Early Detection of Renal Damage Using Kidney Injury Molecule -1 (KIM-1) In Dogs Associated with Dehydration

Noha M. Ryad*, Eman S. Ramadan, Mohammed A. Elkhiat, Noha Y. Salem and Ibrahim A. Saleh
Department of Internal Medicine and infectious diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

BACKGROUND: Dehydration may be associated with a declined kidney function and serious signs of kidney injury. The kidney response differently to dehydration levels which are not yet known. **Aim**: detection of the effect of dehydration on kidney functions levels and kidney injury biomarkers in dehydrated dogs and compare it to normal. **Material and methods**: A total number of 12 dogs were enrolled in this study (6 dehydrated and 6 controls). Inclusion criteria was presence of history of fluid loss for 48-hour, dehydration stage based on clinical assessment and the assessment of urine specific gravity. Blood samples were withdrawn from each animal on Ethylenediamine tetraacetic acid tube for hematology and plain tube to estimate total protein, albumin, BUN and creatinine. Ultrasonography was conducted on non-sedated dogs using a micro convex 5–8 MHz transducer. Urine samples were acquired through catheterization to determine urine specific gravity, then urine sediment was derived by centrifugation and commercially available Enzyme-linked immunoassay test was utilized to assess the levels of kidney injury molecule-1. **Results**: Hematological parameters showed non-significant elevation in packed cell volume in association with significant elevation in mean corpuscular volume were recorded in dehydrated dogs. Non-significant elevation in blood urea nitrogen, creatinine, and total protein were recorded in dehydrated dogs compared with control dogs. Urinalysis showed significant elevation in urine specific gravity and KIM-1 (P value ≤0.05) in urine samples of dehydrated dogs compared to control dogs. **Conclusion**, dehydration state might be impact the reading of urinary KIM-1 even in the absence of apparent azotemia.

Keywords: Dehydration, KIM-1, Dogs, Kidney injury.

Introduction

Water is an essential element in life, plays a pivotal role in maintaining physiological equilibrium in living organisms [1]. Dehydration a condition characterized by an inadequate amount of water in the body, poses a serious threat to various physiological functions, particularly those of the kidneys [2]. The canine kidney is a vital organ responsible for maintaining water balance, is particularly susceptible to the effects of dehydration [3]. Dehydration can result from various factors, including inadequate water intake, excessive fluid loss through vomiting or diarrhoea, or environmental conditions such as heat stress [4].

In recent years, the focus on understanding the mechanisms underlying dehydration-induced renal damage has grown, prompting investigations into specific biomarkers [5]. One such biomarker that has gained popularity is Kidney Injury Molecule-1 (KIM-1), a transmembrane protein expressed in renal tubular cells [6]. KIM-1, originally identified as a marker of tubular injury in humans, has been recognized as a
promising indicator of renal damage in various species, including dogs [7]. The physiological significance of KIM-1 in the kidney is becoming more clear, KIM-1 confers on epithelial cells the ability to distinguish and phagocytose dead cells that are present in the post-ischaemic kidney and contribute to the obstruction of the tubule lumen that characterizes acute kidney injury (AKI) [8]. In a healthy kidney, KIM-1 is absent, though, after hypoxia, a huge number of epithelial tissues of renal proximal convoluted tubules can be identified by urine test. This elevated Kim-1 expression is an effective indicator for renal ischemia and hypoxic injury [7]. KIM-1 confers on epithelial cells the ability to distinguish and phagocytose dead cells that are present in the post-ischaemic kidney and contribute to the obstruction of the tubule lumen that characterizes acute kidney injury (AKI) [8]. In a healthy kidney, KIM-1 is absent, though, after hypoxia, a huge number of epithelial tissues of renal proximal convoluted tubules can be identified by urine test. This elevated Kim-1 expression is an effective indicator for renal ischemia and hypoxic injury [7]. KIM-1 confers on epithelial cells the ability to distinguish and phagocytose dead cells that are present in the post-ischaemic kidney and contribute to the obstruction of the tubule lumen that characterizes acute kidney injury (AKI) [8]. In a healthy kidney, KIM-1 is absent, though, after hypoxia, a huge number of epithelial tissues of renal proximal convoluted tubules can be identified by urine test. This elevated Kim-1 expression is an effective indicator for renal ischemia and hypoxic injury [7]. KIM-1 confers on epithelial cells the ability to distinguish and phagocytose dead cells that are present in the post-ischaemic kidney and contribute to the obstruction of the tubule lumen that characterizes acute kidney injury (AKI) [8]. In a healthy kidney, KIM-1 is absent, though, after hypoxia, a huge number of epithelial tissues of renal proximal convoluted tubules can be identified by urine test. This elevated Kim-1 expression is an effective indicator for renal ischemia and hypoxic injury [7].

Scanty papers discussed the relation between dehydration in canine patients and expression of urinary KIM-1, therefore, this study seeks to elucidate the specific impact of dehydration on KIM-1 in dogs.

Material and Methods

Ethical approval

This study was performed in accordance with the ethical standards of the institution Animal Care and Use Committee, faculty of Veterinary Medicine Cairo University, Egypt. This study was granted ethical Vet CU 25122023858

Animals and sampling

This study enrolled twelve (n=12) mongrel dogs, (8 males and 4 females), six (n=6) dehydrated dogs and six (n=6) control dogs. Dog’s age range was between 2-4 years, weight between 15-20 Kg.

Inclusion criteria was presence of history of fluid loss for 48-hour caused by vomiting and diarrhea, on physical examination, patient showed rapid and weak pulses, dryness of mucous membranes and moderate loss of skin turgor, dehydration status was assessed based on physical and clinical examination to be moderate (7%), and the assessment of urine specific gravity.

Blood samples were withdrawn from each animal on Ethylenediamine tetraacetic acid (EDTA) tube for hematology and plain tube was used for serum separation to estimate total protein, albumin, Blood urea nitrogen (BUN) and creatinine using specified test kits (Spectrum-diagnostic, Egypt).

Urine samples were acquired through the catheterization technique illustrated by Taylor (2016) [14]. The refractometer was employed to determine the specific gravity of the urine. The urine sediment was derived by subjecting the samples to a 10-minute centrifugation at 2500 g, subsequently; 0.25 ml of each canine urine sediment was frozen at -80°C.

Following the collection of all samples, commercially available Enzyme-linked immunoassay (ELISA) test from BT LAB (E0147Ca, China) were utilized to assess the levels of KIM-1, the test was conducted in accordance with the manufacturer’s guidelines.

Ultra-sonographic examination

Ultrasonographic assessments were conducted on non-sedated dogs using the Esaote MyLabOne equipped with a micro convex 5–8 MHz
transducer. Dogs were positioned in right lateral recumbency for left kidney examination and left lateral recumbency for right kidney examination. Scanning below the transverse process of the first to third lumbar vertebrae was performed on both the left and right kidneys to identify any alterations in tissue structure.

**Statistical analysis**

Data are presented as mean ± SE, data set of control and dehydrated dogs were analyzed using Student T-test, P value ≤ 0.05 considered significant.

**Results and Discussion**

Hematological parameters are depicted in Table 1. There were no recorded changes in hemoglobin, MCHC and WBCs in dehydrated patients compared to control group. Non-significant elevation in PCV in association with significant elevation in MCV was recorded in dehydrated dogs compared to control dogs. For serum biochemistry, there was no recorded change in albumin. Non-significant elevation in BUN, creatinine, and total protein were recorded in dehydrated dogs compared with control dogs.

Non-significant elevation in packed cell volume (PCV) in association with significant elevation in mean corpuscular volume (MCV) were recorded in dehydrated dogs, the finding of this work were consistent with other reports [15] where there was no significant difference in PCV between euhydrated dogs and severely dehydrated one. Meanwhile, significantly higher MCV values was observed between this groups and these findings were attributed to dehydration ratio.

Non-significant elevation in BUN, creatinine and total protein were recorded in dehydrated dogs compared with control dogs (Table 1). For clinical detection of decreased Glomerular Filtration Rate (GFR), significant loss of nephrons and drops in overall kidney function are necessary, with assessments of up to 50% or more decline in function occurring before azotemia develops [16]. The traditional clinical kidney parameters serum blood urea nitrogen (BUN) and serum creatinine (Cr) were relatively insensitive in our study, which indicates the lower sensitivity and deprived predictive value of these conventional biomarkers at the early stage of kidney injury. The traditional diagnostic indicator serum creatinine was only significantly increased in case of severe kidney injury [7]. Recently, serum creatinine, the gold standard for testing AKI, has a number of drawbacks that impact the condition’s early detection and prognosis.

Examination of urine sample showed significant elevation in urine specific gravity and KIM-1 in dehydrated dogs compared to control dogs as depicted in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Dehydrated dogs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10$^6$/μl)</td>
<td>6.143±0.3538</td>
<td>5.130± 0.3396</td>
<td>0.0980</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.30± 0.6557</td>
<td>14.63± 0.6060</td>
<td>0.2022</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>39.77± 2.063</td>
<td>44.75±2.287</td>
<td>0.1812</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>69.74± 1.508</td>
<td>84.40± 4.992</td>
<td>0.0228</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.80± 2.166</td>
<td>32.75± 0.60</td>
<td>0.6127</td>
</tr>
<tr>
<td>WBCs (10$^3$/μl)</td>
<td>7.233± 1.185</td>
<td>8.225± 0.770</td>
<td>0.4947</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>9.333± 0.4216</td>
<td>14.71± 2.251</td>
<td>0.0781</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.825± 0.0769</td>
<td>1.09± 0.123</td>
<td>0.1238</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>6.488± 0.4351</td>
<td>7.245± 0.6410</td>
<td>0.3660</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.574± 0.2007</td>
<td>3.429± 0.1171</td>
<td>0.5954</td>
</tr>
</tbody>
</table>

P value ≤0.05 is considered significant.
Urinalysis showed significant elevation in urine specific gravity and KIM-1 in urine samples of dehydrated dogs compared to normal control dogs. In order to improve the ability to predict the occurrence of AKI more and more efforts were made to discover novel urinary biomarkers prior to serum creatinine [17]. Euvhydration is the state of having ideal body water content, and it is directly maintained by the kidneys. In human medicine; Dehydration is the process of losing body water; while hypohydration is the state of inadequate body water brought on by acute or chronic dehydration [18]. There is no universal gold standard assessment for dehydration so dehydrated state can be reliably identified by monitoring changes in two or more markers over time [19]. The kidneys get a significant amount of their cardiac output through the renal artery, which divides into numerous smaller arteries and arterioles before being filtered by the nephron, the kidney’s functional unit [20]. The state of dehydration causes a cascade of hormonal responses to motivate water conservation including the activation of the renin-angiotensin- aldosterone system and the release of vasopressin [21]. One of the important consequences of increased plasma renin is the production of angiotensin II, Activating the angiotensin II results in many physiological actions that are important during dehydration [20]. Dehydration may be associated with a compromised kidney function and potential signs of kidney injury [22]. A case of dehydration resulting in lowering extracellular volume [23]. And It has been hypothesized reduced renal blood flow and circulatory blood volume have been proposed as potential causes of kidney attenuation, ischemic kidney stress, and even “temporary” kidney injury [24]. Acute kidney injury (AKI) is defined as a rapid reduction in kidney function (e.g., GFR), with increasing concentrations of nitrogenous waste products in the plasma (e.g., creatinine, urea) [25]. Prerenal cause of AKI is the most prevalent type of AKI, resulting from kidney hypoperfusion brought on by decreases in actual or effective arterial blood volume [26]. This hypoperfusion triggers the production of vasopressin, raises RSNA, and activates the renin-angiotensin-aldosterone pathway.

Urine specific gravity was high in the examined patients; increased urine specific gravity is due to an increased urine concentration because of dehydration. There is a decrease in renal blood flow and GFR owing to dehydration, and the renal vasculature develops an ischemia environment [20]. These results suggest that dehydration develops a state of kidney injury as a consequence of induced kidney stress.

In the present study, KIM-1 was elevated in urine sample without corresponding increase in serum creatinine. In the study performed by Lippi, et al., [27] they observed a fast elevation in urine KIM-1 2-hours following kidney injury and this elevation was sustained for up to 48 hours while serum creatinine did not start to rise except after 12 -24 hr post injury. Traditionally changes in kidney function are quantified via measures of GFR and urine output to identify the severity of AKI. Nowadays there are recent biomarkers that could identify kidney damage at an earlier stage. KIM-1 is a transmembrane glycoprotein that is not expressed in healthy group however expressed in urine in case of dehydration. During AKI, KIM-1 is mainly upregulated in proximal tubule cells, which is reflected by increases in urinary KIM-1 [28]. Thus, increases in urinary KIM-1 likely indicate proximal tubule injury, although the etiology is nonspecific. Therefore, KIM-1 might be a specific predictor for early AKI. The potential diagnostic and prognostic value of this biomarker needs to be validated further in the future. It has been hypothesized that In this case, the elevations in AKI biomarker are transient and of a lower magnitude than those observed in clinical situations. However, an increase in AKI biomarker in this non-clinical situation likely reflects an increased potential to develop AKI because some underlying pathophysiological processes taking place [29]. This explanation is compatible with the theory that small and transient increases in AKI biomarkers are indicative of acute kidney stress [30]. Accordingly, a higher risk of developing AKI is indicated by elevations in AKI biomarkers, and the degree of this risk is correlated with the elevations in AKI biomarkers.

Ultrasound examination showed non-significant changes between the dehydrated group and the control one.

Ultrasonography of kidney is considered a noninvasive diagnostic modality that is not dependent on kidney function and it has minimal side effects, it also allows depiction of renal internal architect [31]. In the present study there were no alterations detected via ultrasound examination, Nelson and Couto (2019) [31], reported that the presence of normal ultrasound picture of kidney does not rule out the possibility of renal disorder.
Conclusion

In conclusion, dehydration state might be impact the reading of urinary KIM-1 leading to its elevation, this elevation might be detected in earlier course of condition and before triggering elevation in traditional renal biomarkers (BUN and Creatinine). The increase in AKI biomarker in this non-clinical situation likely reflects the increased potentiality to develop AKI.

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Conflicts of interest

There are no conflicts to declare

Funding statement

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References


EARLY DETECTION OF RENAL DAMAGE USING KIDNEY INJURY MOLECULE -1 (KIM-1)  


الكشف المبكر عن تلف الكلى الناجم عن الجفاف باستخدام جزيء إصابة الكلى (KIM-1)  

في الكلاب

نها محمود رياض, أ. أ. إبراهيم عبد الستار, نهي يوسف سالم, محمد عوني الخياط, إيمان شوقي رمضان

قسم أمراض الباطنة والمعدية - كلية الطب البيطري - جامعة القاهرة - مصر.

المتعرض: قد يتراوح الجفاف مع انخفاض وظائف الكلى وعلامات خطيرة لإصابة الكلى. استجابة الكلى بشكل مختلف لمستويات الجفاف التي لم تعرف بعد. الهدف: الكشف عن تأثير الجفاف على مستويات وظائف الكلى والمؤشرات الحيوية لإصابة الكلى في الكلاب المصابين بالجفاف ومقارنتها بالطبيعية. المواد والطرق: تم تسجيل 12 كلباً في هذه الدراسة (6 كلاب مجففة و6 كلاب مراقبة). وكانت معايير الإشغال وجود تاريخ من فقدان السوائل لمدة 48 ساعة، ومرحلة الجفاف على أساس التقييم السريري وثقل النوعي للبول. تم تسجيل عينات BUN والكريدينين، و BUN/creatinine. ثم تم تسجيل على عينات البول من خلال الطرد المركزي لتحديد النسبة المئوية للبول، ثم استخلاص KIM-1، ونسبة الكرياتينين والبروتينات في البول، و BUN والكريدينين، و BUN/creatinine. تم استخدام اختبار ELISA المتوازن لقياس مستويات KIM-1 في البول. النتائج: أظهرت مراقبة الدم ارتفاعاً غير ملحوظاً في NCV، والكريدينين، والبروتينات في الكلاب المجففة. تم تسجيل ارتفاع غير ملحوظ في نسبة BUN والكريدينين والبروتينات في الكلاب المجففة. أظهر تحليل البول ارتفاعاً غير ملحوظاً في الثقل النوعي للبول. وعلى قراءة البوليول KIM-1 على حسب حالة الجفاف، قد يكون تأثير KIM-1 على حالة مرضية وظيفة الكلى بحالة عدم وجود أوزومي واصحة.

الكلمات المفتاحية: الجفاف، الكلاب، إصابات الكلوي، KIM-1.