTION

Acute kidney injury (AKI), is a relatively frequent problem, occurring in approximately 20% of hospitalized patients with cirrhosis. Although serum creatinine may underestimate the degree of renal dysfunction in cirrhosis, measures to diagnose and treat acute kidney injury (AKI) should be made in patients in whom serum creatinine rises abruptly by 0.3 mg/dl or more (≥26.4 μmol/l) or increases by 50% or more from baseline or a urine output of less than 0.5 ml/kg per hour for more than 6 hours (Mehta, 2007). The most common causes of AKI in cirrhosis are prerenal azotemia (volume responsive prerenal AKI), acute tubular necrosis, and hepatorenal syndrome (HRS) (Weismuller, 2008). Patients with cirrhosis can develop all types of AKI but they can additionally develop HRS, a type of prerenal AKI that is not responsive to volume expansion and is seen exclusively in patients with severe liver dysfunction (Port, 2007).

HRS is a unique potentially reversible form of AKI secondary to renal vasoconstriction that results from splanchnic vasodilatation (Pomier-Layrargues, 2003).

AKI is mostly secondary to infection, hypo-volemia (gastrointestinal haemorrhage, aggressive diuresis, or diarrhea), use of vasodilators, and other factors that cause renal vasoconstriction such as nonsteroidal anti inflammatory drugs or intravenous contrast agents (Paramesh, 2004).
Acute tubular necrosis (ATN) is more common than HRS as a cause of AKI, accounting for about a third of the cases. It is mainly caused by an ischemic insult to the renal tubules as a result of a hypotensive event after bleeding or severe sepsis. However, the use of aminoglycosides, which are directly toxic to renal tubules, was found to be the most important predictor of ARF in cirrhosis (Hampel, 2001). A change in the serum creatinine is not sensitive for an early diagnosis of acute kidney injury. Nowadays there are urinary biomarkers for the detection of acute kidney injury such as matrix metalloproteinase-9 (MMP-9), N-acetyl-b-D-glucosaminidase (NAG), and kidney injury molecule-1 (KIM-1) (Cholongitas, 2007). N-acetyl-b-D-glucosaminidase (NAG), which is a hydrolytic lysosomal enzyme with high molecular weight and very low physiological activity. It originates principally in proximal tubules and normally cannot pass through the glomerular filtration. NAG has been reported to be a very sensitive and reliable marker of renal failure. Therefore, estimation of this biomarker is being done in various conditions involved with renal injury or dysfunction. Some of the uses of urinary NAG include: nephritic syndrome, nephrotoxic drugs, urinary tract infection, heavy metal poisoning, kidney transplants, vesicoureteric reflux, and diabetes mellitus (Lim, 2008).

NAG relatively large size (130 kDa) prevents glomerular filtration with the result that urinary NAG represents enzyme leakage from proximal tubular cells into the tubular lumen. It is stable in urine across a range of pH and temperature, and it is easily quantified by commercially available colorimetric or spectrophotometric methods (Han, 2002). NAG can be detected in AKI, and that the concentration of this marker is significantly higher in urine samples from patients with AKI compared with urine samples from patients with chronic kidney disease (CKD) and normal controls in cross-sectional study (Han, 2008).

MATERIAL AND METHODS

The present study included 40 patients with liver cirrhosis and ascites who developed acute kidney injury (AKI) and 10 healthy age and sex matched control persons.

The forty patients had decompenased liver cirrhosis and ascites and lower limb oedema and ultrasonographic evidence of liver cirrhosis. Those patients had serum creatinine level higher than 1.5 mg/dl after admission with no sustained improvement in renal functions (decrease in serum creatinine less than 1.5 mg/dl after diuretic withdrawal and expansion of plasma volume with 1.5 L of plasma expander. There was no ultrasonographic evidence of obstructive uropathy or intrinsic parenchymal renal disease. Those patients developed acute rise of creatinine more than 50 % of baseline within 48 hours after hospital admission.

Patients with serum creatinine less than 1.5 mg/dl, shock, ongoing bacterial infection, fluid losses, current treatment with nephrotoxic medications and ultrasonographic evidence of obstructive uropathy or intrinsic parenchymal renal disease were excluded from the study. All cases included in the study were subjected to full history taking and clinical examination.

Blood urea and creatinine levels 12 hours and 48 hours after admission due to acute decompensation of liver cirrhosis, Creatinine clearance. Fasting and 2 hours post prandial blood glucose, Liver biochemical tests as serum albumin, bilirubin total and direct, Urine analysis, Serum Na and K levels. Serum uric acid level and Viral markers as HBsAg and HCVAb. 'Urinary N-acetyl-b-D glucosaminidase (NAG)' 12 hours and 48 hours after admission due to acute decompensation of liver cirrhosis.

The N-acetyl-β-D-glucosaminidase (NAG) assay kit (provided by Bio Quant, San Diego CA) was used for determination of NAG in patients urine samples.

Statistical calculation was done; group data were expressed as mean, standard deviation. Group comparisons were performed using Chi-Square test for qualitative variables. Statistical significance of differences between group means was defined at (P <0.05).

Results:

This study included 40 patients with liver cirrhosis and ascites that developed acute kidney injury (AKI) 28 males and 12 females (M:F = 2.33:1) and their ages ranged from 42 to 67 ( mean ± SD = 59 ± 5.3 years) and 10 healthy age and sex matched control persons. The precipitating factors of AKI were tapping of large volume of ascites in 25 patients, spontaneous bacterial peritonitis in 9 patients, haematmesis in 6 patients.

In cirrhotic patients group there was statistically significant difference between blood urea 12 hours after admission ranged from 19 to 180 mg/dl (mean±SD=79.8 ± 39.1) and blood urea 48 hours after admission ranged from 107 to 246mg/dl (mean ± SD = 169 ± 38.3) with P value = 0.0001.

Serum creatinine 12 hours after admission ranged from 0.5 to 1.9mg/dl (mean ± SD = 1.4 ± 0.4) and serum creatinine 48 hours after admission ranged from 1.7 to 4.8 mg/dl (mean ± SD = 3.3 ± 0.9) was also statistically significant P value = 0.0001.
Urinary NAG levels 12 hours (mean ± SD = 88.7 ± 39.9) and 48 hours (mean ± SD = 100 ± 44.1) for patients of study group revealed statistically significant P value = 0.0001.

Comparison between urinary NAG, creatinine and urea 12 and 48 hours after admission showed that urinary NAG rises early before their rise (figure 1).

Urine analysis was negative for albumin in this group. Serum uric acid ranged from 3.4 to 15.2 mg/dl (mean ± SD = 7.3 ± 2.6).

Also liver function tests and electrolytes were done and revealed serum albumin level for this group ranged from 1.2 to 3 g/dl (mean ± SD = 2.2 ± 0.5), total bilirubin ranged from 1.7 to 7.2 mg/dl (mean ± SD = 3.55 ± 1.4), direct bilirubin ranged from 0.5 to 3.3 mg/dl (mean ± SD = 1.5 ± 0.7), ALT ranged from 11 to 57 U/l (mean ± SD = 29.1 ± 13.1), AST ranged from 11 to 78 U/l (mean ± SD = 41.9 ± 14.8), PT ranged from 16.3 to 35 sec., (mean ± SD = 20.8 ± 4.3), PC ranged from 12 to 60% (mean ± SD = 43.8 ± 10.5), INR ranged from 1.4 to 5.7 (mean ± SD = 2 ± 0.7), serum sodium ranged from 110 to 133 meq/l (mean ± SD = 125 ± 5.5), serum potassium ranged from 4 to 5.9 meq/l (mean ± SD = 4.9 ± 0.5) and random blood sugar ranged from 80 to 177 mg/dl (mean ± SD = 119.1 ± 25).

Control group examined for urinary NAG level 12 & 48 hours which ranged from 20 to 29 IU/L (mean ± SD = 23.3 ± 3.4) as shown in figure (4).

The result revealed statistically significant differences between both groups regarding mean blood urea levels 12 & 48 hours after admission as, mean serum creatinine levels 12 & 48 hours after admission as, mean creatinine clearance as, mean haemoglobin level, mean platelet count, mean random blood sugar, mean serum uric acid, mean serum albumin, mean bilirubin (total & direct), mean ASTas, mean (PT& PC & INR), mean (Na and K) and mean urinary NAG levels 12 and 48 hours after admission with statistically significant P value = 0.0001 for all previous laboratory data.
Fig. 2: Showing Comparison Between Urea and NAG at 12 & 48 Hours for Study Group.

Fig. 3: Showing Comparison Between Creatinine and NAG at 12 and 48 Hours for Study Group.

Fig. 4: Showing Comparison Between NAG at 12 and 48 Hours for Study and Control Groups.

**Discussion:**
Liver disease is endemic in Egypt. Patients with chronic hepatitis C are growing in number even with measures taken to reduce transmission. Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% among regions and demographic groups (Han, 2004).
Renal failure complicates patients with liver disease. It varies from AKI to CKD. Renal failure is a challenging complication of liver cirrhosis complicating chronic hepatitis C, this is primarily related to reduction in systemic vascular resistance due to splanchnic vasodilatation triggered by portal hypertension also in some patients, with cirrhosis, intrinsic renal diseases may be present that are related not to alternations in systemic hemodynamics but rather to etiological factors underlying the liver disease such as glomerulonephritis associated with hepatitis B or hepatitis C infection (Bass, 2007).

The traditional laboratory approach for detection of renal deterioration does not allow for early detection of renal impairment. It needs serial measurements of serum creatinine concentrations at different time which can lag detection of AKI early before complications arise.

Recently, several protein biomarkers have been evaluated as non-invasive indicators of kidney injury such as Matrix Metalloproteinase-9 (MMP-9), N-acetyl-b-D glucosaminidase (NAG), and Kidney injury molecule-1 (KIM-1) (Lehman, 2009).

N-acetyl-β-D-glucosaminidase (NAG) is one of the glycolytic enzymes distributed in various tissue cells and is found mainly in lysosomal enzymes found mainly in proximal tubular cells that have been shown to be a sensitive proximal tubular injury marker.

Early data showed that NAG assay provides an early indication of tubular dysfunction resulting from renal disease or nephrotoxic damage (Prices, 1992). Recently, urinary (NAG) performed the best marker for mortality risk prediction after AKI (Coca, 2008). Also, (Lisowska, 2010), found that urinary NAG can be one of panel of serum and urine markers released from damaged tubular cells and used for early detection of AKI. Urinary NAG has shown to be a sensitive proximal tubular injury marker (Westhuyzen, 2003), secreted in urine when kidney is damaged; therefore, it is used as marker which can detect kidney damage (Skálová, 2005) and predictor of outcome in primary glomerulonephritis (Bazzi, 2003). Therefore, estimation of this biomarker is being done in various conditions involved with renal injury or dysfunction. Some of the uses of urinary NAG include: nephritic syndrome, nephrotoxic drugs, urinary tract infection, heavy metal poisoning, kidney transplants, vesicoureteric reflux, and diabetes mellitus (Lisowska, 2010).

So far, urinary biomarkers have not been tested to detect AKI in patients with liver disease, although a large number of studies have profiled the release of NAG across a diverse range of clinicopathologic conditions, each purporting to demonstrate subtle proximal renal tubular damage.

This study is designed to detect the role of urinary NAG as a non invasive method for early detection of acute deterioration of kidney functions in patients with decompensated advanced liver cirrhosis and ascites (defined as 50% rise of serum creatinine from baseline within 48 hours). i.e Patients who develop acute decompensation of liver Cirrhosis as in haematemesis, SBP, excessive use of diuretics, tapping of large volume of ascitic fluid and also patients with liver cirrhosis undergoing surgical procedures.

Our study included 40 patients with liver cirrhosis and ascites that developed acute kidney injury (AKI) and 10 healthy age and sex matched control persons.

Comparison between blood urea levels 12 hours and 48 hours (for patients of study group revealed statistically significant P value = 0.001.

Also, comparison between Serum creatinine levels 12 hours and 48 hours for patients of study group revealed statistically significant P value = 0.0001 .

Comparison between Urinary NAG levels 12 hours and 48 hours for patients of study group revealed statistically significant P value = 0.0001. These finding is similar to what was found by Amakasu (1991), who assessed urinary NAG activity in liver cirrhosis with renal impairment and found that NAG activity was markedly elevated in the early phase of renal failure. These results suggested that the measurement of urinary NAG activity was useful for early diagnosis of renal impairment in cirrhotic patients.

Urinary NAG levels 12 hours ,creatinine and urea showed statistically significant rise (P =0.0001) in NAG at 12 hours before creatinine and urea rise however there was no statistical difference when compared at 48 hours.

Our study proved that urinary NAG rise before the urea and creatinine at 12 hours in patients with liver cirrhosis who developed AKI leading to hepatorenal syndrome (figure 1a,1b,2&3). These findings was similar also to what is found by Han et al who found that NAG increased within 6h of pediatric cardiac surgery, remaining elevated through 48 h. Although higher in patients who developed AKI, diagnostic utility was modest 12 h post- CPB and its sensitivity greater than 80% .

Also, it coincides with the data that urinary MMP-9, NAG, and KIM-1 can be detected in AKI, and that the concentration of each marker is significantly higher in urine samples from patients with AKI compared with urine samples from patients with chronic kidney disease (CKD) and normal controls in cross-sectional study (Han, 2002).
Our data can be explained by that renal damage is facilitated by low tubular flow and this can lead to urinary NAG secretion from proximal tubules. Another explanation is that the circulating endotoxins in case of hepatorenal syndrome induce renal vasoconstriction and damage (vasomotor nephropathy) which also can lead to tubular flow and this can lead to urinary NAG secretion from proximal tubules. Hepatorenal syndrome can be considered a form of pre-renal AKI, because functional renal failure develops from diffuse vasoconstriction in vessels supplying kidney which may lead to low tubular flow and this can lead to urinary NAG secretion from proximal tubules (Venkat, 2010).

In the current clinical practice, identification and classification of AKI is based on elevations in serial serum creatinine concentrations, which are delayed and therefore unreliable in the acute settings. Due to the lag of creatinine rise in AKI in patients with liver cirrhosis for at least 48 hours so our recommendation for assessment of kidney functions in these patients by urinary NAG measurement as it is a reliable and non-invasive method for early detection of AKI in these patients so early management either conservative measures or preparation for liver transplantation as it is the only radical treatment for hepatorenal syndrome.

Urinary NAG is considered relatively simple, cheap, fast and non-invasive method in the detection and follow up of renal damage under various conditions. Our study proved with no doubt that urinary NAG rises early before urea and creatinine when measured at 12 hour and the marked creatinine rise was evident at 48 hours from insult, a time at which usually intervention carries a worse prognosis. Urinary NAG provided a very sensitive and reliable indicator of renal damage in patients with liver cirrhosis who developed HRS.

**Conclusion:**

We conclude that urinary NAG is a reliable biomarker for detection of AKI in patients with chronic liver disease and that it allows early detection of AKI before serum urea and creatinine. Our study showed that there is a statistically significant increase in urinary NAG when measured in patients with liver cirrhosis at 12 hours and at 48 hours from exposure to factors that can precipitate hepatorenal syndrome. So, we concluded that serial measurement of urinary NAG may allow early detection of AKI in patients with liver cirrhosis long enough before serum creatinine and urea.

**REFERENCES**


