

## Evaluation The role of Trefoil Factor1 as early stage biomarker in patients with Nephrolithiasis

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### Abstract

Nephrolithiasis is a public health problem that affect large scale of peoples around the world. naturally, there are many substances ,proteins, lipids, glycosaminoglycans and inorganic compounds, work as kidney stone inhibitors in the normal renal tubular fluid that inhibit crystal growth, aggregation, and/or adhesion to renal surfaces, among this inhibitors we investigate the concentration of trefoil factor 1 (TFF1) as a stone formation inhibitor and kidney injury biomarker in serum of 75 kidney stone patients and 15 healthy subjects using ELISA technique. The results shows no significant changes within patients groups while shows that TFF1 levels were significantly increased in stone patients when compared with the healthy controls ( $P \leq 0.05$ ), which support the fact that it could use as early marker of kidney injury (KI) .The results also revealed no significant changes within patients groups while there was a significant changes between patients and controls for both blood urea and serum creatinine levels in renal stone patients in comparison to the apparently healthy control subjects ( $P \leq 0.05$ ). Further investigation are needed to show the inhibitory effect of TFF1 in urine of kidney stone patients.

### Introduction

Nephrolithiasis remains a public health problem around the world, affecting 1–20% of the adult population [1]. Of all types of renal stones, calcium oxalate (CaOx) is the most common composition found by chemical analysis [2]. To date, the pathogenic mechanisms of stone formation is not under stood. One long standing hypothesis is that stone formation is related to intratubular crystal nucleation, growth, and aggregation [3]. The urine from patients with nephrolithiasis is commonly supersaturated with calcium and oxalate ions [4], favoring CaOx crystal nucleation and growth (5). Nucleated crystals can be retained in the kidneys of these patients by adhering to renal tubular epithelial surfaces [6,7]. Within the environment of supersaturated calcium and oxalate ions, the stone can then be formed. In contrast, nucleated crystals are not retained in the normal kidney [8]. Calculation of the flow rate of renal tubular fluid and the rate of crystal growth in normal subjects suggests that nucleated crystals are eliminated from the normal kidney before they attach to tubular epithelial surfaces [9, 10].

Additionally, there are urinary substances known as “stone formations inhibitors” in the normal renal tubular fluid that inhibit intratubular crystal growth, aggregation, and/or adhesion to renal epithelial surfaces [11]. These substances include proteins, lipids, glycosaminoglycans, and inorganic compounds. Abnormality in function and/or expression levels of these molecules, especially proteins, in the urine and renal tubular fluid has been proposed to be associated with stone formation [12–14].

The trefoil factor family (TFF) peptides are important proteins involved in the regeneration and repair of the urinary tract [15]. TFF peptides are secretory products of various mucine-producing epithelial cells and promote restitution and regeneration processes of mucous epithelia via induction of cell migration, resistance to proapoptotic stimuli, and angiogenesis [15,16]. During restitution, mucosal continuity is restored by elongation and migration of epithelial cells to cover denuded areas of damage. Though TFF peptides have many been investigated in the

gastrointestinal tract, they were also detected in the urinary tract with TFF3 as the most abundant followed by TFF1 [17]. In preclinical studies TFF3 has already been established as a urinary biomarker for kidney toxicity in animal models [18] and has been successfully shown to be up regulated in chronic kidney injury CKD patients [19,20].

The aim of the study is to evaluate the ability to use TFF1 peptide levels as early stage biomarker in urine of renal stone patients.

**Methods**

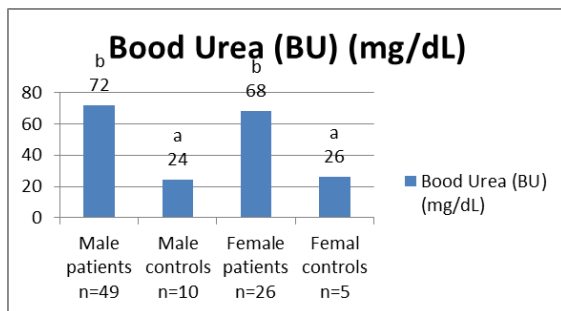
A cross section study done in Tikrit city for the period from September 2017 to January 2018. The current study included 75 patients with kidney stone diseases 49 were males and 26 were females there ages range was 35-50 years. All patients were screened and followed up in out-patients clinics in Salah - Aden general hospital. Also, 15 healthy volunteers (5femals and 10 males) served as controls. A venous blood and urine sample was obtained from all patients and healthy volunteers. Blood was centrifuged at 3000 rpm for 10 min at -20°C. Aliquots were transferred into tubes, snap frozen and stored at -20°C until further use. TFF1 was determined using enzyme-linked immunosorbent assay (ELISA) kits (Human TFF1 Elabscience - USA) according to the manufacturer’s instructions, ELISA were concluded in tikrit university central laboratory .

**Statistical Analysis:**

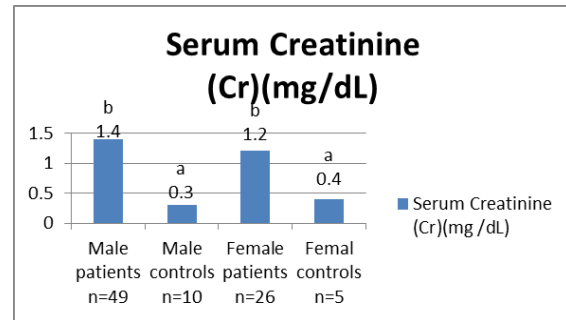
Statistical analysis the results was performed using *Statistical Package for the Social Sciences* software (SPSS), for windows 7.All data were presented as mean ±S.D (standard deviation). ANOVA test were used to compare between means of variables between males and females and within the same gender group, *p* values less than 0.05 were used as significant value.

**Result and discussion**

Figure 1 and Figure 2, shows no significant changes within patients groups while there was a significant changes between patients and controls, there was a significant increase in the levels of blood urea (BU) and serum creatinine (Cr) in the renal stone patients in comparison to the apparently healthy control subjects ( $P \leq 0.05$ ).



**Figure 1** Boood Urea levels in Healthy Controls and renal calculi patients. (the deferent liters mean there is a significant differences at  $p \leq 0.05$ )



**Figure 2** Serum Creatinine levels in Healthy Controls and renal calculi patients. (the deferent liters mean there is a significant differences at  $p \leq 0.05$ )

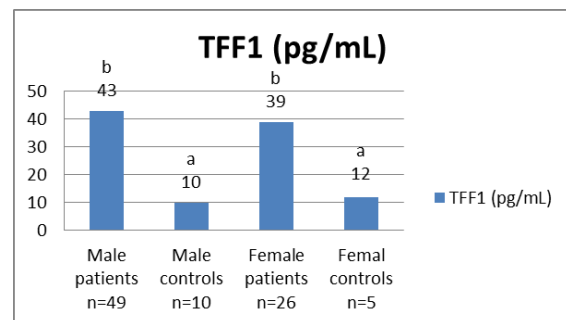
Renal stone injury diseases is a gradual, progressive and irreversible loss of normal functioning of kidneys[21].

Noor *et al.* (2014) explained that there is a steady and continued decreased in renal clearance or glomerular filtration rate (GFR), which leads to the accumulation of urea, creatinine and other chemicals in the blood [22].

The kidney stone may lead to Chronic renal failure, in this case the increase of serum urea is proportional to the progression of the disease, but it is highly influenced by a catabolic state or an excessive protein ingestion, leading to a higher production of other waste substances of protein catabolism[23].

As the excretory function of kidney is impaired, urea and creatinine excretion is hampered leading to its increased levels in blood, so significant elevation in blood urea and serum creatinine levels are observed in kidney patients in the present study [24].

Figure 3 shows no significant changes within patients groups while shows that the TFF1 levels were significantly increased in stone patients when compared with the healthy controls ( $P \leq 0.05$ ).



**Figure 3** TFF1 levels in Healthy Controls and renal calculi patients. (the deferent liters mean there is a significant differences at  $p \leq 0.05$ )

Lebherz *et al.* (2015), pronounced an increase in TFF1 urine levels with the onset of kidney diseases KD[25]. KD has a multifactorial origin and is associated with increased cell damage caused by uremic toxins, inflammation, and oxidative stress [26,27]. Persistent inflammation triggers sustained renal damage and contributes to the progression of kidney disease to end-stage renal failure. To minimize cell damage and limit ongoing cell death

counterregulatory mechanisms are initialized in order to hold progression of renal dysfunction[25].

TFF peptides are evolutionarily a highly conserved group of proteins which participate in epithelial protection and restitution. They promote cell migration as well as angiogenesis, limit proapoptotic stimuli, and facilitate leukocyte migration [15]. All TFF peptides are essential for epithelial restitution and can induce cell migration, but they differ in other accessory functions. An example is the tumor suppressive function of TFF1, which has been proven in an animal gastric cancer model [28].

The increased TFF1 urine concentrations are in accordance with the findings from other studies by Astor et al.(2011) and Du *et al* (2014), they stated that the damage was due to ongoing epithelial damage, TFF1 expression is up regulated in chronically inflamed tissues to ensure epithelial restitution and regeneration [19,20].

Rinnert *et al* (2010) stated that TFF peptides are synthesized all along the urinary tract, with TFF3

expression as the most pronounced. Therefore, it is not surprising to find elevated TFF3 levels in patients suffering from KD, an affliction associated with ongoing renal inflammation and epithelial damage [17].

### Conclusions and Recommendations

Our results indicate that TFF1 has the potential to early identify individuals at risk, and that changes in TFF peptide expression might predict disease progression.

The simultaneous evaluation of patients with different causes of KD might conceal important findings in certain afflictions, Therefore, further clinical testing and longitudinal surveys with more patients are necessary .

Finally, the ELIZA test kit that used for determining the TFF1 levels are relatively expensive therefore the development of a new cheaper test method to determine the TFF1 are needed .

**Table 1 Blood Urea and Serum Creatinine levels in Healthy Controls and renal calculi patients**

Biochemical Parameters	Healthy Controls (Mean ± SD) n.=15		patients (Mean ± SD) n.=75		p. value
	Male n=10	Female n=5	Male n=49	Female n=26	
Blood Urea (BU) (mg/dL)	24 ±5	26 ±3	72 ±12 *	68 ±8	0.05
Serum Creatinine (Cr) (mg /dL)	0.3 ±0.02	0.4 ±0.03	1.4 ±0.08*	1.2 ±0.1	0.05

**Table 2 TFF1 levels in Healthy Controls and renal calculi patients**

Biochemical Parameters	Healthy Controls (Mean ± SD) n.=15		Patients (Mean ± SD) n.=75		p. value
	Male n=10	Female n=5	Male n=49	Female n=26	
TFF1 (pg/mL)	10 ±4	12 ±5	43 ±7	39 ±3	0.05

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## تقييم دور الـ Trefoil Factor 1 كمؤشر حيوي في المراحل المبكرة للإصابة الكلوية في مرضى

### التحصي الكلوي

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### الملخص

إن تحصي الكلية هو مشكلة صحية عامة تؤثر على غالبية الشعوب في جميع أنحاء العالم. وبطبيعة الحال، هناك العديد من المواد، والبروتينات، والدهون، و glycosaminoglycans والمركبات غير العضوية، تعمل كمثبطات للتحصي في السائل الأنبيوي الكلوي الطبيعي تقوم بتنشيط نمو البلورة، والتجمع، و/ أو الالتصاق على الأسطح الكلوية، من بين هذه المثبطات تقوم بالتحري عن تركيز TFF1 (trefoil factor 1) كمثبط لتشكل الحصى وإصابة الكلى في مصل 75 مريض بحصى الكلى و 15 من الأشخاص الأصحاء باستخدام تقنية الاليزا. أظهرت النتائج عدم وجود فروق معنوية ضمن مجموعة المرضى بينما أظهرت وجود ارتفاع معنوي في معدلات الـ TFF1 في المرضى مقارنة مع المجموعة الضابطة عند مستوى معنوية (P ≤ 0.05) التي تدعم حقيقة أنه يمكن أن تستخدم كعلامة مبكرة لإصابة الكلى. كما أظهرت النتائج أيضاً عدم وجود فروق معنوية ضمن مجموعة المرضى في مستويات كلا من يوريا الدم وكرياتينين المصل بينما أظهرت وجود ارتفاع معنوي في التراكيز في المرضى مقارنة مع المجموعة الضابطة عند مستوى معنوية (P ≤ 0.05). هناك حاجة إلى مزيد من الأبحاث لإظهار التأثير الكابح للـ TFF1 في البول في مرضى حصى الكلى.