Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Apr 20; 8(B):262-267.
https://doi.org/10.3889/oamjms.2020.4160
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Nephrology*

Assessment of Urinary Kidney Injury Molecule-1 as an Indicator of Early Renal Insult in Children with Cystic Fibrosis

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Edited by: Sinisa Stojanoski
Citation: Shahin W, Badr A, Ahmed R, Alattar M, Alfalaki M, Rabie W. Urinary Kidney Injury Molecule-1 is a Useful Early Indicator of Renal Insult in Children with Cystic Fibrosis. Open Access Maced J Med Sci. 2020 Apr 20; 8(B):262-267. https://doi.org/10.3889/oamjms.2020.4160
Keywords: Cystic Fibrosis; Urinary β2–Microglobulin; Urinary kidney injury molecule-1 (KIM-1); Renal; Children
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Received: 07-Dec-2019
Revised: 07-Mar-2020
Accepted: 19-Mar-2020

Funding: The author(s) unveil that this work was totally funded by the contributing authors, with no financial assistance from any commercial or non-profit funding agencies
Competing Interests: The author(s) declare that there is not any commercial affiliation or consultancy of any of the authors that could be construed as a conflict of interest with respect to the submitted data.

Abstract

BACKGROUND: The risk of acute kidney injury in cystic fibrosis (CF) patients is due to renal tubular affection by *CFTR* gene.

AIM: Our study aimed at early detection of renal impairment in CF patients, to enable careful monitoring and adjustment of nephrotoxic medications.

METHODS: Fifty patients with CF were enrolled in our study; they were age- and sex-matched to 40 healthy control children. All subjects were screened by urine analysis, measurements of kidney function tests, fractional excretion of sodium, β2-microglobulin (beta-2-M) excretion, and renal ultrasound examination. Urinary kidney injury molecule-1 (KIM-1) was assayed using ELISA technique.

RESULTS: Both urinary beta-2-M and KIM-1 concentrations were significantly higher in CF patients compared to the control group ($p < 0.001$). The duration of the disease was significantly positively correlated with the urinary beta-2-M and KIM-1 levels ($r = 0.6$ and 0.7, respectively; $p < 0.01$).

CONCLUSIONS: Our results showed that urinary KIM-1 can be considered as a sensitive early indicator of acute renal injury.

Introduction

Cystic fibrosis (CF) is the most common lethal, autosomal, recessively transferred genetic disorder among Whites [1]. The *CFTR* gene encoding the CF transmembrane conductance regulator (*CFTR*) protein, spanning 1480 amino acids [2]. Epithelial cells of airways, the gastrointestinal tract (including the pancreas and biliary system), the sweat glands, and the genitourinary system mostly express *CFTR* gene [3]. *CFTR* gene dysfunction is the major cause of severe chronic lung disease in children, resulting in a wide and variable spectrum of manifestations and presenting complications [4]. Moreover, CF individuals are at risk of acute kidney injury (AKI) and the development of chronic renal disease, mainly due to exposure to multiple possibly nephrotoxic agents including aminoglycosides, nonsteroidal anti-inflammatory drugs, and immune suppressants. These factors contribute to the risk of AKI and ultimately the development of chronic renal disease [5]. A type-I transmembrane glycoprotein known as kidney injury molecule-1 (KIM-1) is primarily expressed on the surface of T-cells and possesses two extracellular domains [6]. Normal kidney shows low expression of KIM-1, however, it is significantly increased following kidney injury in cells of proximal tubules. Following kidney injury, the extracellular domains of KIM-1 separate from the cell surface and pass through urine in a metalloproteinase-dependent process [7]. Rapid increase in urinary KIM-1 develops on tubular injury, the degree of injury, interstitial fibrosis, and inflammation which are all correlated with this increase. Investigation of kidney diseases is frequently performed through the assessment of serum creatinine and urinary albumin. Yet, these biomarkers are not sensitive enough for early detection of changes in renal function [8]. Urine β2-macroglobulin (β2M) values indicate renal filtration disorders. Measurement of values in both serum and urine can help distinguish a problem of cellular activation from a renal disorder [9]. Increased urine β2M levels reflect tubular disorders of the kidney. In such cases, serum β2M levels are usually normal since the dysfunction is in tubular reabsorption [10].

In view of these facts, there is a desperate need to identify the most appropriate way of measuring and monitoring renal function in these potentially vulnerable patients. Awareness of the importance of renal function

monitoring is vital as by the time the patients' creatinine starts to increase they will have lost 50% of their renal function. With the modern advances in the diagnosis and management of CF patients and its impact on their survival for longer decades, it is imperious that we preserve their renal function, to maintain their aging years [11].

Based on the fact that *CFTR* is expressed in the renal tubules. We hypothesized in this work that there is some degree of renal impairment in young CF patients, even before exposure to provocation factors that will produce definite renal impairment later in life. Our aim was to detect early renal impairment in patients with CF through sensitive urinary biomarkers such as urinary KIM-1 and β_2-microglobulin (beta-2-M). Thus, we can closely monitor those patients and pay much attention to the prescribed medication, especially those with nephrotoxic effects such as Garamycin and Amikacin which are commonly used.

Methods

Study design

The current study included 50 patients with CF. Patients were recruited over a period of 2 years from the pulmonology unit at the Children's Hospital, Cairo University, Egypt. It is a tertiary referral teaching hospital serving a population of 20 million. The CF clinic follows up about 300 patients. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Cairo University, as to be in agreement to with Helsinki Declaration II, Finland; IRB approval number 02-25-2016. Informed consents were obtained from the legal guardians of all participating subjects.

Eligibility criteria

Children already diagnosed as CF patients based on symptoms, signs, and two positive sweat chloride tests ≥60 mmol/L (carried out at our hospital) as recommended by CF Foundation guidelines, using Wescor Macroduct system for collection of sweat by pilocarpine iontophoresis and then quantitative analysis by chloridometer in our laboratory [12]. Both genders were included with age ranges between 1 and 15 years.

Exclusion criteria

Patients with one or more of the following were excluded from the study:

a. Receiving systemic or inhaled Garamycin or Amikacin
b. Those with CF-related diabetes
c. Those with congenital renal anomalies or family history of renal diseases (renal stones and inherited tubular diseases).

Control group

The included patients were age and sex matched to 40 healthy children recruited from the ophthalmology clinic or from the vaccination center as a control group to compare the values of the urinary biomarker used in the study between healthy children and children with CF.

Analytic approach

All the CF patients were subjected to full clinical history taking and thorough clinical examination. They were all subjected to laboratory assessment according to the following protocol:

1. Sampling
   Blood and morning mid-stream urine specimens were collected, aliquoted, and stored at -80°C till the time of the assay
2. Biochemical analysis of urine samples.
   Urinary KIM-1 and urinary beta-2-M were assayed using commercially available ELISA kits (Catalog No: DKM100 and Catalog No: EM5001-1, respectively), following the manufacturer's instructions.

Renal imaging

The ultrasonic renal examination does not require any preparation of the patient and was performed with the patient in the supine position. The kidneys were examined in longitudinal and transverse scan planes with the transducer placed in the flanks. Patients were examined with a linear array transducer with higher center frequencies.

Statistical methods

Data were coded and entered using the statistical package SPSS version 22. Data were summarized using mean±standard deviation for quantitative variables and frequencies (percentages) for categorical variables. Comparisons between categories were made using unpaired t-test in normally distributed quantitative variables. Correlations between quantitative variables were carried out using Pearson's correlation coefficient. $p < 0.05$ was considered as statistically significant.

Results

The mean age of the study population was 4.65 years ranged between 1 and 11 years, whereas

the mean age at first presentation was 0.46 years ranged between 0.08 and 4 years. Twenty-nine patients (58%) were male and 21 (42%) were female, as shown in Table 1. Twenty-two patients (44%) were born from consanguineous marriage and 10 (25%) had sibling with CF in the same family. Baseline data of the studied population are illustrated in Table 1.

Table 1: Demographic data of CF patients

Variables	n=50
Age in years	4.65 (2.82)*
Age of first presentation in years	0.46 (0.61)*
Sweat chloride testing	95.80 (29.12)*
Male	29 (58)#
Female	21 (42)#
Positive consanguinity	22 (44)#
Positive family history	12.5 (25)#

*Data are presented as mean (standard deviation). #Data are expressed as frequency (%).

Regarding the clinical presentation of CF patients chronic cough was the main respiratory symptom (92%) and pulmonary exacerbations were present in most cases (78%) as the primary cause of attending the CF clinic. Pancreatic insufficiency (steatorrhea) is the main GIT symptom (90%). Failure to thrive, defined as weight and height below third centile, is present in most cases (92%) and only 1 patient (2%) had elevated liver enzymes, as shown in Table 2.

Table 2: Clinical manifestations among CF patients at the time of enrollment

Variable	n=50*
Chronic cough	46 (92)
Pulmonary exacerbations	39 (78)
Clubbing	18 (45)
Pancreatic insufficiency	45 (90)
Distal intestinal obstruction	3 (6)
Liver affection	1 (2)
Failure to thrive	46 (92)

*Data are presented as frequency (%).

Renal imaging showed that 49 patients (98%) had normal renal U/S studies and one patient had Grade-1 nephropathy. Routine urine analysis was normal in 84% of the cases, while only 16% showed some albuminuria, oxaluria, and hyperuricosuria, as indicated in Figure 1. The mean values of beta-2-M and KIM-1 in cases and control group presented in Table 3 were the results that showed significant increase of beta-2-M level in patients when compared to normal control with 36.75% and showed significant increase of KIM-1 level in patients when compared to normal control with 19.09% at $p < 0.05$.

Table 3: Urinary biomarker (β2MG and KIM-1) values in patients and control children

Variable	CF patients (n=50)	Control (n=40)	p-value
β2MG (ng/ml)	614.32 (40.08)	449.22 (46.33)	<0.05
KIM-1(ng/ml)	0.57 (0.09)	0.307 (0.043)	<0.001

Data are presented as mean (SD).

These results are illustrated in Figure 2, respectively. The duration of disease was positively correlated with both urinary biomarkers (beta-2-M and KIM-1) ($r = 0.83$ and 0.78, respectively, $p < 0.01$), as

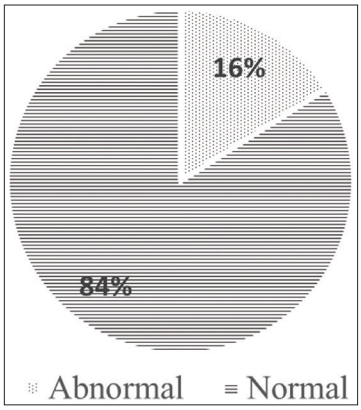

Figure 1: Urine analysis results among cystic fibrosis cases. Routine urine analysis was normal in 84% of the cases, while only 16% showed some albuminuria, oxaluria, and hyperuricosuria

presented in Figure 3. There was a non-significant correlation between both biomarker (beta-2-M and KIM-1) with fractional excretion of sodium (FE_{Na}), pH, bicarbonate, and sweat chloride testing at $p > 0.05$. There was a significant correlation between biomarker (beta-2-M and KIM-1) with the progression of age with $p = 0.01$, as presented in Table 4 and graphically illustrated in Figure 3. Beta-2-M and KIM-1 were positively correlated, $r = 0.531$, $p = 0.01$, as illustrated in Figure 4.

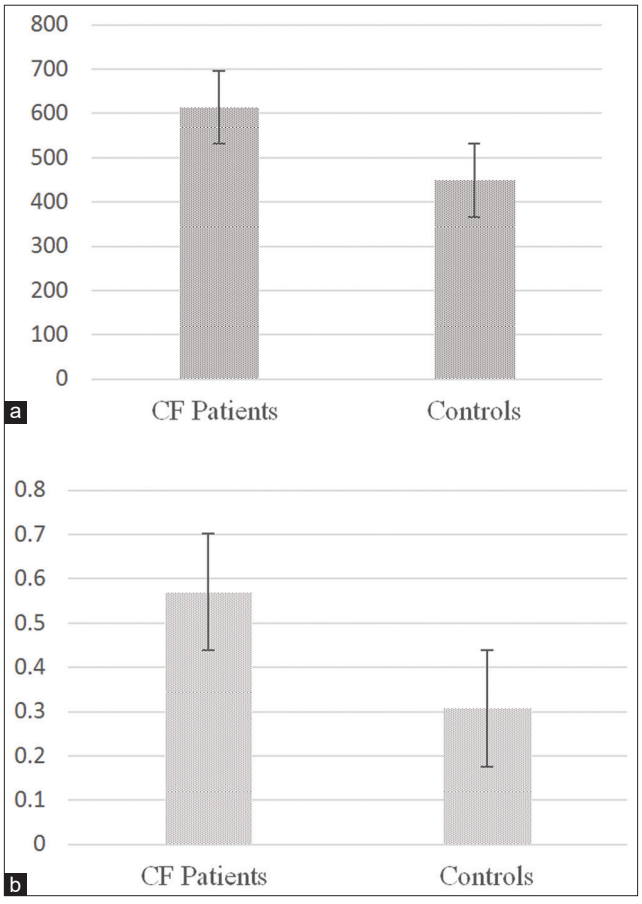

Figure 2: Comparison between beta-2-M and KIM-1 proteins in patients and controls. (a) Results show significant increase of beta-2-M level in patients when compared to normal control. (b) Significant increase in KIM-1 level in patients when compared to normal control.

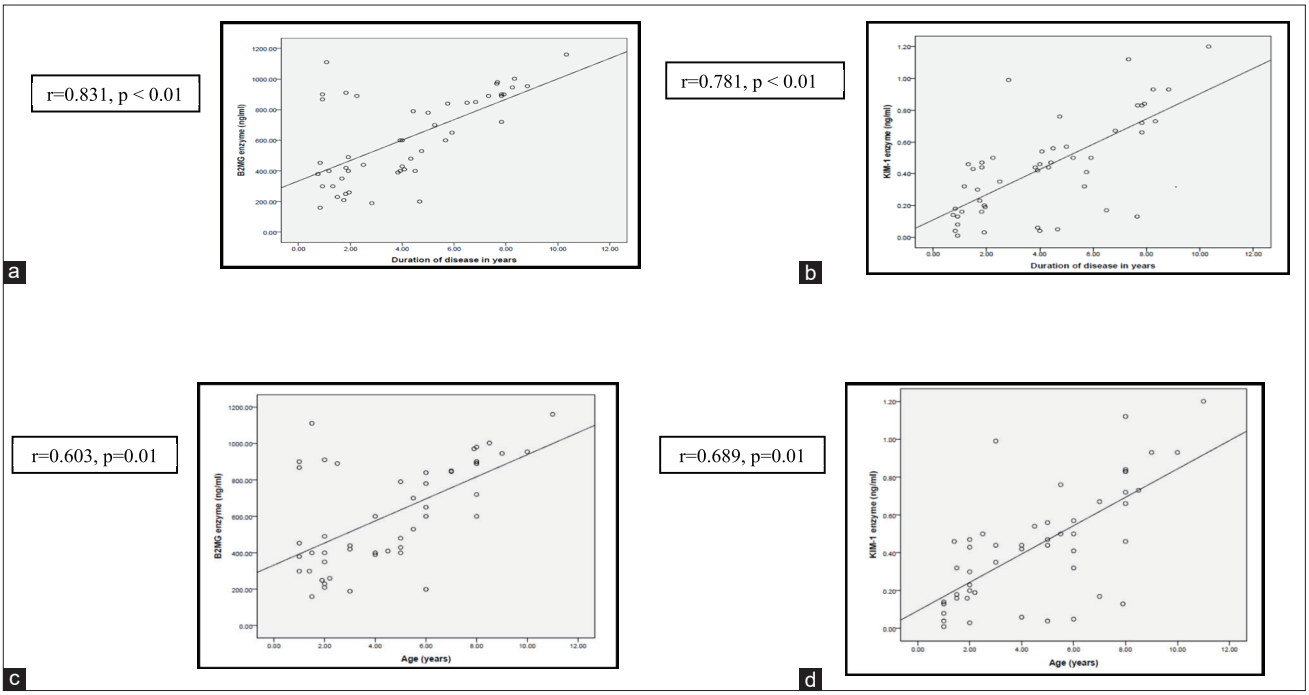

Figure 3: Correlation studies. (a) The duration of disease was positively correlated β2-microglobulin (beta-2-M), (r = 0.83, p < 0.01). (b) The duration of disease was positively correlated kidney injury molecule-1 (KIM-1), (r = 0.78, p < 0.01). (c) Significant positive correlation between urinary beta-2-M elevation and age (r = 0.603, p = 0.01). (d) Significant positive correlation between urinary KIM-1 elevation and age (r = 0.689, p = 0.01)

Discussion

Hypothesis regarding early renal impairment in young children with CF is based on the presence of CFTR in the renal tissue, as well as some few studies that searched for early renal involvement, urolithiasis, and estimated GFR in children with CF [13]. However, there are many factors affecting the renal function in CF due to increased life expectancy as well as the progression of the disease, recurrent infections, and subsequent use of potentially nephrotoxic antibiotics, and it is difficult to tell at a later stage the exact mechanism. Hence, we included young patients aged 1 till 11 years and excluded those receiving gentamycin or any nephrotoxic medications and those with no family history of renal disease. To the best of our knowledge, this is the 1st time in literature to study these two non-invasive urinary biomarkers (i.e., beta-2-M and KIM-1) in children with CF, which surprisingly were significantly high in CF patients compared to controls. All patients had normal serum Na, K, BUN, and creatinine level, signifying that creatinine is not a reliable early indicator during acute changes in kidney function, which goes in line with the study conducted by Boer *et al*., in 2010, showing that serum creatinine levels can vary greatly with personal factors such as age, gender, and muscle mass. Muscle metabolism, certain medications, and hydration status play a role in creatinine level. It will not become elevated obviously unless GFR has decreased by at least 50% below its normal values [14]. Eight patients (16%) had abnormal urine analysis, including albuminuria, oxalate, and uric acid and 42 patients (84%) had normal urine analysis, which shows that urine analysis only is not a reliable indicator of early changes in kidney function, as shown in the study of Shemesh *et al*. who evaluated the AKI after receiving

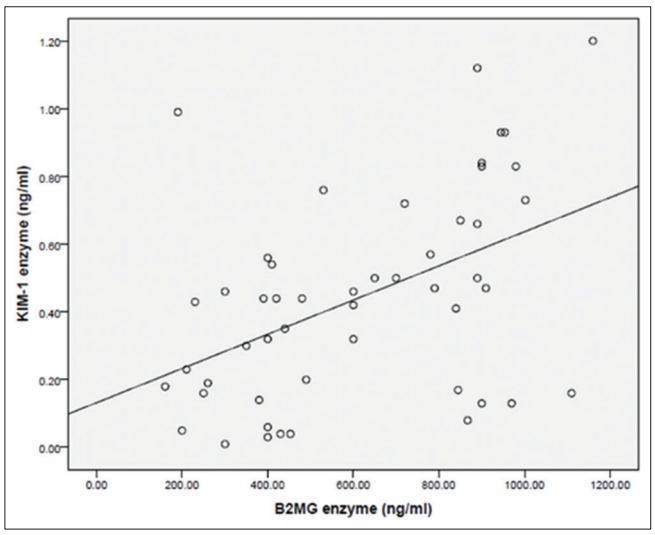

Figure 4: Correlation between urinary kidney injury molecule-1 and β2-microglobulin. This figure denotes a significant positive correlation between both markers, r = 0.531, p = 0.01

Table 4: Correlation studies for biomarker the assayed biomarkers (β2MG and KIM-1)

Variable	Beta-2-M (n=50)		KIM-1 (n=50)	
	r	p-value	r	p-value
Age	0.603	0.01	0.689	0.01
FE_{Na}	0.128	0.377	0.239	0.095
PH	0.161	0.264	–0.217	0.130
Bicarbonate	0.102	0.482	–0.066	0.651
Sweat chloride testing	0.72	0.618	0.05	0.970

three doses of cisplatin which is known nephrotoxic drug and showed that urine analysis was insensitive as an early indicator of acute renal injury [15]. Renal imaging showed that 49 patients (98%) had normal renal U/S studies and one patient had Grade-1 nephropathy, which also showed that renal U/S is not a reliable early indicator during acute changes in kidney function. This finding is similar with the study of Biggi *et al*., 2006, who evaluated the ultrasound parameters in patients with urinary tract infection, which concluded that ultrasound cannot distinguish cystitis from pyelonephritis in those studied children nor it can identify children with renal damage [16]. Beta-2-M is a good biomarker used to assess the glomerular and tubular functions. High level of serum β_2-M indicates glomerular malfunction, while elevated urinary β2M suggests proximal tubular dysfunction. Recently, measurement of urinary β2M has become a well-known method of tubular function assessment. Measurement of β2M in urine sediment is included in RenalVysion™ (Bostwick Laboratories, Uniondale, NY) as a biomarker to evaluate renal tubular function [17]. Our study showed significant elevation of urinary β2M levels in the diseased group compared to control group ($p < 0.05$), with an increase of 36.75% vis-à-vis control group.

KIM-1 is a Type-1 transmembrane protein that is not normally detected in the kidney tissue but is present at very high levels in the epithelial cells of the proximal tubule in human and rodent kidneys usually after ischemic or toxic injury. The KIM-1 ectodomain is stable in urine for long periods of time, it is easily detected in kidney as well as in urine after AKI [18]. Hereby, there was a significant elevation of urinary KIM-1 levels in diseased group compared to the control group, where $p < 0.05$, with an increase of 19.09%. The meta-analysis done by Shao *et al*., in 2014, detected that urinary KIM-1 was an accurate and reliable method of diagnosis in infants and children rather than in adults. They concluded that the comorbid conditions, such as hypertension, diabetes mellitus, and atherosclerosis, which are more common in adults may influence urinary KIM-1 concentrations in adults. Furthermore, it was noted that the urinary KIM-1 is fairly sensitive 74.0% and highly specific 86.0% in diagnosing AKI [19].

These data support our hypothesis that renal injury starts early in life of patients with CF. Furthermore, both urinary levels of beta- 2-M and KIM-1, have significant correlation with the duration of disease in CF children, $p = 0.01$; indicating that there is progression of renal disease in CF patients regardless of the use of nephrotoxic medications. When comparing both biomarker (beta-2-M and KIM-1) with FE_{Na}, blood pH, and bicarbonate, there was no significant correlation between them. This also applies to the values of the sweat chloride testing. By correlating the two biomarkers (beta-2-M and KIM-1), there was a significant direct correlation ($p = 0.01$) between them, where $r = 0.467$.

Conclusions

Renal impairment among CF patients increases with age. Urinary KIM-1 and beta-2-M are good early indicators of renal impairment in CF patients. Further studies with a larger population with different ethnicity are needed to support our study regarding early renal impairment in patients with CF. Thus, close renal monitoring of those patients and avoidance of factors aggravating renal impairment such as nephrotoxic antibiotics and salt depletion in addition to proper diet control are recommended to keep good nutritional status and overall good health condition.

Authors' Contributions

All authors have substantially contributed to the intellectual content of this paper.

Walaa A Shahin, MD: Study design, conception and acquisition of clinical data and clinical application of results, manuscript writing, revising the article for intellectual content, and final approval of the version to be published.

Ahmed Bader, MD: Study design, conception and acquisition of clinical data and clinical application of results, manuscript writing, revising the article for intellectual content, and final approval of the version to be published.

Rawdah Ahmed, MSc: Acquisition of data and samples, genetic analysis and laboratory work, interpretation of results, and data analysis.

Mona Alattar, MD: Revising the article for intellectual content and final approval of the version to be published.

Mona Alfalaki, MD: Revising the article for intellectual content and final approval of the version to be published.

Walaa A Rabie, MD: Study design, genetic analysis, interpretation of the results and data analysis, manuscript writing, revising the article for intellectual content, and final approval of the version to be published.

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