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Is C-type natriuretic peptide level can be an early indicator for acute kidney injury?

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Abstract

Acute kidney injury (AKI) is defined as a reduction of renal function in hours, including both structural damage and loss of function. There are limited number of biomarkers for early detection and staging of severity. C-type natriuretic peptide (CNP) has been detected in at several tissues. We aimed to evaluate plasma CNP and creatinine levels correlated with duration of ischemia in an experimentally induced AKI rat model. Forty male Sprague-Dawley type rats (aged 8 to 12 weeks, weighing 250-350 g) were used. The animals were randomly separated into 4 groups: Group 1 (n:10): Only laparotomy was performed. The left renal artery was clamped for 3, 6 and 9 hours in groups 2 (n:10), 3 (n:10) and 4 (n:10) respectively. CNP and creatinine levels were measured in serum samples from rats. A significant increase in creatinine levels was determined in group 2 according to group 1 ($p=0.006$). The mean plasma creatinine values in group 3 and 4 were decreased compared to group 2 but this difference was not statistically significant ($p=0.0862$). The mean CNP level in Group 2 (39.5 ± 7.93 mg/dl) was found numerically higher than group 1 (37.90 ± 5.38 mg/dl). There was a statistically insignificant decrease in mean CNP levels in group 3 and 4 compared with group 2. Renal ischemia increases the level of CNP. Although the increase in CNP levels is not significant, it can be said that clinical and experimental studies evaluating the timing of ischemia involving different durations should be performed.

Keywords: Acute Kidney Injury, renal ischemia, C-type natriuretic peptide

Introduction

Acute kidney injury (AKI) is very important pathology for morbidity and mortality in clinical practice. For this reason early diagnosis and treatment are necessary to prevent permanent damage. Regardless of the etiology, various pathophysiological processes such as endothelial dysfunction, microcirculation changes, tubular injury, venous congestion and inflammation occur respectively [1]. The most common cause of AKI is ischemia and may results from various diseases such as renal artery stenosis, renal vein thrombosis, vasculitis, atherosclerotic and thrombotic embolism, etc. Furthermore, in some surgical procedures, the risk of renal ischemia may be observed, particularly in relation to the involvement of renal arteries in the dissection of the aorta and vascular surgery procedures [2]. Although not exactly defined in clinical trials, it is shown that clamping renal veins in animal studies also causes kidney injury [3]. The current diagnostic approach of AKI is based on a decrease in an acute glomerular filtration rate as reflected by an acute increase in serum creatinine

levels and / or a decrease in urine output over a period of time [2]. Kidney damage biomarkers have several potential roles in AKI including early detection and staging of severity, and as end points of clinical trials [4-6]. There are several early damage biomarkers as urinary creatinine, kidney injury molecule-1 (KIM-1), Cystatin C, Interleukin 8, Neutrophil Gelatinase, associated lipocalin (NGAL), clusterin, monocyte chemotactic protein, osteopontin etc. But none of these except creatinine were usable in clinical practise [7-10]. In addition to these markers, new studies are carried out in this subject and the searches are continuing.

C-type natriuretic peptide (CNP) is a new member of the family of natriuretic peptides, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Although these ones exhibit similar properties, CNP releasing from vascular endothelium has shorter half-life and low circulating concentration. Natriuretic peptides show their effects by binding to receptors containing guanylate cyclase [11]. There are two forms of Natriuretic peptide receptors A and B. In studies on vascular smooth muscle cells, CNP was determined as an agonist of type B natriuretic peptide receptor [12]. There are several studies showing the effect of natriuretic peptides on different types of ischemia [13-17]. The aim of this study was to evaluate plasma CNP and creatinine levels correlated with duration of ischemia in an experimental model of acute kidney injury.

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Material and Methods

This study was performed on 40 Wistar rats aged 8 to 12 weeks and weighing between 250 to 350 gr after the approval of the Animal Experimental Committee of Gaziosmanpasa University Faculty of Medicine. The rats were kept in a light-controlled room with a 12:12-h light-dark cycle; temperature (22 ± 0.5 °C) and relative humidity (65 %–70 %) were kept constant. They received a rat diet, water and libitum. The rats were deprived of food for 12 h before the experiment. Rats were separated into 4 groups by simple randomisation.

Group 1 (sham group) (n:10): Only laparotomy was performed in this group of rats without cross-clamping the left renal artery. Group 2 (n:10): The left renal artery was clamped for 3 hours following laparotomy in this group of rats. Group 3 (n:10): Following laparotomy in this group of rats, the left renal artery was clamped for 6 hours. Group 4 (n:10): The left renal artery was clamped for 9 hours following laparotomy in this group of rats. At the end of the study 3 cc intracardiac blood was taken from each rats in all groups then euthanized by cervical dislocation. C type natriuretic peptide levels and creatinine levels were measured in serum samples from rats in groups.

Biochemical analysis

The plasma levels of CNP were measured using CNP Elisa Kit (No: 201-11-0056; Sunredbio Biological Technology, China) with elisa method and plasma levels of creatinine were measured

using Creatinine Colorimetric Assay Kit (No: 700460; Cayman Chemical, Ann Harbor, MI) with colorimetric method according to manufacturer's instructions.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation. One way analysis of variance were used to compare the continuous normal data among groups. For post-hoc comparisons between the pair-wise groups, the Tukey HSD test was used. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY). Lower than 0,05 a p-value was accepted as significant.

Results

Mean plasma creatinine levels were measured as 0.53 ± 0.08 mg / dl in Group 1. A significant increase in creatinine levels was seen in group 2 compared with group 1 [0.64 ± 0.09 mg/dl ($p=0.006$)]. The mean plasma creatinine values in group 3 and 4 (0.59 ± 0.05 mg/dl, 0.56 ± 0.04 mg/dl) in six and nine hours kidney ischemia were decreased compared to group 2 but those were not statistically significant (Table 1, Fig 1). The mean CNP level in group 1 was 37.90 ± 5.38 mg/dl. The mean CNP level in Group 2 (39.5 ± 7.93 mg/dl) was found to be numerically higher than group 1, but this was not statistically significant. There was statistically insignificant decrease in mean CNP levels in group 3 and 4 compared to group 2 ($p = 0.862$) (Table 1, Figure 2).

Table 1. Distributions of CNP and creatinine levels according to groups

Age	1 (n=10)	2 (n=10)	3 (n=10)	4 (n=10)	P
CNP	37.90 ± 5.38	39.5 ± 7.93	37.6 ± 9.14	36.1 ± 11.71	0.862
Creatinine	$0.53 \pm 0.08a$	$0.64 \pm 0.09b$	$0.59 \pm 0.05ab$	$0.56 \pm 0.04ab$	0.006

CNP: C-type Natriuretic Peptid

One-way ANOVA test was used. Data are shown as mean $\pm$ standard deviation. For groups, different superscripts (a,b,c) in the same row (ANOVA) indicate a statistical significant difference

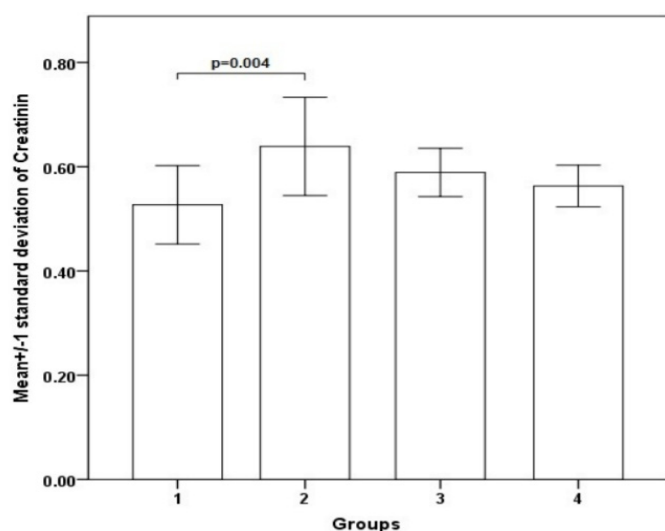


Figure 1. The levels of creatinine according to groups. Bar graph with standard deviation of creatinin

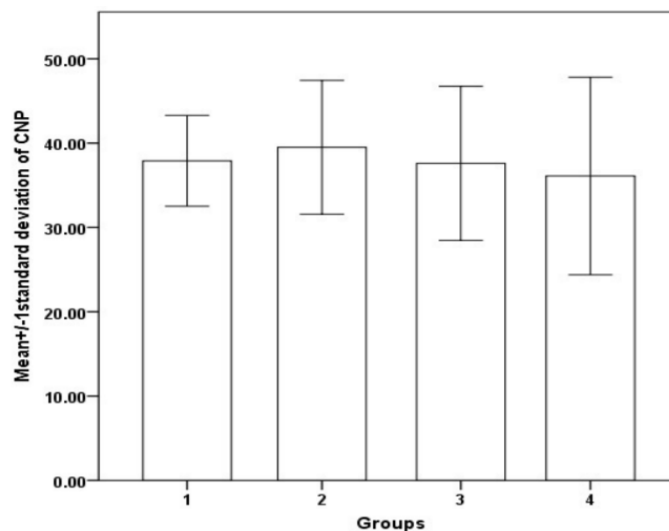


Figure 2. The levels of CNP according to groups. Bar graph with standard deviation of C-Type Natriuretic Peptide

Discussion

Kidney; is highly sensitive to ischemia-related injury resulting in vasoconstriction, endothelial damage and inflammatory process [18]. This sensitivity can be partly explained by structural relationships between the renal tubules and blood vessels in the external medulla of the kidney [19]. Following reduction in effective renal perfusion, epithelial cells can not protect sufficient intracellular ATP for the required processes. This ATP depletion leads to cell damage and can cause cell death by necrosis or apoptosis if it is severe enough [20]. All segments of nephrons may be affected in the ischemic process, but proximal tubular cells are the most frequently affected cells. In addition, the function of the nephron is to filter, concentrate, and reabsorb many substances from the tubular lumen. Because of the deterioration of tubular function in ischemia, toxic substances that cannot be cleaned can reach toxic levels for renal epithelial cells [21].

Although serum creatinine is sensible to acute changes in renal function; age, sex, muscle mass, diet, medications and hydration status may vary its levels. It isn't a direct indicator of tubul damage. Even if the kidneys are structurally intact, it may also increase in renal hypo-perfusion and prerenal azotemia. For these reasons, serum creatinine is considered to be an 'imperfect gold standard' for the diagnosis of AKI [2]. Over the last few years, some new AKI biomarkers have been studied out and confirmed to improve early detection. Some of them are neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), liver-type fatty acid-binding protein, interleukin 18 (IL-18), insulin-like growth factor-binding protein 7, tissue inhibitor of metalloproteinase 2 (TIMP2), calprotectin, urine angiotensinogen (AGT), and urine microRNA [7-10,22]. C-Type natriuretic peptide; although mainly found in the brain [23], it has been later suggested that it is also present in the heart [24]. In addition, it was found in endothelial cells of human coronary arteries, peripheral circulation, arteries and veins in various regions. CNP; it has been shown to play a role in the regulation of vascular tone rather than natriuretic function [23].

The studies about the effects of natriuretic peptides on different types of ischemia can be seen at literature. However, according to our knowledge there were no studies evaluating CNP levels in the acute kidney ischemia model in english literature. In a study, Sward et al. [13] investigated the effect of recombinant human atrial natriuretic peptide (h-ANP) in ischemic acute kidney failure and found h-ANP administration corrected renal function. In a clinical study, it was shown that BNP is associated with inducible myocardial ischemia [14]. Kamakura et al. [16] detected high BNP levels in critical leg ischemia. Furthermore in an experimental study Demirtaş et al. [17] evaluated the relationship between serum CNP levels in acute mesenteric ischemia and showed that CNP levels increased correlating with ischemia time. The use of CNP as an indicator in detecting inducible peripheral ischemia has been investigated in experimental study and it has been reported that plasma CNP is associated with cellular response in ischemic tissues depending on the time [26]. Also; it is believed that CNP is produced in tubular cells and is presented as a local modulator with anti-inflammatuary and anti-proliferative effects under pathological conditions. Determination of urine CNP levels in experimental nephropathies has led us to understand the

relationship with kidney structure and function [27].

In an experimental nephropathy secondary to ureteral obstruction, Hu et al. [28] determined an increase in urinary CNP excretion before changes in urinary protein, albumin, blood urea nitrogen, and creatinine were observed. In the same study, it was observed that CNP levels in the abdominal aorta and renal vein were increased in the first 24 hours but this was not statistically significant and this increase was observed to regress within weeks

In our study, a statistically insignificant increase was observed in serum CNP levels at the 3rd hour of acute renal ischemia but there was a decrease in CNP levels at the 6th and 9th hours.

Renal ischemia causes changes in tubular cell polarity, loss of tubular epithelial barrier cell integrity, necrotic and apoptotic cell death, expression of characteristic genes of embryonic kidney mesenchyma [29]. The damaged kidney epithelium can be completely restored structurally and functionally unlike the heart and brain. The kidney has regenerative capacity after acute ischemic and / or toxic damage. This is manifested by the proliferation and migration of weakly differentiated cells along stripped basal membranes of the damaged tubular segments after ischemia [30]. In animal models after ischemic injury repair; proliferation is seen as a maximum in the flat segments of the proximal tubules in the outer medulla, where damage is prominent. There are several probabilities for the origin of regeneration of epithelial cells.

In response to damage, they may be redefined to dedifferentiating, proliferating, and then mature tubular cells. Bone marrow cells may lead to damaged epithelial cells. Renal mesenchymal stem cells after damage replace epithelial cells [30]. Morphological reparation is manifested by the emergence of differentiated epithelial cells expressing vimentin, a marker for multipotent mesenchymal cells [27-31]. In next step, the cells upregulate and encode specific genes for various growth factors such as IGF-1, hepatocyte growth factor (HGF) and fibroblast growth factor. At the end stage, the cells express differentiation factors are redifferentiated until precisely transformed into polarized epithelium. So, in the process of recovery after ischemia; renal tubular cells repeat the phases and processes takes place during normal kidney development [31-33].

While cell death itself does not produce a regenerative response, epithelial cells in the death process produces signals that initiate the repair process. Cytokines may play a role in the formation of signals for neutrophils and monocytes resulting in infiltration of them to the tissue, and promotes the dedifferentiation and proliferation of epithelial cells. These cytokines may arise from kidney tissue, epithelial and mesenchymal cells or infiltrating cells [33].

Some of genes that support the concept of "recapitulation of phylogenin by ontogenesis" include NGAL, leukemia inhibitor factor, transcription factor Ets-1 and WNT -4. Not all these transcripts are critical for early kidney repair, but also play an important role in the regeneration and repair processes of mature kidneys after ischemic injury [34]. This may be one of the explaining factor why CNP levels decrease depending on the duration of ischemia in this study.

The increase in plasma CNP levels positively correlate with ischemia time in mesenteric ischemia and peripheral ischemia models.

Because of some pathologies as renal artery thrombosis, thrombosed abdominal aortic aneurysms affecting one of the renal arteries and especially aortic dissection including involvement of one of the renal arteries that requires surgical intervention in vascular procedures, we prefer to clamp one of the renal artery. There was not a consensus in animal models about the renal injury performed by clamping only one or bilateral renal artery, only renal vein or pedicle. It is important to mention that renal pedicle (artery and vein together) clamping was used in most murine ischaemic AKI, which is different from patients with AKI. In humans, AKI is induced either by renal artery hypoperfusion during shock and cardiac surgery or by renal vein occlusion by thrombosis and during liver transplantation in which the inferior vena cava is clamped. According to our results and to the regenerative capacity of kidney and compensatory mechanisms of the other kidney we believe that significant results may be obtained by clamping bilateral renal artery clamping. The clamping one of the renal artery is a partial limitation of this study.

Conclusion

Although CNP levels do not correlate with acute kidney injury, better biochemical results may be produced with different acute renal ischemia models including long term follow-up and besides the experimental studies, randomized clinical trials are needed.

Competing interests

The authors declare that they have no competing interest.

Financial Disclosure

The authors received no financial support for this study.

Ethical approval

This study was performed after the approval of the Animal Experimental Committee of Tokat Gaziosmanpasa University School of Medicine with the number of 2018 HADYEK-02

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