Evaluation of renal tubular injury in Egyptian patients with cystic fibrosis

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Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
AKI	Acute kidney injury
ARF	Acute renal failure
β2MG	Beta-2-microglobulin
Carb Hb	Carbamylated hemoglobin
CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFRD	Cystic Fibrosis Related Diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator protein
DIOS	Distal intestinal obstruction syndrome
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ENaC	Epithelial sodium channel
FDA	Food and Drug Administration
FE_{Na}	Fractional excretion of sodium
GFR	Glomerular filtration rate
GST	Glutathione-S-transferase
HPOA	Hypertrophic pulmonary osteoarthropathy
HRCT	High resolution computerized tomography
ICS	Inhaled corticosteroids
IRT	Immune reactive trypsinogen
KIM-1	Kidney injury molecule-1
LDH	Lactate dehydrogenase
MI	Meconium ileus
MMP-9	Matrix metalloproteinase-9
MMW	Middle molecular weight
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NHE3	Sodium hydrogen exchanger 3
NPD	Nasal potential difference

NSAID	Non-steroidal anti-inflammatory drugs
PI	Pancreatic insufficiency
ProAnp	Prohormone of atrial natriuretic peptide
PS	Pancreatic sufficiency
SE	Standard error
SPSS	Software package used for statistical analysis
T1DM	Type (1) diabetes mellitus
T2DM	Type (2) diabetes mellitus
TIM	T cell Immunoglobulin-Mucin
UO	Urine output
UTI	Urinary tract infection
VUR	Vesico-ureteral reflux

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Abstract

Background and objective: Cystic fibrosis is the most common life-limiting autosomal recessive disease among people in the USA and Europe with increased prevalence in Egypt. Pulmonary disease is the most important cause of morbidity and mortality in CF. Affected children are also in danger of acute kidney injury and the development of chronic renal disease due to presence of CFTR gene in renal tubules as well as exposure to multiple potentially nephrotoxic agents including aminoglycosides, non-steroidal anti-inflammatory drugs NSAIDs and immune-suppressants. The aim of our study is to early detection of renal impairment in Egyptian patients with CF and hence careful monitoring and adjustment of medications especially nephrotoxic drugs like Garamycin, Amikacin and Ciprofloxacin which are used repeatedly in these patients.

Subjects and methods: Fifty CF patients were enrolled in our study from the allergy and pulmonology unit in Children Hospital of Cairo University. Diagnosed based on clinical manifestations and elevated sweat chloride testing ≥ 60mg/L. They were age and sex matched to forty healthy children as a control group. All patients are screened for renal affection by measuring urine analysis, kidney function tests, FE_{Na}, β2MG, KIM-1 in urine using ELISA technique and renal U/S.

Results: Out of 50 children there are 30 patients (60%) have high levels of β2MG and 33 patients (66%) have high levels of KIM-1. There were significant differences between β2MG enzyme level and KIM-1 enzyme level in case and control children (P value < 0.05). Serum creatinine and renal U/S were normal for all patients. There was significant Correlation between urinary β2MG, KIM-1 enzymes elevation and duration of disease (P value < 0.01, P value <0.01 respectively).

Conclusion: In conclusion, this study confirms presence of early renal impairment among CF patients and urinary β2–Micro globulin β2MG ,Urinary kidney injury molecule-1 KIM-1 enzymes are a good early indicator of renal impairment in cystic fibrosis patients. Further studies with a larger sample size are needed to evaluate urinary enzymes as sensitive tools useful in the early diagnosis of acute renal injury before conventional laboratory assays become deranged.

Keywords: Cystic fibrosis, urinary β2–Micro globulin, Urinary kidney injury molecule-1 enzyme, Egypt.

Introduction

Cystic fibrosis CF is the most common lethal autosomal recessively transmitted genetic disorder among whites **(Maurya, et al. 2012).**

The few studies available about CF in Arabs are suspecting the presence of many undiagnosed patients and emphasize a higher incidence rate particularly in view of the high consanguinity rate in the range of 25–60%. Furthermore, the population of the region is characterized by large family size, high fertility rates, high maternal and paternal age, and high rate of marriage among members of the same tribes. And in Lebanon, Desgeorges, et al. detected a 50% rate of consanguineous marriage **(Desgeorges, et al. 1997)**. Previous study showed a higher rate of consanguineous marriage reaching to 84% ,with increased prevalence in Egypt **(El-Falaki, et al. 2014)**.

Dysfunction of the cystic fibrosis transmembrane conductance regulator protein CFTR leads to a wide and variable array of presenting manifestations and complications. CF is responsible for most cases of exocrine pancreatic insufficiency in early life and is the major cause of severe chronic lung disease in children. It is also responsible for many cases of hyponatremic salt depletion, nasal polyposis, pansinusitis, rectal prolapse, pancreatitis, cholelithiasis, and no autoimmune insulin-dependent hyperglycemia. Because CF may manifest as failure to thrive and, occasionally, as cirrhosis or other forms of hepatic dysfunction, this disorder enters into the differential diagnosis of many pediatric conditions **(Kliegman, et al. 2015).**

Although CFTR is found in the kidney, mainly in the proximal and distal tubules, and its inactivation can cause low molecular weight proteinuria, its exact role and effect in CF related kidney disease is unknown. Patients with CF are chronically exposed to several potentially nephrotoxic factors, these include bacterial infections; colonization with Pseudomonas aeruginosa, and their

associated immune complexes and the antibiotics (aminoglycosides) which are used in their treatment. In addition, diabetes mellitus, liver disease, may produce renal injury. Nephropathy can also result from abnormalities in salt transport, and the development of CF related diabetes CFRD requiring insulin **(Nazareth and Walshaw 2013).**

These factors put these patients in danger of acute kidney injury AKI and the development of chronic renal disease **(Prestidge, et al. 2011)**.

Aim of the work

We hypothesize in this study that there is some degree of renal impairment in patients with CF that needs special tool to be able to detect it early before conventional labs and clinical manifestations are affected which if occurred it will be at a late stage of renal disease, so our aim is to detect early renal impairment in Egyptian patients with CF and hence we can monitor those patients carefully and adjusting their medication, especially those with nephrotoxicity like Garamycin, Amikacin and Ciprofloxacin which are commonly used in those patients.

CYSTIC FIBROSIS

Definition and Etiology:

Cystic fibrosis CF is an inherited multisystem disorder of children and adults; it is the most common life-limiting recessive genetic trait among whites **(Waters 2012)**.

CF is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator Protein CFTR **(des Georges, et al. 2004)**. CFTR, which is located on chromosome 7, encodes a chloride channel involved in electrolyte exchanges through the plasma membrane of numerous epithelial cell types and leads to a dysfunction of the exocrine glands. Mutations in CFTR genes result in a defective mucociliary clearance with production of viscous and sticky bronchial mucus that facilitates colonization with airborne bacteria and fungal spores **(Müller, et al. 2011).**

Dysfunction of the CFTR leads to a wide and variable array of presenting manifestations and complications. CF is responsible for most cases of exocrine pancreatic insufficiency in early life and is the major cause of severe chronic lung disease in children. It is also responsible for many cases of hyponatremic salt depletion, nasal polyposis, pansinusitis, rectal prolapse, pancreatitis, cholelithiasis, and nonautoimmune insulin-dependent hyperglycemia. Because CF may manifest as failure to thrive and, occasionally, as cirrhosis or other forms of hepatic dysfunction, this disorder enters into the differential diagnosis of many pediatric conditions**(Kliegman et al., 2015)** .

Incidence of Cystic Fibrosis:

The mean incidence of cystic fibrosis among North Americans of European ancestry is 1 in 2,500. Studies carried out in different geographic areas report incidence values ranging from 1 in 1,800 to 1 in 8,500, suggesting that incidence have to be assessed specifically for any population. The average CF incidence in Italy was found to be 1 in 4,238, which is lower than the values reported elsewhere **(Bossi, et al. 2004).**

In developing countries, CF had remained largely unrecognized **.**In Egypt, the first study aiming at evaluating the magnitude of the CF problem in Egypt was done by Abdel Salam and colleagues 1993 and reported a prevalence rate of 1:2664 in 18,560 screened newborns and 1:56 in a series of 224 high risk children **(Abdel-Salam, et al. 1993)**. In another more recent study done by Naguib and colleagues, 61 patients suspected of having CF were screened using the CF Indicator sweat test system for qualitative assessment of the sweat chloride concentration. Of the 61 patients, 12 (20%) had positive sweat chloride screening. Ten of the 12 patients underwent quantitative sweat testing and were positive **(Naguib, et al. 2007)**. Another study was done in 2014 revealing that the prevalence of CF in Egyptian patients is 36%, which indicates that increasing medical awareness about CF had led to diagnosis of more patients**(El-Falaki et al., 2014).**

Some reports have been published about CF patients in the Middle East **(Eskandarani 2002)**. These reports showed the following frequencies, 1:5800 in Bahrain **(Al-Mahroos 1998)**, 1:2650 in Jordan, 1:2560 in Kuwait **(Nazer 1992)**, and 1: 15,876 in United Arab Emirates **(Frossard, et al. 1998)**. Though CF was generally believed to be rare or nonexistent in Saudi Arabia, in one study, 21 Saudi children were diagnosed as having CF, evidenced by typical clinical features and elevated sweat chloride concentrations from seven referral

centers over a period of 10 years **(Nazer, et al. 1989)**. The few studies available about CF in Arabs are suspecting the presence of many undiagnosed patients and emphasize a higher incidence rate particularly in view of the high consanguinity rate in the range of 25–60% **(Alwan, et al. 1997).**

Historical Background of CF:

In 1949, it was postulated that cystic fibrosis must be caused by a defect in a single gene (and therefore a single protein) on the basis of the autosomal recessive pattern of inheritance of the disease. The characterization of the molecular mechanism, therefore, included early attempts to identify the causative protein. High levels of salt in the sweat of patients with cystic fibrosis suggested an abnormality in fluid and electrolyte transport in the sweat gland, and so established that sweat ducts in such patients are impermeable to chloride, Studies of nasal epithelium and analysis of epithelial cells from the airways of patients with cystic fibrosis provided conclusive evidence of a defect in chloride permeability of plasma membranes in the lung **(Dodge and Boulyjenkov 1992).**

These findings, which were confirmed by several laboratories worldwide, led to the hypothesis that a defective chloride channel situated in the apical membranes of the lung surface or glandular epithelium accounts for respiratory failure and this abnormality could explain the other clinical manifestations of cystic fibrosis **(Rowe and Clancy 2006).**

Soon after the discovery of abnormal chloride transport in cystic fibrosis, scientists had identified the gene that is responsible for the disease independently of any prior knowledge of the structure of the cystic fibrosis protein. They named the gene product CFTR, since the predicted protein sequence did not resemble other ion channels. This name posed a problem for