UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Medicina
Programa de Pós-graduação em Ciências da Saúde - Saúde da Criança e do Adolescente

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BIOMARCADORES URINÁRIOS EM PACIENTES PEDIÁTRICOS COM RIM SOLITÁRIO FUNCIONAL CONGÊNITO: UM ESTUDO PILOTO

Linha de pesquisa – Distúrbios dos Rins e Trato Urinário

Belo Horizonte
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BIOMARCADORES URINÁRIOS EM PACIENTES PEDIÁTRICOS COM RIM SOLITÁRIO FUNCIONAL CONGÊNITO: UM ESTUDO PILOTO

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BIOMARCADORES URINÁRIOS EM PACIENTES PEDIÁTRICOS COM RIM SOLITÁRIO FUNCIONAL CONGÊNITO: UM ESTUDO PILOTO

EDUARDA ALMEIDA WAKABAYASHI MACIEL

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(Salmos 126:3)

RESUMO

***Introdução*:** O rim solitário funcional (RSF) é um importante subgrupo dentro do espectro das Anomalias Congênitas dos Rins e Trato Urinário (CAKUT). Níveis séricos de ureia e creatinina ainda são os marcadores mais utilizados na avaliação da função renal, embora não permitam detecção precoce de injúria renal. Nesse sentido, o objetivo deste estudo piloto foi medir a concentração urinária de biomarcadores renais em pacientes pediátricos com RSF e comparar com as medidas dos mesmos marcadores em crianças saudáveis. ***Pacientes e Métodos*:** Este estudo transversal incluiu 30 pacientes pediátricos com RSF congênito (agenesia renal, hipodisplasia renal primária e rim displásico multicístico) e 20 indivíduos saudáveis pareados por idade e sexo (controles). Todos os participantes foram submetidos a coleta de amostra única de urina para mensuração de dezessete marcadores urinários, sabidamente associados a função e/ou lesão renal. Medições de Calbindina, Colágeno IV, FABP1, GSTα, IP-10, KIM-1, Osteoactivina, Renina, TFF-3, TIMP-1, α-1-Microglobulina, Albumina, Clusterina, Cistatina C, EGF, Lipocalina-2 / NGAL e Osteopontina foram realizados usando os painéis 1 e 2 de kits multiplex de lesão renal. Os dados foram analisados no software GraphPad Prism versão 6.0. ***Resultados*:** Os marcadores Cistatina C, Osteopontina, Calbindina, Osteoactivina, TIMP-1, KIM-1, IP-10, Renina, EGF e Clusterina apresentaram valores significativamente menores nas amostras de urina dos pacientes comparado com os controles. Lipocalina-2/NGAL foi a única molécula com resultados significativamente maiores em pacientes comparado com controles. Os demais marcadores apresentaram níveis semelhantes em ambos os grupos. Os níveis séricos de creatinina aumentaram significativamente desde o diagnóstico até a última avaliação ambulatorial antes do fechamento do banco de dados deste estudo, enquanto a taxa de filtração glomerular diminuiu. ***Conclusão*:** em pacientes pediátricos com RSF, o comportamento de algumas moléculas urinárias é diferente do observado em controles saudáveis. Estudos longitudinais devem ser realizados para validar a utilidade clínica desses marcadores.

PALAVRAS-CHAVE: Função renal, biomarcadores urinários, rim solitário congênito, CAKUT, doença renal crônica, pediatria.

ABSTRACT

Background: Solitary Functioning Kidney (SFK) is an important subgroup of the Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) spectrum. Serum urea and creatinine levels are still the most used markers to assess renal function, even though these measurements do not allow early detection of renal injury. In this sense, the aim of this pilot study was to measure urinary concentration of kidney biomarkers in pediatric patients with SFK and to compare with the same biomarkers measurements in healthy children and adolescents. ***Patients and methods:*** This cross sectional study included 30 pediatric patients with congenital SFK (renal agenesis, primary renal hypodysplasia, and multicystic dysplastic kidney) and 20 healthy individuals paired for gender and age (controls). All the participants underwent a single urine collection to measure seventeen urinary biomarkers, known to be related to kidney function and/or lesion. ***Results:*** Cystatin C, Osteopontin, Calbindin, Osteoactivin, Tissue inhibitor of metalloproteinase 1, Kidney injury molecule-1, Protein induced by interferon, Renin, Epithelial Growth Factor and Clusterin were significantly reduced in urine samples of patients in comparison with controls. Lipocalin-2/NGAL was the only molecule with significantly increased levels in patients when compared with controls. The remaining molecules were similar in both groups. Serum levels of creatinine increased significantly from baseline to the last outpatient visit before closing the database of this study, whereas estimated GFR reduced. ***Conclusion:*** In pediatric patients with congenital SFK, the behavior of some urinary molecules is different from healthy controls. Longitudinal studies should be performed to validate the clinical utility of these biomarkers.

KEYWORDS: Renal function, urinary biomarkers, solitary functioning kidney, CAKUT, chronic kidney disease, pediatrics.

LISTA DE ILUSTRAÇÕES

Artigo de revisão:

Artigo original:

LISTA DE ABREVIATURAS E SIGLAS

B2M – Beta-2-microglobulina

CAKUT – Congenital Anomalies of the Kidney and Urinary Tract

Cysc C – Cistatina C

DRC – Doença renal crônica

EGF – Fator de crescimento epidérmico (Epidermal growth factor)

FABP1 – Proteína ligadora de ácidos graxos 1 (Fatty acid binding protein 1)

GST-alfa – Alfa-glutationa s-transferase (Alpha-glutathione S-transferase)

IP-10 – Proteína 10 induzida por interferon gama (Interferon-gamma-induced protein 10)

ITU – infecção do trato urinário

KIM 1 – Molécula de injúria renal 1 (Kidney injury molecule 1)

MCP-1 – Proteína quimiotática de monócitos 1

NGAL – Lipocalina associada a gelatinase e neutrófilos (Neutrophil gelatinase-associated lipocalin)

OPN – Osteopontina (Osteopontin)

RSF – Rim solitário funcional

TFF-3 – Fator trifólio 3 (Trefoil Factor 3)

TFG – Taxa de filtração glomerular

TIMP-1 – Inibidor tecidual de metaloproteinase 1 (Tissue inhibitor of Metalloproteinase 1)

UFMG – Universidade Federal de Minas Gerais

NOTA EXPLICATIVA

A apresentação da presente dissertação foi organizada sob a forma de redação de artigos científicos, de acordo com a resolução 03/2010 aprovada pelo Programa de Pós-Graduação em Ciências da Saúde – Área de Concentração em Saúde da Criança e do Adolescente, da Faculdade de Medicina da Universidade Federal de Minas Gerais, disponível em: https://ftp.medicina.ufmg.br/cpg/programas/saude_crianca/arquivos/2013/resolucao_03_2010_regulamenta_formato_de_teses_e_dissertacoes.pdf

O artigo original desta dissertação avaliou os níveis urinários de biomarcadores de função/lesão renal que incluíram Calbindina, Colágeno IV, FABP1, GSTα, IP-10, KIM-1, Osteoactivina, Renina, TFF-3, TIMP-1, α-1-Microglobulina, Albumina, Clusterina, Cistatina C, EGF, Lipocalina-2/NGAL e Osteopontina em pacientes com rim solitário funcional congênito acompanhados no ambulatório de nefrouropatias da Unidade de Nefrologia Pediátrica do Hospital das Clínicas da UFMG. O artigo foi submetido para publicação no Jornal de Pediatria e aguarda parecer dos revisores.

O artigo de revisão discute os aspectos do rim solitário funcional congênito, abordando sua fisiopatologia, diagnóstico, complicações, prognóstico, seguimento clínico e papel dos novos marcadores urinários no acompanhamento desses pacientes. O referido artigo foi aceito para publicação na *Current Medicinal Chemistry*.

As referências bibliográficas estão dispostas ao final de cada artigo ou seção, conforme as normas de Vancouver (Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication - www.ICMJE.org).

SUMÁRIO

1. INTRODUÇÃO

O desenvolvimento renal tem início entre a quarta e quinta semanas de gestação e caracteriza-se por interações complexas entre o ducto mesonéfrico e o mesênquima meta-néfrico [1–4]. Como resultado desse processo, os néfrons são formados até a 34ª a 36ª semana de gestação, sem a possibilidade de formação adicional de unidades funcionais após o nascimento. Isso significa que o número total de néfrons de um recém-nascido (cerca de 300.000 a 1.100.000 de néfrons por rim - com alta variabilidade interindividual) deve durar por toda a vida do indivíduo [5–7].

As anomalias do trato urinário (CAKUT) representam um subgrupo de prevalência considerável dentre as anomalias congênitas mais comuns [8, 9]. Por este motivo, constituem uma das principais causas de morbidade na população pediátrica e representam coletivamente um fator de risco relevante para o desenvolvimento de doença renal crônica em crianças e adultos jovens [10,11–14].

Dentre as inúmeras condições que integram o grupo das CAKUT, o rim solitário funcional (RSF) destaca-se como um importante subgrupo. Esta condição caracteriza-se pela ausência anatômica ou funcional de um rim desde o nascimento e seus principais fenótipos são a agenesia renal unilateral, a hipodisplasia renal primária e o rim displásico multicístico [8,15]. A incidência de RSF é de cerca de 0,05% e a associação com outros CAKUT é relativamente frequente. Estudos incluindo pacientes pediátricos com rim único reportam incidência de CAKUT associado em 26-47% dos casos [4,5,10,14,16].

Alguns estudos indicam que pacientes com RSF congênito estão propensos a desenvolver hipertensão, proteinúria, glomeruloesclerose e doença renal crônica [15,17-20]. No entanto, há controvérsia a este respeito e a incidência desses desfechos clínicos ainda não é bem caracterizada na literatura [12,15,17,21,22]. Poucos trabalhos avaliaram resultados em longo prazo de pacientes com CAKUT, o que se deve em parte à falta de uma classificação homogênea e às dificuldades na separação dos diferentes subfenótipos. Porém, sabe-se que RSF com CAKUT ipsilateral tem pior prognóstico que RSF sem CAKUT associado [8,14,16, 22-26].

Uma das teorias desenvolvidas para explicar a pior sobrevida renal de pacientes com RSF considera que a redução na massa renal, que é característica deste condição, resulta em um ciclo vicioso de hiperfiltração glomerular, que pode ocasionar, em longo prazo, injúria renal – expressa como hipertensão, microalbuminúria e/ou doença renal crônica [27].

Enquanto alguns autores consideram que o mecanismo da hiperfiltração compensatória seja uma alteração adaptativa benéfica para o indivíduo, por evitar a redução da função renal que

inevitavelmente ocorreria nesses pacientes, estudos mais recentes alertam sobre o potencial deletério desses mecanismos compensatórios [13,17,28], considerando que a sobrecarga glomerular contínua favoreceria lesão renal prematura e consequente progressão para doença renal crônica [29,30].

Sabe-se que a taxa de mortalidade de pacientes com injúria renal aguda grave que necessitam de diálise não diminuiu significativamente nos últimos 50 anos e que a concentração sérica de creatinina, embora amplamente utilizada na prática clínica, representa uma medida tardia e pouco confiável para identificação de alterações agudas da função renal [31,32–35]. Nesse sentido, a busca por novos biomarcadores capazes de predizer precocemente a ocorrência de dano renal tem assumido grande importância [30,36,37], especialmente em pacientes com risco aumentado de evolução desfavorável.

O campo de pesquisa dos biomarcadores de lesão renal é amplo, porém pouco estudado. Nesse contexto, o presente estudo pode ser considerado um trabalho preliminar que se propôs a fazer uma busca mais ampla de potenciais marcadores urinários que devam ser alvo de investigações futuras.

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2. REVISÃO DE LITERATURA

Review Article

CONGENITAL SOLITARY FUNCTIONING KIDNEY: A REVIEW

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List of abbreviations

AKI - acute kidney injury

BMI - body mass index

BUN - blood urea nitrogen

β2M - β-2-microglobulin

CAKUT - Congenital Anomalies of the Kidney and Urinary Tract

CKD - chronic kidney disease

DMSA - dimercapto-succinic acid

EGF - Epidermal growth factor

ESRD - end-stage renal disease

FABP-1 - Fatty acid binding protein

GFR - glomerular filtration rate

GST-α - α-glutathione S-transferase

IL-1β - interleukin-1 beta

IP-10 - Protein induced by interferon

KIM-1 - Kidney injury molecule 1

M-CSF - macrophage colony-stimulating factor

MAG-3 - mercapto acetyl tri glycine

MCDK - multicystic dysplastic kidney

MCP-1 - monocyte chemotactic protein-1

MRI - magnetic resonance imaging

NGAL - neutrophil gelatinase-associated lipocalin

PUJO - pelviureteric junction obstruction

SFK - Solitary functioning kidney

SNGFR - single nephron glomerular filtration rate

TFF-3 - Trefoil factor 3

TIMP-1 - Tissue inhibitor of metalloproteinase 1

TNFα - tumor necrosis factor alpha

URA - unilateral renal agenesis

US - ultrasonography

UTI - urinary tract infection

VUR - vesicoureteral reflux

Abstract: *Background*: Solitary functioning kidney (SFK) is a subgroup of the Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT). Although in the past the prognosis of these patients was considered good, numerous studies have shown different levels of kidney damage associated with this condition. Serum creatinine measurement is still the most used marker to assess renal function, even though the limitations are widely known. *Objective*: The present review aimed to summarize and update the scientific literature on congenital SFK, discussing its pathophysiology, diagnosis, complications, prognosis, role of novel urinary biomarkers, treatment and follow-up. *Results:* The natural history of congenital SFK is still an unresolved issue due to several factors. Although it has not yet been proven in humans, *Brenner*'s hyperfiltration hypothesis is the most concrete theory to explain the poor renal outcomes of patients born with one functioning kidney. The search for novel urinary biomarkers capable of assessing renal function and predicting renal outcomes has already started, but there are still few studies with this specific population. Among the most studied markers, Cystatin C, EGF and NGAL have shown potential usefulness for the follow-up of these patients. The treatment still relies on the search for kidney injury and general renoprotective measures. *Conclusion:* Further research with longer follow-up duration is needed to better understand the natural course of congenital SFK and the role of novel urinary biomarkers in this specific population. Thus, it will be possible to improve the prognosis of these patients.

Keywords: solitary functioning kidney; CAKUT; children; renal function; hyperfiltration; urinary biomarkers; chronic kidney disease.

1. INTRODUCTION

Congenital anomalies of the kidneys and urinary tract (CAKUT) are the spectrum of disorders caused by defects in embryonic development of the urinary tract, which can affect the kidneys, the collecting system, bladder, and/or urethra [1]. Excluding genetic anomalies, they represent the most common group of congenital alterations [2] and, collectively, are the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) in childhood [3–8]. These abnormalities result from the interaction between genetic and environmental factors. It is believed that maternal diseases (such as gestational diabetes), as well as the use of medications during pregnancy, are capable of influencing the process of renal morphogenesis [9–11].

Among the subtypes of CAKUT, the solitary functioning kidney (SFK) stands out. It is considered a relatively common birth defect and has an estimated incidence of 1 in 1000-1500 births [12–15]. The main phenotypes of congenital SFK include renal agenesis, primary renal hypodysplasia, and multicystic dysplastic kidney (MCDK) [2, 16].

Being born with a single functioning kidney implies having a smaller kidney mass than healthy individuals. Despite the attempted adaptation with compensatory hyperplasia and hypertrophy of the remnant nephrons since the intrauterine period, the total amount of functioning renal tissue remains reduced. Until a few years ago, the prognosis of these patients was considered to be good and the compensatory mechanism of hyperfiltration was seen as a beneficial adaptation [11, 17, 18]. However, in the past two decades, numerous studies with pediatric patients with SFK have shown different levels of kidney damage [2, 6, 19–22]. These changes are associated with arterial hypertension and proteinuria [11], renal tissue injury [14, 23], and a significantly higher risk for dialysis in adult life [3, 20, 24].

In addition to the fact that the natural history of children with SFK is not fully established, there is still no consensus on the best method to assess and monitor these patients [3, 11]. Serum creatinine and creatinine clearance, still used to estimate GFR in clinical practice, are known to be inaccurate measures, especially in patients with SFK [6, 25]. Other urinary molecules are under investigation as potential markers of renal function and injury [26–28].

This study aimed to summarize and update the scientific literature on congenital SFK, reinforcing the importance of studying this condition, as well as discussing the pathophysiology, diagnosis, complications, prognosis, urinary biomarkers, treatment and follow-up.

2. SOLITARY FUNCTIONING KIDNEY

2.1 Definition, Epidemiology and Pathophysiology

Congenital SFK is a common abnormality in the spectrum of CAKUT and is defined as the anatomical or functional absence of a kidney from birth [16, 26]. Recent studies have estimated its incidence at about 0.05% [17] and the association with ipsilateral CAKUT in a variation of approximately 26-47%, including vesicoureteral reflux (VUR), pelviureteric junction obstruction (PUJO), megaureter or a duplex kidney [6, 7, 11, 17, 23, 29].

Human kidney development begins at the fifth gestational week. The process results from complex interactions between the growing ureteral bud of the mesonephric duct and the metanephric mesenchyme [7, 30–33]. In normal human kidney development, the metanephros contributes to the mesonephric bud, from which the ureteric buds arise, forming the collecting system of the kidney [34]. The outgrowth of the ureteric bud occurs as a consequence of the secretion of glial cell line-derived neurotrophic factor by the undifferentiated mesenchyme of the metanephrogenic blastema [35]. Urine production starts around 10 weeks of gestation and it is the major contributor to amniotic fluid from about 14 weeks [34]. Nephrons are formed until 34 to 36 gestational weeks. The possibility of additional nephron formation after 36 weeks is very unlikely. At birth, there are approximately 900,000 nephrons per kidney, with high interindividual variability [11, 36, 37]. The total nephron number remains stable, but nephron size is expected to increase during childhood [34].

Congenital SFK is attributed to three major conditions: multicystic dysplastic kidney (MCDK), unilateral renal agenesis (URA) and primary renal hypodysplasia [6, 11, 38, 39]. The first two conditions occur as a consequence of abnormal interactions of the metanephric blastema and the ureteric bud in the uterus, while renal hypodysplasia is defined as a rudimentary kidney with less than 5% of relative function [16, 17].

MCDK is a severe form of renal dysplasia in which cysts of different sizes comprise the affected kidney resulting in the absence of normal functioning renal tissue. It is considered the most common cystic renal disease in children and has an estimated incidence of 1 in 4300 births [16, 26, 40]. On the other hand, URA is characterized by complete absence of unilateral renal tissue and ureter in fetal life [41]. Primary renal hypodysplasia is caused by abnormal involution of primitive renal tissue, leading to a non-functioning kidney. Observational studies in patients with SFK have shown the predominance of this anomaly in males and in the left side of the body. However, the reasons for these findings are not fully understood [6, 11, 18, 20, 26, 42].

The assessment of fetal anatomy using ultrasonography has become standard practice, allowing the diagnosis of many structural abnormalities in the fetus before birth [34]. In a case of URA, the fetus' bladder may be normal, while the contralateral kidney may appear hypertrophied. As

for MCDK, the dysplastic kidney seems to be large and bright, with multiple cysts placed in the renal parenchyma, reducing the cortico-medullary differentiation [34].

Most cases of URA are indeed renal aplasia due to the difficulty in detecting this anomaly by ultrasound. As URA and unilateral renal aplasia are hardly distinguished in clinical practice, both are referred to as URA, whose estimated occurrence is 1 in every 1000 births [6, 7, 11, 18, 23, 43]. In cases not detected intra-uterus, the misdiagnosing is even higher due to the possibility of spontaneous involution of MCDK. It is estimated that around 5% of MCDKs will be completely involuted before birth [11, 40]. Despite all the difficulties to differentiate these three conditions, URA is pointed as the predominant cause of congenital SFK [7, 43].

Kidney development is also susceptible to the influence of genetic and environmental factors, including medications administered during pregnancy, intrauterine growth restriction, stress, premature birth, and maternal diseases, like diabetes. Additionally, negative effects on nephrogenesis and glomerular filtration rate (GFR) can be observed following drug administration in the prematurely born neonate with SFK. Aminoglycosides, nonsteroidal anti-inflammatory and anti-epileptic drugs are commonly used and can disturb nephrogenesis [11, 36].

The natural history of congenital SFK is still an unresolved issue due to several factors. First, most studies available in the literature are observational and took place in tertiary hospitals. Therefore, besides the numerous limitations that do not allow generalizations to the entire population, there may also have been a "selection" of more seriously affected patients. Second, the lack of genetic and molecular data to diagnose the subtypes of SFK compromises the assessment of clinical outcomes. Several biases and confounding factors occur when grouping SFK patients for data analysis. The arbitrary assumption of SFK as a single and homogeneous group can place together patients with very different clinical courses [3].

2.1.2 The hyperfiltration theory

In the 1980s, Brenner *et al.* conducted experiments in animal models with renal mass reduction and described the 'hyperfiltration hypothesis'. According to this theory, a reduced number of functional nephrons leads to compensatory glomerular hypertension and enlargement of the remnant nephrons, resulting in glomerular hyperfiltration. This process and the consequent hemodynamic and structural changes can produce glomeruloesclerosis, leading to a vicious cycle of additional reduction in nephron number [39, 44, 45].

The pathophysiological process of hyperfiltration starts at the single nephron glomerular filtration rate (SNGFR) level. Increased glomerular capillary pressure leads to stretching of the

capillary wall and consequent progressive damage to podocytes [27, 46]. In addition, the epithelial cells of the proximal tubule develop a hypermetabolic state secondary to hypertrophy and this process releases reactive oxygen species [47]. Some data suggest that tubular injury is present in the pathological process of SFK patients [26, 46–49]. Among the theories that try to explain tubular involvement, one proposes that excessive reabsorption of ultrafiltered proteins may induce apoptosis/necrosis of tubular cells. However, the studies about tubular lesions in congenital SFK are scarce.

Even though there may be additional nephron formation in cases of congenital SFK that occurs before the end of nephrogenesis, the total nephron number will be still lower than in two-kidney healthy individuals [36, 46, 50]. Therefore, patients with congenital SFK are, by definition, a clear model of reduction in renal mass since birth [51]. In this sense, the hyperfiltration theory applies to the natural history of congenital SFK, putting these patients at risk for renal tissue injury [6, 11, 52]. The primary goal of glomerular hypertrophy is to minimize the functional consequences of structural nephron loss in an attempt to maintain homeostasis [37, 46, 52]. This adaptation may be beneficial at birth, once it preserves glomerular filtration rate from an inevitable reduction [11, 16, 21, 53]. However, over time, it is believed that adaptive hyperfiltration in the non affected kidney turns maladaptive, because of continuous glomerular overload, which results in loss of glomerular barrier function, proteinuria, declining glomerular filtration rate and increased risk of hypertension and premature kidney damage [11, 21, 27, 36, 44, 45].

The hyperfiltration theory remains unproven in humans, mainly because of the inability to measure SNGFR and total nephron number *in vivo* [6]. However, several studies state that contralateral kidney hypertrophy process is expected in congenital SFK beginning as early as at 20 to 22 gestational weeks [16, 18, 26]. Shirzai *et al.*, Wang *et al.* and Balki *et al.* evaluated children with SFK and observed an incidence of compensatory hypertrophy of the single kidney in 81.8%, 82% and 86%, respectively [52, 53, 46].

It is worth noting that literature data are still controversial regarding the consequences of the hypertrophy mechanism and the role of other factors in renal survival. Despite the possible deleterious effects in the long-term, which are associated to the development of CKD [6, 11, 27, 46], some authors have not found an association between hypertrophy and poor renal outcome [2, 16, 53]. In fact, these authors believe in the hypothesis that the absence of compensatory hypertrophy is associated with poor renal outcome [17, 23]. It is considered that the additional nephron formation confers a certain degree of protection to the overloaded kidney [14, 22]. In a cohort of patients with congenital SFK followed from the first months of life until the beginning of adulthood, Marzuillo and colleagues observed that none of the patients who developed kidney injury had evidence of early-

in-life renal length higher than 2 standard deviations. The authors attributed this finding to the protective role of hyperplasia [22]. Prospective studies are necessary to elucidate this question.

Figure 1 summarizes the sequences of events related to glomerular hyperfiltration.

2.2 Congenital versus Acquired SFK

In the past, the prognosis of patients with congenital SFK has been erroneously considered similar to those with acquired SFK, including transplant donors and survivors from Wilms' tumor. Actually, congenital and acquired SFK represent completely different entities [3, 51]. First of all, in the congenital anomaly the interaction between genetic defects and environmental aspects affect the development of the kidney and the urinary tract since fetal life and continues throughout the entire life of the individual [3, 21]. In addition, the incidence of CAKUT on the remnant kidney is considerable and varies from 26% to 47% [6, 7, 11, 17, 29]. In the case of living kidney donors and nephrectomized patients, the remaining kidney is usually healthy.

On the other hand, it is suggested that the adaptive potential of congenital SFK is better than acquired SFK [14, 21]. This stands on the fact that (1) as nephrogenesis ceases by the 36th week of gestation, acquired SFK can adapt through hypertrophy, but not through hyperplasia [11, 14], (2) the response time is shorter in cases of acquired SFK, once it is necessary to rapidly make up for the substantial and abrupt loss of filtration surface area [21] and (3) the hypertrophy rates in acquired SFK patients are lower than what is observed in congenital SFK, which is noted through the rapid decrease in GFR seen in nephrectomized patients after surgery [46]. In this way, acquired SFK is considered to be more vulnerable to worse outcomes [26]. However, studies with transplant donors have shown preserved renal function even after more than 25 years of nephrectomy [21].

It still remains unclear which condition implies the worse prognosis, whether being born with a single kidney or undergoing nephrectomy later in life. Further prospective studies with long follow-up time are needed.

2.3. DIAGNOSIS

Renal scintigraphy using radioactively labeled dimercapto-succinic acid (DMSA) or mercapto acetyl tri glycine (MAG-3) is still considered the gold standard for SFK definitive diagnosis [54, 55]. Disadvantages of this method include exposure to ionizing radiation, high cost and frequent need for sedation, especially in young children [54].

Because of these limitations, the most used imaging method for identifying and evaluating congenital SFK is ultrasonography (US). US is a non-invasive, relatively cheap and safe method [54–56], although its accuracy is observer-dependent.

The inclusion of US in routine prenatal practice has contributed to increase the overall detection of CAKUT [7, 34, 41]. Prenatal US allows the suspicion of congenital SFK, which must be confirmed postnatally [39]. Grabnar *et al.* and Krill *et al.* suggested that ultrasound alone may be sufficient to diagnose solitary kidney. They have shown that the estimated accuracy of abdominopelvic US in diagnosing SFK was around 98% [54, 55].

Voiding cystourethrogram is an invasive test, which is recommended when there is a suspicion of high-grade vesicoureteral reflux, since this anomaly is present in approximately 20% of congenital SFK patients [7]. Finally, magnetic resonance imaging (MRI) of the kidney and urinary tract can provide detailed anatomical information. Nowadays, its use is limited to patients with undefined diagnosis or when anatomic details are needed, for example, in case of surgery planning. Advances in MRI techniques may, in the future, enable more accurate assessment of inflammation and fibrosis, as well as nephron counts [39].

2.4 COMPLICATIONS AND PROGNOSIS

Despite being a controversial issue with unanswered questions, many studies share the same concern: congenital SFK is not a benign condition [11, 15, 51, 57]. The magnitude of the risk of living with a congenital SFK is still a matter of debate [2, 39]. Even though the pathophysiological mechanism of hypertrophy and hyperfiltration justifies some expected complications, it seems not to be the only factor responsible for the outcomes [37].

Children with congenital SFK have higher risk of premature kidney damage, hypertension, proteinuria and progression to CKD [6, 11, 15, 19, 27, 37, 46, 51, 58–61]. Normally, poor renal outcomes associated with SFK are attributed to glomerular alterations, secondary to hyperfiltration-mediated injury [27, 44, 45]. It is considered that albuminuria and hypertension are reliable markers of this process [21, 46, 62]. Patients who already have proteinuria and hypertension may be at increased risk of developing complications [49].

Sanna-Cherchi *et al.* conducted a longitudinal study with a cohort of patients with CAKUT up to 30 years of age and reported that almost 1/3 of patients with SFK had low renal survival, requiring renal replacement therapy (hemodialysis or peritoneal dialysis) at some point during the follow up. Compared with a reference group, the risk for an impaired renal outcome was even higher when vesicoureteral reflux (VUR) was present (hazard ratio, 7.50; 95% confidence interval, 2.72 to

20.68) [20]. In a study conducted by Ardissino *et al.*, a non-linear decline in the probability of kidney survival was observed with a marked reduction in puberty. The curve's point of decline was identified at 11.6 years of age in male patients and at 10.9 years in females. In these individuals, the probability of renal replacement therapy was estimated to be 51.8% during the second decade of life [24].

The KIMONO (KIdney of MONofunctional Origin) study [6], the largest cross-section study of SFK, comprising more than 200 cases of congenital and early-acquired SFK, reported that 32% of all enrolled patients showed signs of kidney injury, defined by the presence of hypertension and/or albuminuria and/or use of renoprotective medication, at a mean age of 9.5 years. Furthermore, it was reported that estimated GFR of patients showed a slow decline from 9 years of age onwards, whereas microalbuminuria was identified from 16 years onwards. The mean age for developing kidney injury in children of both types of SFK was 15 years old and patients with ipsilateral CAKUT (about 34% of children with SFK) had a higher incidence of kidney injury.

However, there are still conflicting results among studies that evaluated the long-term outcomes of congenital SFK [2, 17, 22, 38, 52, 63]. Some authors found relatively good prognosis. La scola *et al.* evaluated 142 children with congenital SFK and found decreased estimated GFR rate in only 12% of children at a median age of 2.4±2.6 years, while Poggiali *et al.* reported 11% of renal injury among 162 children with congenital SFK [2, 17].

Despite the controversies, there is a consensus that some factors can influence the natural course of congenital SFK and consequently its prognosis. Among the risk factors, the presence of additional CAKUT and the absence of compensatory kidney hypertrophy have been investigated in several studies [2, 17, 22, 46, 57, 64]. Using logistic regression models, Westland *et al.* showed that increasing age and the presence of ipsilateral CAKUT are independent risk factors for kidney injury, while kidney length was inversely associated with this outcome [23]. Using cox regression models, Marzuillo and colleagues reported that additional CAKUT was the only significant risk factor for kidney injury [22]. The logical basis for the association of ipsilateral CAKUT with worst prognosis is the fact that these patients are under a greater risk of recurrent urinary tract infections (UTIs) or other events that may contribute to decrease the number of nephrons [57]. As the incidence of additional CAKUT is considerable [6, 14], patients with this condition should be closely monitored [3, 23, 51, 52].

In the cohort study conducted by Poggiali and colleagues, risk factors for clinical outcomes related to renal injury also included recurrent UTIs [2]. Schreuder *et al.* found that higher body mass index (BMI) was related to microalbuminuria, indicating that obesity may constitute an additional risk factor for patients with SFK [59]. Low birth weight is also considered a factor of worse prognosis due to the even more reduced renal mass of these individuals [23, 59]. Additionally, there is evidence

that puberty is a critical period for developing kidney injury and progressing to CKD. Although the underlying mechanisms have not yet been identified, renal deterioration is believed to be related to sex hormones, as well as to increased body metabolic demands [6, 11, 23, 24].

Numerous factors, either modifiable or non-modifiable, can influence the evolution of patients with SFK. For this reason, it is important to distinguish individuals at high risk for kidney injury and CKD from those at low risk before injuries take place. Ideally, this definition should happen in the neonatal period, when congenital SFK is identified [26]. In a recent study, McArdle *et al.* reported that, although hypertension, proteinuria and reduced GFR are available markers to detect renal injury in children with SFK, these alterations occur at later stages of the disease. Thus, novel biomarkers must be investigated to allow early detection of individuals at high risk for developing kidney injury earlier in life [26].

Table 1 summarizes the findings of the main original studies about patients with SFK.

Table 1: Main original studies about patients with solitary functioning kidney

Author	Year	Ref.	Study design	Number of patients	Main Findings
Poggiali et al	2019	2	Retrospective Cohort	162	There is an overall low risk of renal injury for the majority of the infants with congenital SFK. Risk factors such as lower baseline renal function, presence of contralateral CAKUT, reduced renal length of the functioning kidney at birth and recurrent UTIs during follow-up may contribute to this outcome.
Westland et al	2011	6	Retrospective Cohort	206	Children with SFK have an increased risk of developing hypertension, albuminuria and CKD in later life. Furthermore, when SFK and ipsilateral CAKUT are both present, children have also a higher risk to develop renal injury in adulthood.
Grapin et al	2021	15	Cross-sectional	210	Kidney function declines with age in patients with SFK

La Scola et al	2016	17	Retrospective Cohort	146	Insufficient renal length was a potential risk factor for CKD in children with CSK.
Argueso et al	1992	19	Retrospective Cohort	157	Patients with SFK due to URA are at a higher risk of developing proteinuria, hypertension and renal insufficiency even in the absence of structural anomalies of the kidney
Sanna-Cherchi et al	2009	20	Retrospective Cohort	312	Patients with CAKUT have potential poor renal survival during the years
Jaoudé et al	2011	21	Retrospective Cohort	92	In short and medium-term analysis, SFK may not impact renal function, but it may affect at long-term. Any condition of reduced nephron endowment potentially predisposes the patients to a higher risk of hypertension and renal impairment in later life
Marzuillo et al	2021	22	Retrospective Cohort	56	Until early adulthood, the prognosis of congenital SFK appears to be milder than it was thought. CAKUT associated with congenital SFK is the most important risk factor for kidney injury in these patients.
Westland et al	2013	23	Retrospective Cohort	407	Substantial proportion of children with an SFK develops renal injury during childhood. The risk to develop renal injury is independent of SFK type but increases with the presence of ipsilateral CAKUT, age, and a small renal length.
Ardissino et al	2012	24	Cross-sectional	935	Puberty is an independent risk factor for the progression of CKD.

Srivastava et al	2020	27	Cross-sectional	144	Increased urinary PGE2 from elevated SNGFR and consequently increased FFSS during early stage of CKD precedes overt microalbuminuria and is a biomarker for early hyperfiltration-induced injury in individuals with SFK.
Davidovits et al	2017	38	Retrospective Cohort	32	Congenital SFK is apparently associated with little or no renal damage in infancy or childhood. Compensatory enlargement of the functioning kidney begins *in utero* and might serve as a prognostic factor for normal renal function after birth.
Balkı et al	2021	46	Retrospective Cohort	42	Children with SFK are at risk of renal injury due to glomerular hyperfiltration. Even patients with compensated functional kidneys may show signs of renal damage at early ages.
Taranta-Janusz et al	2014	49	Cross-sectional	52	Urinary activities of HEX, the isoenzymes HEX A and HEX B, and FUC, GAL, MAN, and GLU are elevated in children with SFK.
Kolvek et al	2014	51	Prospective Cohort	42	A substantial proportion of children with SFK develop renal injury during childhood, especially those with CAKUT in the SFK. Close follow-up of albuminuria, blood pressure and eGFR are warranted to identify chronic kidney disease at its early stages.

Shirzai et al	2014	52	Cross-sectional	50	Children with SK have increased 24-h urinary MA excretion in the long term, and need prolonged follow-up to detect early deterioration of renal function and to prevent end-organ damage later in life.
Westland et al	2014	58	Cross-sectional	47	Based on ABPM, one in five children with a SFK has hypertension. As the majority of these subjects were not hypertensive during office BP measurements, ABPM should be considered in the clinical management of SFK patients.
Schreuder et al	2008	59	Retrospective Cohort	66	Microalbuminuria and/or hypertension is present in 50% of patients with congenital SFK. Patients with SFK warrant a systematic follow-up of blood pressure, proteinuria and renal function, especially in those with low birth weight.
La Scola et al	2020	60	Retrospective Cohort	126	A CSK *per se* can be associated with an increased risk of hypertension at the pediatric age. Therefore, ABPM, which has proved valuable in the screening of hypertension, is warranted in children with a CSK, even if laboratory and imaging assessment is otherwise normal.
Stefanowicz et al	2012	61	Cross-sectional	47	WTs have similar eGFR to individuals with URA and are more likely to have arterial hypertension. The patients with URA have signs of tubular damage.

Zambaiti et al	2019	62	Retrospective Cohort	40	Patients with a small/absent dysplastic kidney have an increased risk to develop hypertrophy and hypertension compared to patients with a large residual, regardless of nephrectomy. ABPM revealed absent dipping in most patients with SFK.
Marzuillo et al	2017	63	Retrospective Cohort	322	The prevalence of renal damage was 3.9%. Among congenital anomalies of the kidney and urinary tract, congenital solitary functioning kidney represented the major risk factor.
Matsell et al	2021	64	Retrospective Cohort	230	Children with URA are more likely to have an associated genetic syndrome, a non-renal anomaly and associated CAKUT than MCDK cases. They are also at higher risk of developing hypertension, proteinuria and kidney injury over time.

SFK: Solitary Functioning Kidney; CAKUT: Congenital Anomalies of the Kidney and the Urinary Tract; UTI: Urinary Tract Infection; CKD: Chronic Kidney Disease; CSK: Chronic Solitary Kidney; PGE2: Prostaglandin E2; SNGFR: Single Nephron Glomerular Filtration Rate; FFSS: Fluid Flow Shear Stress; HEX: N-acetyl β-hexosaminidase; FUC: α-fucosidase; GAL: β-galactosidase; MAN: α-mannosidase; GLU: β- glucuronidase; eGFR: Estimated Glomerular Filtration Rate; MA: Microalbuminuria; ABPM: Ambulatory Blood Pressure Measurements; BP: Blood Pressure; WTs: Wilms Tumor survivors; URA: Unilateral Renal Agenesis; MCDK: Multicystic Dysplastic Kidney.

2.5 KIDNEY BIOMARKERS

According to the *National Institutes of Health working group* "biomarker" is the term used to define a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [65]. An ideal kidney biomarker must fulfill some characteristics, including high sensitivity, high specificity, early detection (identify kidney injury before elevation of creatinine clearance) with high

accuracy, availability in easily accessible sources (blood and/or urine), low cost and capacity of prognostic prediction [66–69].

The idea of developing novel biomarkers capable of identifying and monitoring kidney diseases is based on the fact that early diagnosis is the most efficient way to prevent CKD progression [66]. This purpose gains even more strength considering that, in the current scenario, serum levels of creatinine and urea are still considered the gold standard for assessing renal function, although their limitations are widely known [70–74].

In this sense, the last two decades have been marked by an exponential increase in searches for more accurate markers to improve the care of patients with or at risk of kidney disease [28, 66, 75]. However, in specific populations, such as children with solitary functioning kidney (SFK), the studies are scarce and the behavior and usefulness of some molecules remain to be determined.

Urine has some advantages over serum/plasma. Besides being a simple, inexpensive and not necessarily invasive collection, urine is in close contact to the site of the injury. As disadvantages, we can mention the fact that the urinary concentration of some substances varies according to the level of hydration and also that urine needs to be frozen until be analyzed.

Several alternatives to serum creatinine and urea have emerged recently [67]. Among them, some have already been widely evaluated in the pediatric population, including Cystatin C, Lipocalin-2/NGAL, Albumin, Epidermal growth factor (EGF), Kidney injury molecule 1 (KIM-1), Tissue inhibitor of metalloproteinase 1 (TIMP-1), and Alpha-1-microglobulin while others are still under investigation regarding their role in the assessment of kidney function and/or damage. In this review, we focused on these urinary markers.

Creatinine

In daily pediatric care, serum creatinine and equations that use creatinine to estimate GFR are the routine way to assess kidney function. The most used equation is the Schwartz formula, which considers serum creatinine, height, and a numerical constant (k value) [76, 77]. Despite being widely used, serum creatinine presents a number of disadvantages, which makes it an inaccurate and low sensitive marker [25, 69, 71, 72, 75].

Creatinine is the product of nonenzymatic breakdown of creatine in muscle. It is produced at a relative constant rate, which is directly influenced by muscle mass. Creatinine is freely filtered in the glomerulus, but its proximal tubular secretion accounts for 10%-20% of its excretion, which can cause overestimation of true GFR. In addition, while some medications can increase the serum creatinine concentration, the action of extra-renal factors (such as creatinine’s degradation by gut bacteria) can cause its reduction [25, 66].

Among many limitations, the major one is the lag time between kidney damage and a resultant rise in serum/plasma creatinine levels. It takes an approximately 50% of kidney parenchymal loss before any increase in serum/plasma creatinine levels is noticed [67, 70, 73, 78]. Furthermore, the measurement of serum/plasma creatinine levels does not allow the identification of the site and extent of kidney injury. In addition, it is not capable of differentiating structural kidney damage and functional hemodynamic alterations. Finally, the reliability of the measurement is also limited by the fact that creatinine metabolism is influenced by many factors, including diet, age, sex, race, muscle mass, hydration status and medications [66, 69, 73, 75, 78].

All of these limitations are even more concerning in pediatric patients with a single kidney. Westland and colleagues studied a group of 77 children with SKF to determine the performance of six common equations in estimating GFR, compared with the gold standard method (inulin single injection) [25]. The authors found out that improved combined serum cystatin C/creatinine/BUN–based equation by Schwartz *et al.* (eGFR-CkiD2) was the most precise, while urinary creatinine clearance presented the worst results, leading to overestimation of GFR and consequently underestimation of CKD stage [79]. The authors discouraged the use of creatinine clearance in SFK patients. Unfortunately, despite acceptable accuracy, all the six equations misclassified CKD stage in this specific group of patients. All these limitations stimulated the search for new biomarkers capable of identifying kidney damage in patients with SFK.

Cystatin C

Cystatin C is an endogenous low molecular weight protein that inhibits the cysteine-protease enzyme. It is produced by all nucleated cells at a constant rate. In the healthy kidney, cystatin C is filtered by the glomerulus, and then is almost entirely reabsorbed in the proximal tubule, without secretion [66, 69, 72, 73, 78–83]. For this reason, the molecule is not identified in urine in significant amounts. Considering this mechanism, measurement of urinary cystatin C is a potentially valuable tool for diagnosing tubular damage and dysfunction, while serum concentration of cystatin C can reflect alterations in GFR [68, 70, 80, 81]. Unlike creatinine, cystatin C does not appear to be significantly affected by diet, sex or muscle mass [66, 69, 70] and its inter-individual variation accounts for 25% of its biological variability compared to 93% for creatinine [79]. However, the measurement of cystatin C has some limitations: hypo- or hyperthyroidism can affect its production, as well as high-dose steroid therapy [73, 84]. While the serum concentration of cystatin C has been widely studied as a parameter of GFR [25, 68, 70, 85], little attention has been focused on its urinary dosage. Hellerstein and coworkers evaluated a cohort of 82 children and adolescents diagnosed with various renal diseases, including SFK, and observed that the fractional excretion of cystatin C (FE

Cyst C) increased in proportion to the decrease in GFR. The authors concluded that the urinary [cystatin C/Creatinine] ratio is a reliable screening tool for detecting decreased GFR that does not require serum measurements [85]. Despite promising data, larger studies are needed to confirm its utility as a renal function marker in the SFK population.

Lipocalin-2/NGAL

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein that belongs to the lipocalin family. It is involved in innate immunity and is produced by hepatic, intestinal, pulmonary tissue, and renal tubular cells [66, 73, 75, 82]. In the kidneys, NGAL is synthesized in the distal nephron and in the proximal tubules, and secreted into urine by the thick ascending limb of the loop of Henle and the collecting ducts. In healthy subjects, circulating NGAL is filtered through the glomerulus and reabsorbed by the proximal tubule [69, 73, 80]. This molecule is one of the best-studied urinary biomarkers and is considered an early and sensitive predictor of acute kidney injury (AKI), as it rapidly accumulates in the renal tubules and urine in case of kidney injury. This positive association with kidney injury has been already documented and investigated in various renal diseases [66, 69, 73, 80–83, 86]. Urinary NGAL has also been assessed as a potential marker of progression from AKI to CKD [73, 83], and as an early biomarker of tubulointerstitial fibrosis in glomerular diseases [87, 88].

Albumin

It is well established that microalbuminuria is an early marker of kidney damage and its measurement is part of the screening for CKD [46, 66, 89]. Along with lower GFR, the measurement of urinary albumin excretion has predictive value over mortality in children with CKD [90]. Normally, in healthy kidneys, albumin is filtered in the glomerulus and reabsorbed in the proximal tubules. Thus, the presence of albuminuria, although usually indicates glomerular damage, may also reflect an alteration at proximal tubular level [66]. In patients with SFK, albuminuria is considered one of the first markers to evidence hyperfiltration damage [21, 46, 52]. In this sense, some authors recommend that microalbuminuria screening should be started in the first years of SFK, considering that increased urinary albumin excretion precedes changes in GFR and blood pressure [46, 52]. Srivastava *et al*. Evaluated a cohort of children with congenital SFK and observed an elevation of urinary albumin compared with healthy controls [27]. Interestingly, although comparatively higher, the microalbuminuria levels of more than half of these patients were within the reference range, reflecting an early-stage alteration of the glomerular filtration barrier. These data corroborate the recommendation of periodic assessment of microalbuminuria in patients with SFK.

The microalbuminuria measurement also has limitations. The rate of albumin excretion does not seem to be constant throughout the day and has considerable variation on a day-to-day basis [21]. These physiological variations can justify discrepant results in the studies that evaluated microalbuminuria in SFK patients [46, 52, 59, 89].

EGF

Human Epidermal Growth Factor (EGF) is a peptide synthesized as a large glycosylated precursor (prepro-EGF). It binds to the EGF receptor and can be found in most extracellular fluid and secretions including plasma, saliva, amniotic fluid and milk. However, the main source of EGF synthesis and secretion are the thick ascending limb of Henle and distal tubules, explaining the higher concentration of this molecule in the urine [27, 28, 91].

The most important role of EGF is the repair of urothelial tissue after an injury and it is considered an important biomarker of tubular damage [27, 91, 92]. Nonetheless, it is also associated with compensatory renal growth, renal excretion of water and electrolytes, cystic kidney diseases, renal and urothelial malignancy, maintenance and repair of urothelium, and tubular transport [91, 93]. A decrease in urinary EGF concentration is related to CKD progression in various kidney diseases, both in children and adults [27, 94].

Bartoli *et al.* Evaluated the behavior of this biomarker in a group of children with CAKUT, which also included SFK patients [28]. In this study, the urinary ratios of uEGF/uMCP-1 (indicator of regenerative vs inflammatory response) and uEGF/uβ2M (indicator of regenerative response vs. Tubular damage) were compared between patients and healthy controls. The authors found that both ratios were significantly reduced in patients when compared with the controls. The findings suggest a tendency to progressive renal damage, without the normal potential for regeneration of the tubular epithelium.

KIM-1

Kidney Injury Molecule-1 (KIM-1) is a type 1 transmembrane protein whose expression is almost undetectable in healthy kidneys and urine. In case of ischemic or toxic tubular injury, its expression is rapidly upregulated [69, 71, 75, 81, 86, 87].

Urinary KIM-1 increases as early as 24 hours after AKI [81, 96]. This elevation precedes serum creatinine increase and GFR reduction [78]. KIM-1 is thought to participate in both kidney injury and regeneration processes. The molecule activates apoptosis in proximal renal tubular epithelial cells and induces tissue repair and removal of dead cells in the lumen. It is hypothesized

that, after the initial stage of kidney injury, the persistent stimulus to tissue repair leads to excessive cell proliferation that also causes damage to the renal epithelium [75, 78, 81].

The potential of KIM-1 as a predictor of AKI and CKD has been evaluated in different conditions among the pediatric population [81, 95, 96].

TIMP-1

Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a glycoprotein produced in mesenchymal tissue, including glomerular cells [97]. Its expression is upregulated in kidney injuries. The role of this molecule in the pediatric population has already been evaluated in different kidney conditions, including obstructive uropathies, pyelonephritis, nephrotic syndrome, hydronephrosis and glomerulosclerosis [97–101]. TIMP-1 is considered a potential biomarker of fibrosis progression, as the molecule inhibits matrix-degrading enzymes leading to fibrogenesis [97, 102].

Alpha-1-microglobulin

Alpha-1-microglobulin is a low-molecular-weight protein (27kDa) synthesized in the liver, which is freely filtered by the glomeruli and reabsorbed at proximal tubules. Its urinary excretion is increased in proximal tubule injuries [47, 48].

Alpha-1-microglobulin is a useful marker of tubular dysfunction in various renal diseases [103]. Gluhovschi *et al.* Assessed a group of adult SFK patients with and without urinary tract infection [48]. The authors found increased levels of alpha-1-microglobulin in patients with SFK in comparison with the controls, but without statistical significance. They concluded that this result may reflect the adaptive phenomenon of hyperfiltration at the tubular level. In a sample of adult patients with congenital and acquired SFK, Gadalean and colleagues detected increased urinary levels of alpha-1-microglobulin in a subset of patients [47]. The authors concluded that progressive tubular lesions may occur in these patients and high levels of the molecule indicate more advanced stages of injury. Despite promising results in adults with SFK, the role of alpha-1-microglobulin in children with a single kidney still needs to be further evaluated.

Other biomarkers

Numerous other markers are under investigation, including Interferon-gamma-induced protein 10 (IP-10), Glutathione S-transferase alpha (GST), osteopontin, beta-2-microglobulin, and trefoil factor 3 (TFF-3). However, data in pediatric patients with SFK are still not available.

Table 2 summarizes the studies on urinary biomarkers in pediatric patients with SFK.

Author	Year	Ref.	Sample	Urinary Biomarkers	Study design	Main findings
Hellerstein et al	2004	85	82 children and adolescents with various kidney diseases (14 with SFK). *SFK etiology was not informed.	Cystatin C	Cross-sectional study	The ratio of cystatin C/creatinine has a 90% specificity for identification of pediatric patients with eGFR <=60 ml/min/ 1.73 m2 with a false- positive rate of 16.1%
Stefanowicz et al	2012	61	47 children and young adults with SFK and no other defects of the urinary tract (17 with URA; 30 unilateral nephrectomised WTs)	Cystatin C, NGAL, NAG, B2M	Cross-sectional study	The urinary excretion of Cystatin C, NGAL and NAG was similar in both groups. URA patients had higher B2M excretion than WTs.
Balki et al	2021	46	42 adolescents with congenital SFK (22 with URA; 20 with MCDK)	Albumin, creatinine, β2M	Cross-sectional study	Increased urinary albumin and β2 microglobulin were found in 17% and 12% of the patients, respectively.
Srivastava et al	2020	27	60 children with congenital SFK (URA or MCDK)	Albumin, EGF, PGE2	Cross-sectional study	Increased urinary PGE2 ($p = 0.024$) and urinary albumin ($p = 0.019$), but not EGF ($p = 0.412$), were observed in SFK children, compared to controls. These three urinary analytes were independent of each other.
Bartoli et al	2019	28	80 children with CAKUT (40 hypodysplasia, 22 URA; 10 MCDK; 8 nephrectomy)	MCP-1, EGF, β2M and FAS-L	Cross-sectional study	Urinary ratios of uEGF/uMCP-1 (indicator of regenerative vs inflammatory response) and uEGF/uβ2M (indicator of regenerative response vs. Tubular damage) were significantly reduced in patients when compared with the controls.

Shirzai et al	2014	52	50 children with congenital and acquired SFK	Albumin and β2M	Cross-sectional study	All children in the SFK and control groups had normal renal function (eGFR > 90 ml/min/1.73m2). Albumin levels in 24-h urine was higher in patients living with SFK more than 5 years (p = 0.01).
Schreuder et al	2008	59	66 children and adolescents with congenital SFK (URA and MCDK)	Albumin	Retrospective cohort study	Children with congenital renal mass reduction had a lower eGFR, compared with controls. Twenty-three percent of these patients presented microalbuminuria (urinary albumin > 20mcg/min).
Cachat et al	2013	89	155 children and young adults with various kidney diseases (42 with congenital or acquired SFK)	Albumin	Cross-sectional study	In the SFK group there was a weak but significant correlation between urinary albumin and filtration fraction.

Solitary functioning kidney (SFK); estimated glomerular filtration rate (eGFR); unilateral renal agenesis (URA); Wilms tumor (WT); neutrophil gelatinase-associated lipocalin (NGAL); N-acetyl-beta-glucosaminidase (NAG); β-2-microglobulin (β2M); multicystic dysplastic kidney (MCDK); epidermal growth factor (EGF); prostaglandin E2 (PGE2); congenital anomalies of the kidney and urinary tract (CAKUT); monocyte chemotactic protein-1 (MCP-1); FAS-ligand (FAS-L).

2.6. TREATMENT AND FOLLOW-UP

There is no specific treatment for congenital SFK. The management is conservative if complications are absent [62]. The purpose of monitoring these patients is to identify risk factors and complications as soon as they appear, and so, intervene quickly. Unfortunately, individuals with a SFK and preserved renal function have traditionally not been followed during adulthood. Therefore, guidelines for the management and follow up of this condition have not been established yet [11, 15]. Nevertheless, the need for regular clinical follow-up, with multidisciplinary approach, is well accepted and encouraged [2, 11, 19].

According to Poggiali *et al.*, patients at high risk for renal injury (ie, patients with a baseline creatinine higher than 0.58 mg/dl, a reduced contralateral renal length (< 50th percentile), and presence of ipsilateral CAKUT) should undergo closer surveillance for proteinuria, hypertension, and

renal function [2]. On the other hand, patients without these risk factors can be followed by a general pediatrician with guidance regarding healthy habits to avoid other modifiable risks, such as obesity and diabetes.

Corbani and colleagues proposed a follow-up model that includes imaging studies for size and function assessments and periodic blood/urinary tests, as well as blood pressure measurements. In patients with no sign of kidney injury at the time of diagnosis, follow-up with laboratory analyses and measurements of blood pressure should be performed every 2 years until puberty (or 14 years old) and then, if the patient does not develop complications, every 3–5 years. For patients with additional CAKUT, the assessment of these parameters should be done annually [3].

Westland and colleagues, in turn, proposed a different approach [11]. The authors suggested that blood pressure and microalbuminuria should be evaluated at least one time per year in patients without other CAKUT, two times per year in those with additional CAKUT and two-to-four times per year in patients with GFR < 60 ml/min/1.73m^2 or hypertension or in use of medication for proteinuria.

It is important to keep in mind that, as congenital SFK results from embryological disorder, it may be accompanied by subtle dysplastic alterations that may not be identified by standard image and laboratory tests [60]. In this sense, all patients with congenital SFK require regular follow-up. Considering that most studies highlight that deterioration may not be detectable until late adolescence, it is essential that the transition of care from pediatric to adult nephrology services be done properly [20, 23, 27].

3. CONCLUSIONS AND PERSPECTIVES

Pediatric patients with SFK are a subgroup of congenital renal anomalies that deserves special attention. In recent years, there has been an increase in the number of studies dedicated to elucidate the pathophysiology and prognostic factors associated with this condition. However, there is still a lot to be learned. Further research with longer follow-up duration is needed to better understand the natural course of congenital SFK and the role of novel urinary biomarkers in this specific population. More knowledge about this subject means a potential improvement in the outcome of these patients.

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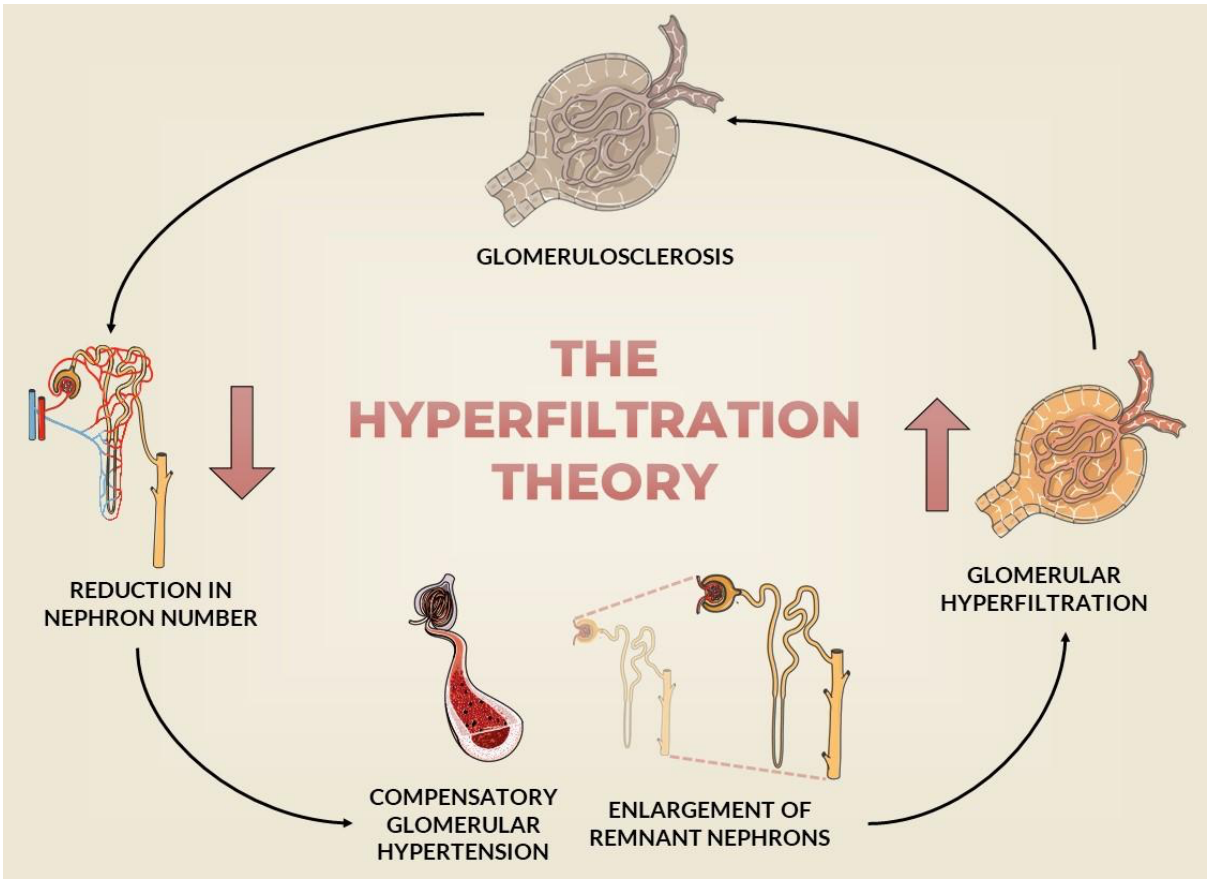

Figure 1

Schematic view of glomerular hyperfiltration-related changes. The reduction of nephron number stimulates the remnant nephrons to increase glomerular filtration rate. The process is associated with increase in glomerular perfusion pressure (glomerular hypertension) and enlargement of remnant nephrons, resulting in glomerular hyperfiltration. These changes lead to release of inflammatory molecules, endothelial lesion, oxidative damage, ultimately determining glomerulosclerosis.

Scheme made with figures from “Urinary System”, by Servier Medical Art (smart.servier.com), used under CC BY 3.0 / Some figures had saturation and transparency modifications.

3. OBJETIVOS

3.1. Objetivo geral

O objetivo geral deste projeto foi medir marcadores de função e lesão renal em pacientes pediátricos com rim solitário funcional congênito e comparar com as medidas dos mesmos marcadores em crianças saudáveis, pareadas por idade e sexo.

3.2. Objetivos específicos

- Quantificar os níveis urinários de dezessete marcadores de função e lesão renal (Clusterina, Cistatina C, Osteopontina, α1-Microglobulina, EGF, Lipocalina-2/NGAL, Albumina, Calbindina, Osteoactivina, Renina, TFF-3, TIMP-1, Colágeno IV, FABP1, GSTα, IP-10, KIM-1) em crianças com rim único e crianças hígidas;
- Comparar os níveis urinários dos marcadores supracitados entre as amostras do grupo com rim único e grupo controle;
- Avaliar possíveis associações e/ou correlações entre os biomarcadores estudados e desses biomarcadores com variáveis clínicas e laboratoriais.

4. PACIENTES E MÉTODOS

4.1. Delineamento

Em estudo de coorte prospectivo previamente realizado por nosso grupo de pesquisa, foram coletadas amostras de urina de pacientes portadores de RSF congênito acompanhados na Unidade de Nefrologia Pediátrica do Hospital das Clínicas da UFMG [1]. Tais amostras se encontravam disponíveis no laboratório, congeladas a -80ºC, em quantidade e qualidade adequadas para novas análises. Foram registrados também, em protocolo próprio, os dados clínicos, os exames laboratoriais e de imagem à admissão e durante toda a evolução, o tratamento e os dados evolutivos de cada um dos pacientes pediátricos portadores de RSF congênito.

A coleta de amostras de urina nos pacientes com RSF e nas crianças saudáveis (grupo controle) ocorreu em um único momento, respeitando-se os critérios de inclusão e exclusão. O presente estudo utilizou essas amostras de urina para mensuração dos biomarcadores urinários de interesse. Foram coletadas e analisadas amostras de urina de 30 pacientes com rim único e 20 crianças saudáveis (grupo controle), as quais se encontravam em acompanhamento no Ambulatório de Pediatria geral da mesma instituição.

4.2. Critérios de inclusão e exclusão

- **Critérios de inclusão:** Crianças e adolescentes portadores de RSF congênito (idade inferior a 20 anos no momento da coleta de urina), confirmado por exames de imagem, em acompanhamento em serviço especializado de Nefrologia Pediátrica, mediante consentimento em participar da pesquisa. O grupo controle incluiu crianças e adolescentes saudáveis, pareados em idade e sexo com os pacientes portadores de RSF, que aceitaram participar da pesquisa.
- **Critérios de exclusão:** Em relação aos casos de RSF congênito, foram excluídos pacientes com malformações em outros sistemas ou malformações múltiplas, pacientes com doenças crônicas ou outras comorbidades (exceto CAKUT associado) e pacientes que abandonaram o acompanhamento, não sendo possível coletar amostra urinária para análise dos biomarcadores. Em relação ao grupo controle, foram excluídas crianças com doenças infecciosas e/ou alérgicas agudas no momento da coleta.

4.3. Variáveis clínicas e laboratoriais de interesse

Em relação aos pacientes com RSF, as seguintes variáveis foram avaliadas à admissão no serviço e/ou na última consulta ambulatorial dentro do período do estudo: gênero, idade, dados antropométricos, pressão arterial, relação proteína/ creatinina em amostra de urina e/ou proteinúria de 24 horas, creatinina plasmática, ritmo de filtração glomerular estimado, presença de outras alterações do trato urinário associadas (CAKUT associado) e achados ultrassonográficos ao diagnóstico.

Em relação ao grupo controle, foram avaliados história pregressa, exame clínico, idade, gênero, dados antropométricos, pressão arterial e relação proteína/ creatinina em amostra de urina no momento da coleta de urina para medida dos biomarcadores.

Em nossa instituição, as medidas de creatinina foram feitas pelo método de Jaffé até novembro de 2011. Por este motivo, a TFG foi estimada pela fórmula convencional de Schwartz [2] para os dados obtidos até esse período. Após novembro de 2011, a creatinina passou a ser medida pelo método enzimático (espectrometria de massa de diluição de isótopos). Portanto, a fórmula de Schwartz modificada [3] foi adotada para estimar a TFG a partir de então.

4.4. Aspectos éticos

Este projeto de pesquisa foi aprovado pelo Comitê de Ética e Pesquisa da Universidade Federal de Minas Gerais (vide ANEXOS) e os pais ou responsáveis legais das crianças assinaram um termo de consentimento livre e esclarecido (vide ANEXOS). O protocolo do estudo não interferiu em nenhuma recomendação médica ou no tratamento dos pacientes com RSF.

4.5. Procedimentos do Estudo

4.5.1. Coleta e processamento do material biológico

As amostras de urina foram coletadas em recipientes apropriados estéreis e centrifugadas a 405g, 4ºC por 5 minutos para decantação. Visando evitar alterações circadianas e efeito pós-prandial, todas as coletas foram realizadas sempre no período da manhã. Os materiais biológicos foram processados e armazenados em freezer a -80 °C até o momento das análises de biomarcadores.

4.5.2. Estudo de biomarcadores em amostras biológicas

Neste estudo foi realizada a mensuração das moléculas Calbindina, Colágeno IV, FABP1, GSTα, IP-10, KIM-1, Osteoactivina, Renina, TFF-3, TIMP-1, α-1-Microglobulina, Albumina, Clusterina, Cistatina C, EGF, Lipocalina-2/NGAL, Osteopontina, por meio do método de *multiplex*

kit of kidney injury panels 1 e 2 (Merck Milipore, MA, USA), conforme as recomendações do fabricante nas amostras de urina de pacientes com RSF e controles saudáveis.

Resumidamente, microesferas de captura revestidas com anticorpos monoclonais específicos para cada analito foram adicionadas aos poços, junto com as amostras de urina e os padrões. Após incubação e lavagem, foi adicionada uma mistura de anticorpos secundários ligados a biotina. Em seguida, a estreptavidina conjugada à proteína fluorescente foi adicionada aos poços e incubada por um breve período. Após a lavagem, o sobrenadante foi descartado e o precipitado contendo as microesferas foi ressuspendido em uma solução tampão. A leitura dos padrões e das amostras foi realizada no analisador de microesferas MAGPIX (Luminex Corporation, Texas, EUA) e os resultados foram analisados no programa Milliplex Analyst (MilliporeSigma), sendo representados em pg/mL. O ensaio de biomarcadores de lesão renal foi realizado em duplicatas. Esses ensaios foram realizados simultaneamente e com o mesmo lote de reagentes para evitar a variabilidade entre os ensaios. A variabilidade intra-ensaio foi inferior a 3%.

4.6. Análise estatística

Para cada grupo, as variáveis qualitativas foram descritas segundo frequências e porcentagens. A distribuição das variáveis quantitativas (contínuas) foi verificada com o teste de Shapiro-Wilk. As variáveis quantitativas gaussianas (normais) foram descritas de acordo com a média e o desvio-padrão e as variáveis quantitativas não gaussianas foram descritas como mediana e intervalo interquartílico.

A associação entre variáveis dicotômicas foi avaliada por meio do teste do qui-quadrado ou pelo teste exato de Fisher, quando apropriado. A comparação entre dois grupos (pacientes com rim único funcional e controles saudáveis) foi feita pelo teste t de Student para dados não pareados ou Mann-Whitney, de acordo com a distribuição. Já a comparação entre as variáveis avaliadas à admissão no serviço e na última consulta ambulatorial foi feita pelo teste t de Student para dados pareados ou teste de Wilcoxon, conforme a distribuição. As correlações entre as variáveis foram analisadas pelo teste de Pearson ou pelo teste de Spearman. Todos os testes estatísticos foram bilaterais usando um nível de significância de $\alpha = 0.05$. Para construção do banco de dados e realização das análises estatísticas, utilizamos o programa GraphPad Prism versão 6.0 (GraphPad, La Jolla, CA, USA).

4.7. Referências

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5. RESULTADOS E DISCUSSÃO - ARTIGO ORIGINAL

URINARY BIOMARKERS IN PEDIATRIC PATIENTS WITH SOLITARY FUNCTIONING KIDNEY: A PILOT STUDY

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List of abbreviations

AKI – acute kidney injury

BP – blood pressure

CAKUT – Congenital Anomalies of the Kidney and Urinary Tract

CKD – chronic kidney disease

Cr – creatinine

EGF – Epidermal growth factor

eGFR – estimated glomerular filtration rate

ESKD – end stage kidney disease

FABP-1 – Fatty acid binding protein

GST-α – α-glutathione S-transferase

IP-10 – Protein induced by interferon

KIM-1 – Kidney injury molecule 1

MCDK – multicystic dysplastic kidney

OPN - osteopontin

SFK – Solitary functioning kidney

SNGFR – single nephron glomerular filtration rate

TFF-3 – Trefoil factor 3

TIMP-1 – Tissue inhibitor of metalloproteinase 1

ABSTRACT

Background: Solitary Functioning Kidney (SFK) is an important subgroup of the Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) spectrum. Serum urea and creatinine levels are still the most used markers to assess renal function, even though these measurements do not allow early detection of renal injury. In this sense, the aim of this pilot study was to measure urinary concentration of kidney biomarkers in pediatric patients with SFK and to compare with the same biomarkers measurements in healthy children and adolescents. **Methods:** This cross sectional study included 30 pediatric patients with congenital SFK (renal agenesis, primary renal hypodysplasia, and multicystic dysplastic kidney) and 20 healthy individuals paired for gender and age (controls). All the participants were submitted to a single urine collection to measure seventeen urinary biomarkers, known to be related to kidney function and/or lesion. **Results:** Cystatin C, Osteopontin, Calbindin, Osteoactivin, Tissue inhibitor of metalloproteinase 1, Kidney injury molecule-1, Protein induced by interferon, Renin, Epithelial Growth Factor and Clusterin were significantly reduced in urine samples of patients in comparison with controls. Lipocalin-2/NGAL was the only molecule with significantly increased levels in patients when compared with controls. The remaining molecules were similar in both groups. Serum levels of creatinine increased significantly from baseline to the last outpatient visit before closing the database of this study, whereas estimated GFR reduced. **Conclusion:** In pediatric patients with congenital SFK, the behavior of some urinary molecules is different from healthy controls. Longitudinal studies should be performed to validate the clinical utility of these biomarkers.

KEY WORDS: Solitary functioning kidney, renal function, urinary biomarkers, CAKUT

Introduction

Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are highly prevalent birth defects [1]. Solitary Functioning Kidney (SFK) is an important condition in the spectrum of CAKUT [2]. SFK can be congenital or acquired. The main phenotypes of congenital SFK include renal agenesis, primary renal hypodysplasia, and multicystic dysplastic kidney (MCDK) [3].

Despite the prognosis of pediatric patients with congenital SFK is considered to be good [4], recent reports have shown that long-term clinical outcome is not as good as it was thought before. The reduced functional nephron number may increase glomerular filtration rate per nephron to maintain a normal renal function. This process leads to a vicious cycle of progressive single nephron hyperfiltration, glomerular hypertension and glomerulosclerosis. These changes are associated with

arterial hypertension and proteinuria [5], renal injury [5,6], and a significantly higher risk for dialysis in adult life [7].

Although new markers of tubular injury in children with SFK are known, including lysosomal exoglycosidases and their isoenzymes, the clinical significance is not determined [8]. In addition, the natural history of children with SFK is not fully established. There is still a lack of consensus on the best method to evaluate and to treat these patients [4]. In this context, the identification of additional biomarkers of kidney function or lesion assumes importance [9]. Therefore, the aim of this pilot study was to measure urinary concentration of kidney biomarkers in pediatric patients with SFK and to compare with the same measurements in sex and age-matched healthy individuals. A second objective was to assess whether there is any correlation between the analyzed biomarkers and the estimated glomerular filtration rate (eGFR) in patients with SFK.

Methods

Study design

This is a cross sectional study including 30 pediatric patients with congenital SFK and 20 healthy individuals paired for gender and age (control group). For the purpose of the present study, we searched data of the patients followed up at our institution from 1998 to 2019. The collection of urine samples for the measurement of biomarkers was carried out in 2016. Blood pressure, proteinuria, serum creatinine and eGFR data were collected at the last outpatient visit before closing the database of this study.

Inclusion and exclusion criteria

The participants were required to meet the following inclusion criteria: SFK diagnosis by imaging method, age under 20 years at the time of urine collection and no other comorbidities, except associated CAKUT. The studied group included the following phenotypes: renal agenesis, primary renal hypodysplasia, and MCDK. The diagnosis of these phenotypes was according to ultrasound findings, as previously detailed [3]. Patients with bilateral severe renal hypodysplasia, multiple malformations and who abandoned postnatal follow-up were excluded.

The control group was composed of 20 healthy children and adolescents, without previous or family history of kidney disease, normal clinical examination and preserved kidney function.

Ethical aspects

The study was approved by the Ethics Committee of the Federal University of Minas Gerais. The parents or individuals responsible for the patients and controls gave written informed consent to

participate. The study protocol did not interfere with any medical prescriptions.

Study protocol

After birth, all patients diagnosed with SFK go through an initial imaging and laboratory evaluation and, afterwards, ultrasound scans, clinical examination and laboratory reviews are performed at 6-month intervals during the first 2 years and yearly thereafter [3]. Blood pressure (BP), proteinuria, plasma creatinine concentration, eGFR and the primary cause of SFK were evaluated. The analysis of the patients with SFK focused on two periods: baseline data at admission to our institution and data at the time of the last outpatient visit within the study period. The urine collection to measure biomarkers occurred between these two moments.

All urine specimens were collected in sterile recipients from morning samples, then transferred to 15 ml plastic tubes and immediately centrifuged (3800 rpm, 5 minutes, 4ºC). The supernatant was transferred to 1.5mL microtubes and stored in the -80ºC freezer until further analysis. BP was measured in the sitting position with appropriate sphygmomanometer cuff using the standard method. Urinary albumin/creatinine ratio between 30 to 300 µg/ mg was considered microalbuminuria.

Since creatinine measurements were made by Jaffe method until November 2011 in our institution, GFR was estimated by the conventional Schwartz formula [10] for data obtained until this period. After November 2011, creatinine was measured by IDMS (isotope dilution mass spectrometry) traceable method. Therefore, the modified Schwartz formula [11] was adopted to estimate GFR.

Biomarkers measurements

A panel of seventeen urinary biomarkers related to kidney function and/or lesion was utilized to evaluate pediatric patients with SFK. The patients and controls were submitted to a single urine collection to measure Calbindin, Collagen IV, Fatty acid binding protein (FABP-1), α-glutathione S-transferase (GST-α), Protein induced by interferon (IP-10/CXCL10), Kidney injury molecule 1 (KIM-1), Osteoactivin, Renin, Trefoil factor 3 (TFF-3), Tissue inhibitor of metalloproteinase 1 (TIMP-1), α-1-Microglobulin, Albumin, Clusterin, Cystatin C, Epidermal growth factor (EGF), Lipocalin-2/NGAL and Osteopontin by using the Human Kidney Injury Magnetic Bead Panel 1 and Panel 2 kits (Merck Millipore Corporation, MA, USA). The measurements were performed according to information from the manufacturer (Millipore Corporation, MA, USA).

Along with urine samples and standard solutions, capture microspheres coated with specific monoclonal antibodies for each analyte were added to the wells. A mixture of secondary biotinylated

antibodies was added after incubation and washing. Subsequently, streptavidin conjugated to the fluorescent protein was incubated for a brief period. After the second washing, the supernatant was discarded and the precipitate containing the microspheres was resuspended in a buffer solution. The standards and samples were acquired in the MAGPIX microsphere analyzer (Luminex Corporation, Texas, USA) and analyzed with the Milliplex Analyst program (MilliporeSigma), as previously described elsewhere [12]. The biomarkers were expressed as pg/ml. All samples were evaluated in a single assay to avoid interassay variability.

Statistical analysis

Data were analyzed using the software GraphPad Prism version 6.0. The qualitative variables were expressed in absolute frequencies and percentages. Gaussian distribution was verified using the Shapiro Wilk test. Non-parametric variables were shown as median and interquartile range, whereas normal distributed variables as mean and standard deviation. For comparisons between variables at baseline and at the time of the last ambulatory visit, we used paired Student T-test or Wilcoxon test according to the distribution. For comparisons between patients and controls, unpaired Student T-test and Mann Whitney test were respectively performed for parametric and non-parametric variables. The Spearman correlation coefficient was used for correlation analysis between biomarkers and eGFR.

RESULTS

Population

This study included 30 patients with SFK, whose age ranged from 6 to 19 years at the time of urine collection. These patients were subjected to a single urine collection to measure the biomarkers.

Among the patients, there was a predominance of males (66.6%) with an age at diagnosis of 4.1±8.2 months. The main cause of SFK was MCDK (n=22, 73.3%), followed by renal hypoplasia (n=6, 20%). Associated urinary tract malformations were observed in 16.6% of the patients. At the last ambulatory visit within the study period, five patients presented elevated blood pressure (above 90th percentile). A total of 16 patients (53.3%) underwent proteinuria evaluation and eight patients (50%) presented some degree of proteinuria.

The control group was formed by 20 healthy individuals, sex and age matched to patients. All controls have normal BP, serum levels of creatinine and eGFR within the reference range.

Comparisons between patients and controls are shown in Table 1. Age and gender distribution were similar in both groups, controls had eGFR significantly higher ($p<0.0001$) and serum creatinine levels significantly lower ($p=0.0034$) than patients.

Biomarkers

1. *Creatinine and eGFR*

Patients' creatinine measurements at baseline were compared with creatinine evaluated at the last outpatient visit before closing the database of this study and showed a significant increase (median, p25-p75; at baseline: 0.40 mg/dl, 0.32-0.53 vs. at last visit: 0.60 mg/dl, 0.54-0.85; Wilcoxon test, p=0.001). Similarly, eGFR significantly decreased from baseline to the last visit measurement (mean±standard deviation; at baseline: 165.7±65.48 vs. at last visit: 102.4±27.38; paired t test, p=0.0001).

2. *Urinary biomarkers*

Cystatin C, OPN, Calbindin, Osteoactivin, TIMP-1, KIM-1, IP-10 and Renin, were significantly reduced in urine samples of patients in comparison with controls, as shown in Figure 1a. Cystatin C levels presented a 45% reduction in SFK children compared to controls (median, p25-p75; controls: 3.04, 1.93-15.16 vs. patients: 1.67, 0.89-4.89, p=0.03). Urinary concentrations of OPN were also lower in patients than in controls (controls: 172.60, 80.86-552.60 vs. patients: 28.75, 21.92-433.70, p=0.04). Urinary levels of Calbindin were 60.4% lower in patients than in controls (controls: 0.45, 0.21-342 vs. patients: 0.18, 0.11-0.45, p=0.03). Osteoactivin urine concentrations in the SFK group was 37.5% lower than in controls (controls: 0.24, 0.15-0.43 vs. patients: 0.15, 0.13-0.22, p=0.03). TIMP-1 measurements were also lower in the SFK group than in controls (controls: 0.28, 0.17-1.63 vs. patients: 0.16, 0.15-0.25, p=0.02). Urinary concentrations of KIM-1 were 30% lower in patients compared to controls (controls: 0.10, 0.07-0.15 vs. patients: 0.07, 0.05-0.11, p=0.04). IP-10 levels were significantly lower in patients than in controls (controls: 0.01, 0.01-0.07 vs. patients: 0.01, 0.01-0.01, p=0.0068). Urinary levels of Renin were also significantly smaller in patients than in controls (controls: 0.04, 0.03-0.05 vs. patients: 0.03, 0.03-0.04, p=0.006).

EGF and Clusterin showed significantly reduced values in SFK patients if compared to controls, as shown in Figure 1b. EGF measurements were 92.2% lower in patients compared to controls (controls: 35.34, 6.01-58.14 vs. patients: 2.74, 0.90-23.60, p=0.0078). Urinary levels of Clusterin were 58.1% lower in patients than in controls (controls: 1392, 321-6693 vs. patients: 582.8, 43.84-1127, p=0.04).

Among all the tested molecules, Lipocalin-2/NGAL was the only one that showed significantly increased levels in patients when compared with the controls. As also shown in Figure 1b, urinary concentrations of Lipocalin-2/NGAL exhibited a 10-fold increase in SFK patients if compared to controls (controls: 2.47, 0.90-4.66 vs. patients: 24.83, 0.59-33.86, p=0.04).

GST-α, FABP-1, Collagen IV, TFF-3, albumin and alpha-microglobulin did not significantly differ in patients and controls (Table 2).

3. *Correlation analysis*

Correlation analysis were performed between urinary biomarkers and the eGFR evaluated at the last ambulatory visit. OPN and eGFR showed a significant positive correlation (Spearman r value=0.432, p=0.02), whereas a significant negative correlation was found between eGFR and KIM-1 (Spearman r value= -0.409, p=0.03). No other significant correlations were detected (Table 3).

DISCUSSION

The CAKUT spectrum is a major cause of morbidity in children [7] with a prevalence of around 4.3 per 1000 births in 2019 in Europe [available in https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence_en]. CAKUT are responsible for 30 to 50 percent of cases of chronic kidney disease (CKD) in pediatric patients [13]. The SFK is an important subgroup of the CAKUT spectrum and a significant fraction of these children is at risk for progression to CKD [5]. The risk of developing renal injury in later life is even worse in those patients with a SFK and ipsilateral urinary tract malformation [14]. Serum levels of urea and creatinine and the eGFR are the most used markers for monitoring kidney function. However, these markers do not allow early detection of renal injury [15,16,17,18]. Thus, the evaluation of new biomarkers to identify as quickly as possible any sign of kidney dysfunction and/or initial injury is an important research goal, especially in the pediatric population. Cells of the renal tubule epithelium synthesize and secrete many molecules to the urine. The measurement of urinary levels of these molecules emerges as a sensitive and non-invasive method for the assessment of renal tubular function [8,19].

The present study was designed to evaluate urinary levels of several molecules related to diverse pathways in kidney metabolism in pediatric patients with SFK in comparison to healthy individuals matched for age and gender. The general idea was to search for a potential and early biomarker of kidney dysfunction or injury. To date, this is the first study in the literature to measure this panel of seventeen molecules in urine samples of pediatric patients with SFK.

Almost forty years ago, Brenner and coworkers [20] described the classic hyperfiltration hypothesis. In an experimental study, reduction in the number of functional nephrons was associated with “adverse adaptations” at the remnant glomeruli, especially hypertrophy, and compensatory glomerular hyperfiltration. As a consequence, the nephrectomized animals presented higher levels of BP, proteinuria and also progressively declined the GFR. Individuals with congenital SFK have, by definition, a reduction in renal mass since intra-uterus and the unique kidney is usually bigger already

at birth [5,14,21,22]. The hyperfiltration hypothesis remains unproven in human studies, probably because of the inability to measure single nephron GFR and total nephron number *in vivo* [14,15]. However, patients with congenital SKF are expected to develop compensatory glomerular hyperfiltration during childhood [23]. Even though this adaptation may seem advantageous at first glance, once it minimizes the GFR reduction in the first years of life, the prolonged increase in nephron size may lead to renal tissue fibrosis, vasoconstriction and tubular cell nephrotoxicity [5,23]. These mechanisms significantly increase the risk for hypertension, proteinuria, and eventually CKD [8,14].

In our analysis, EGF, Clusterin and Calbindin showed significantly reduced values in SFK patients when compared to healthy controls. Azukaitis and colleagues [24] performed a post hoc analysis of the *Cardiovascular Comorbidity in Children with CKD study* in order to assess the association and the predictive value of urinary EGF excretion as a marker of CKD progression. The authors also evaluated if the ratio between urinary EGF and Creatinine (uEGF/Cr) is even better to predict the progression of CKD in the pediatric cohort. They concluded that low urinary levels of EGF predict CKD progression and appear to reflect the extent of tubulointerstitial damage. In addition, lower uEGF/Cr was associated with an increased risk of CKD progression independent of age, sex, baseline eGFR, primary kidney disease, proteinuria, and systolic blood pressure. In regard to urinary levels of Clusterin and Calbindin, only one study in the literature evaluated these molecules in a pediatric population. Wu *et al.* [25] measured urinary concentrations of 12 potential end stage kidney disease (ESKD) biomarkers in pediatric patients with systemic lupus erythematosus associated or not with lupus nephritis. The authors found that urinary levels of Clusterin and Calbindin were significantly elevated in patients with tubulointerstitial lesions. Also, they showed an association between Clusterin elevation and ESKD prediction. These results are divergent to our findings. However, the severity of kidney tissue injury is certainly higher in lupus nephritis than in SFK. In addition, considering that Clusterin and Calbindin reflect tubular injury, the differences in comparison with our results are probably due to the fact that in patients with SFK the initial mechanism of injury is glomerular hyperfiltration rather than tubular damage. On the other hand, our finding of reduced urinary levels of EGF in SFK patients might suggest an early tubulointerstitial injury. Indeed, the molecular mechanisms and the sequence of events related to kidney tissue injury in SFK are still unknown. Therefore, we believe that urinary biomarkers may dynamically change according to the stage of kidney tissue injury.

Cystatin C dosage was significantly reduced in urine samples of SFK patients in comparison with healthy controls. Cystatin C is filtered by the glomerulus, and then is almost entirely reabsorbed in the proximal tubule, without secretion [18], which justifies the fact that, normally, the molecule is

not identified in urine in significant amounts. Considering this mechanism, measurement of urinary cystatin C is a potentially valuable tool for diagnosing proximal tubular damage and dysfunction [26]. Our findings are in contrast to previous studies which identified increased urinary cystatin C excretion related to kidney injury [17,18,27]. Hellerstein and colleagues [27], in fact, observed an association between Cystatin C elevation and decreasing creatinine clearance, while Herget-Rosenthal et al. [17] found increased urinary excretion of cystatin C associated with proteinuria and tubulointerstitial disease, independent of reductions in GFR. These contradictory findings are probably a reflection of differences in selected patients. Our study focused on children with congenital SFK, whereas Hellerstein et al. [27] and Herget-Rosenthal et al. [17] studied patients with glomerular and tubulointerstitial disorders with and without heavy proteinuria. Woodson et al. [18], in turn, used animal models of solitary kidney to measure cystatin C. These animal models are more compatible with acquired SFK rather than the congenital form.

In congenital SFK, the total nephron number is still lower than in two kidneys, despite the compensatory increase in nephron number [5]. Therefore, the expected amount of filtered Cystatin C can be lower, while the proximal tubular function is probably normal, allowing the reabsorption of filtered Cystatin C in SFK patients.

Lipocalin-2/NGAL is one of the most studied urinary biomarkers and is considered to be an early acute kidney injury (AKI) predictor [28]. Nowadays, the positive association of NGAL with kidney tissue injury is well established [18,26]. Among all the tested molecules Lipocalin-2/NGAL was the only one that showed significantly increased levels in SFK patients when compared with controls. However, the behavior of this molecule in SFK remains to be determined.

TIMP-1 and IP-10 were significantly reduced in urine samples of patients in comparison with controls. Our findings differed from other studies that assessed these biomarkers in diverse renal diseases [30-33]. The main differences between these studies and ours, including type of kidney injury, clinical characteristics of the patients and study design, preclude the comparison of results. Although not statistically significant, urinary albumin levels, a known marker of glomerular damage [34-36], had a trend to be higher in patients than in controls.

eGFR showed a statistically significant decrease from baseline to the time of the last ambulatory visit, suggesting a decrease in renal function over the years. In our patients, urinary concentrations of OPN and KIM-1 were lower than in controls. Correlation analysis showed a significant positive correlation between OPN and eGFR and a significant negative correlation between eGFR and KIM-1. This result was expected, considering that OPN is a marker of glomerular damage and KIM-1 reflects tubular injury [25,29]. We did not find any studies analyzing renin and

osteoactivin urinary measurements in pediatric patients with renal diseases. The remaining molecules evaluated in this study were not statistically different.

Our study has several limitations. First, the small sample size and, therefore, a lack of statistical power. Second, the cross-sectional design, which does not allow the dynamic evaluation of the molecules. On the other hand, the strength of our study is the originality since this is the first to analyze this panel of seventeen biomarkers in pediatric SFK.

In pediatric patients with congenital SFK, the behavior of some urinary molecules is different from that of healthy individuals. Considering that this population has an augmented risk to develop renal injury in later life, the search for biomarkers is of relevance. Our study is a preliminary investigation on urinary molecules in SFK. Future prospective and larger studies are required to validate the clinical utility of these biomarkers.

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5.2. APPENDIX - TABLES AND FIGURES

Table 1
Participants' characteristics and kidney function at the last ambulatory visit

	Patients (n=30)	Controls (n=20)	p value
Age (years) (Mean ± SD)	12.27 ± 3.82	14.25 ± 3.24	0.0626
Sex (masculine) (N(%))	20 (66.6)	12 (60)	0.8400
Creatinine (mg/dL) (Mean ± SD)	0.66 ± 0.21	0.52 ± 0.05	0.0034
eGFR (ml/min) (Mean ± SD)	102.4 ± 27.38	131.5 ± 8.55	<0.0001

eGFR, estimated glomerular filtration rate.

Table 2
Comparison between urinary biomarkers collected in patients with solitary functioning kidney and healthy individuals (control group).

Molecules	Patients*	Controls*	p value
GST-alpha	0.02 (0.02-0.04)	0.05 (0.02-0.13)	0.093
FABP-1	10.76 (8.2-14.12)	9.92 (9.07-18.43)	0.3794
Collagen IV	0.53 (0.28-1.48)	0.61 (0.34-2.00)	0.5156
TFF-3	1.23 (0.65-2.26)	1.01 (0.57-5.73)	0.9515
Albumin	1464 (0-3565)	73.62 (1.62-20309)	0.7666
Alpha-microglulin	74.66 (39.32-103.7)	81.86 (69.29-127.6)	0.1469

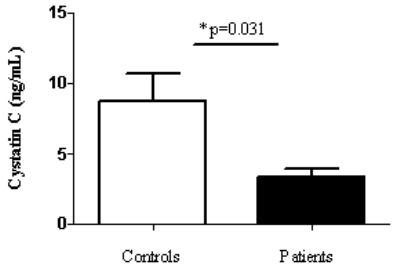
GST-α, α-glutathione S-transferase; FABP-1, fatty acid binding protein; TFF-3, trefoil factor-3.
*The values were quantified in pg/ml and expressed as median and (25-percentile - 75-percentile).
p values were obtained by Mann-Whitney test.

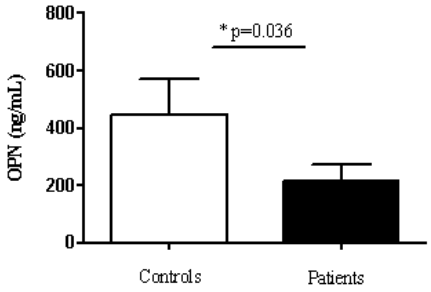
Table 3
Correlation analysis between eGFR and urinary biomarkers

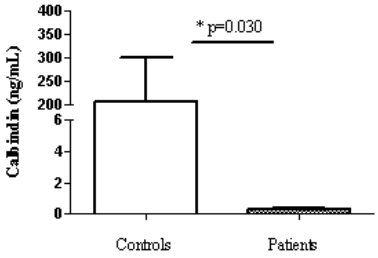
Molecule	Spearman coefficient (r)	p value
Calbindin	-0.3090	0.1168
Collagen IV	-0.3294	0.0869
FABP-1	-0.3274	0.0890
GST-α	-0.3469	0.0705
IP-10	-0.1667	0.3965
KIM-1*	**-0.4090**	**0.0307**
Osteoactivin	-0.2822	0.1457
Renin	0.0215	0.9136
TFF-3	-0.1755	0.3717
TIMP-1	-0.0867	0.6608
α-1-Microglobulin	0.3589	0.0660
Albumin	0.2856	0.1407
Clusterin	0.3056	0.1138
Cystatin C	0.3243	0.0923
EGF	0.2053	0.2946
Lipocalin-2/NGAL	0.3226	0.0941
Osteopontin*	**0.4322**	**0.0216**

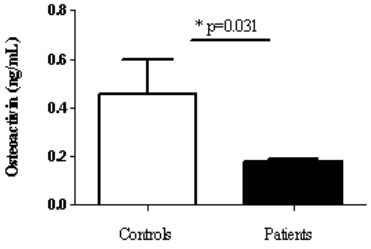
eGFR, estimated glomerular filtration rate. *p value < 0.05

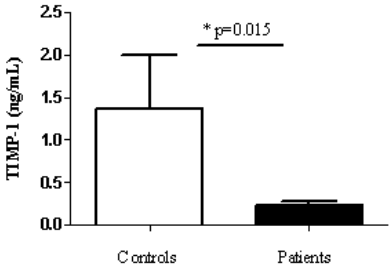
Figure 1a

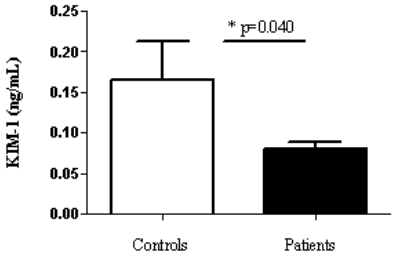

Cystatin C (ng/mL)
15
10
5
0
*p=0.031
Controls
Patients

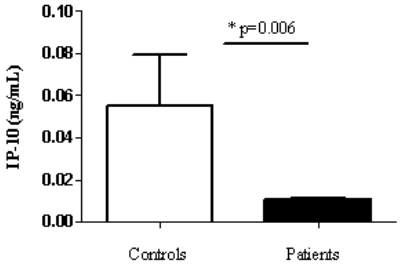

OPN (ng/mL)
800
600
400
200
0
*p=0.036
Controls
Patients


400
350
300
250
200
6
4
2
0
*p=0.030
Controls
Patients


0.8
0.6
0.4
0.2
0.0
*p=0.031
Controls
Patients


TIMP-1 (ng/mL)
2.5
2.0
1.5
1.0
0.5
0.0
*p=0.015
Controls
Patients


KIM-1 (ng/mL)
0.25
0.20
0.15
0.10
0.05
0.00
*p=0.040
Controls
Patients


IP-10 (ng/mL)
0.10
0.08
0.06
0.04
0.02
0.00
*p=0.006
Controls
Patients

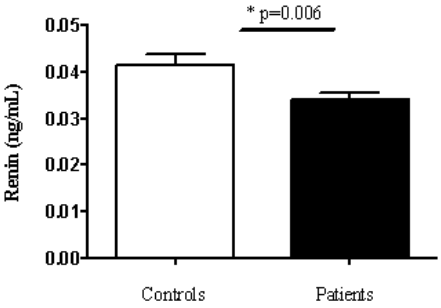

Renin (ng/mL)
0.05
0.04
0.03
0.02
0.01
0.00
*p=0.006
Controls
Patients

Figure 1a- Comparison between urinary biomarkers in patients with solitary functioning kidney (patients) and healthy individuals (controls). *Cystatin C*: patients (n=30) vs. controls (n=20); Mann-Whitney test, *p=0.031. *Osteopontin (OPN)*: patients (n=30) vs. controls (n=20); Mann-Whitney test, *p=0.036. *Calbindin*: patients (n=29) vs. controls (n=12); Mann-Whitney test, *p=0.030. *Osteoactivin*: patients (n=30) vs. controls (n=13); Mann-Whitney test, *p=0.031. *Tissue inhibitor of metalloproteinase 1 (TIMP-1):* patients (n=28) vs. controls (n=12); Mann-Whitney test, *p=0.015. *Kidney injury molecule 1 (KIM-1)*: patients (n=30) vs. controls (n=12); Mann-Whitney test, *p=0.040. *Interferon induced protein (IP-10):* patients (n=30) vs. controls (n=13); Mann-Whitney test, *p=0.006. *Renin*: patients (n=28) vs. controls (n=13); Mann-Whitney test, *p=0.006.

Figure 1b

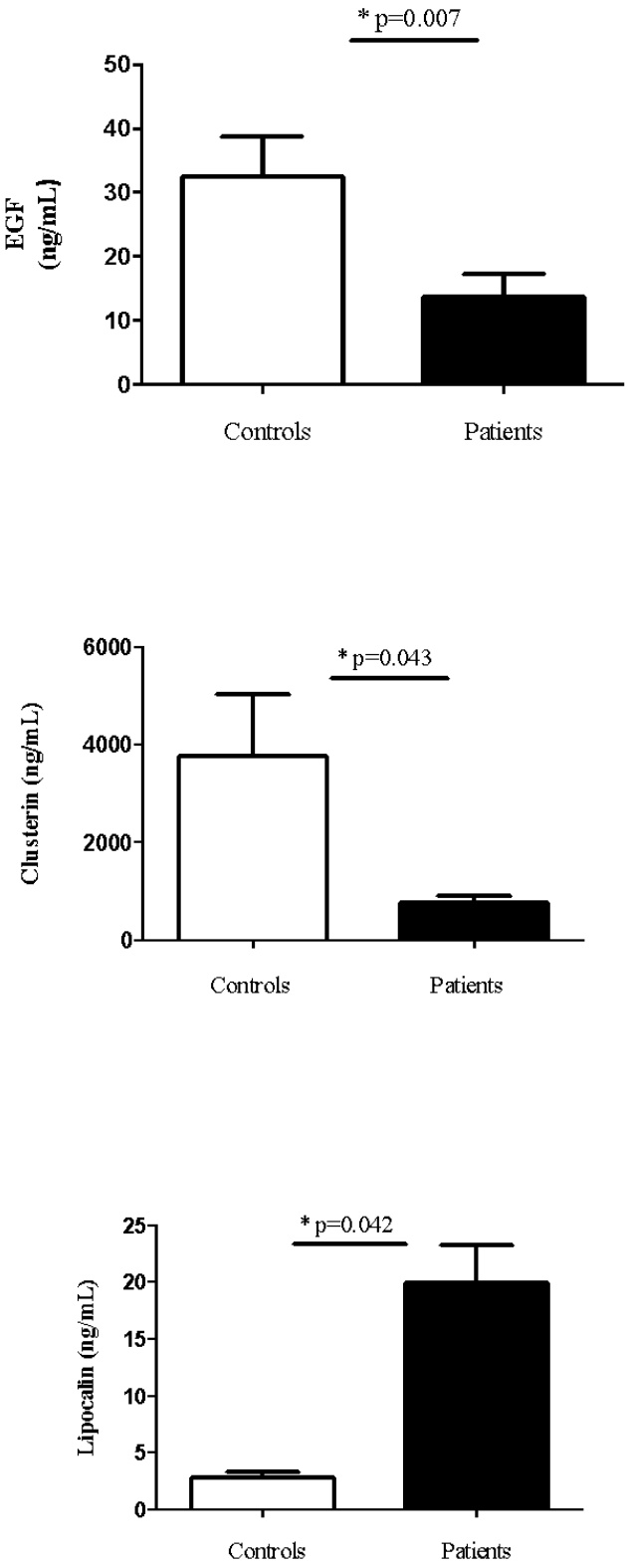


Figure 1b - Comparison between urinary biomarkers in patients with solitary functioning kidney (patients) and healthy individuals (controls). *Endothelial growth factor (EGF):* patients (n=29) vs. controls (n=18); Mann-Whitney test, *p=0.007. *Clusterin:* patients (n=30) vs. controls (n=20); Mann-Whitney test, *p=0.043. *Lipocalin-2 (NGAL)*: patients (n=30) vs. controls (n=17); Mann-Whitney test, *p=0.042.

6. CONCLUSÕES E CONSIDERAÇÕES FINAIS

Os pacientes pediátricos com rim solitário funcional (RSF) fazem parte de um subgrupo de anomalias renais congênitas que merece atenção especial. Muitos estudos já mostraram a necessidade de acompanhamento longitudinal desses pacientes, considerando o risco aumentado de desfechos desfavoráveis [1–4]. Apesar disso, o seguimento desse subgrupo ainda necessita de recursos propedêuticos capazes de identificar as alterações da função renal com maior acurácia.

Com o intuito de detectar precocemente a ocorrência de injúrias renais agudas e de estimar com mais precisão a função deste órgão tão importante, iniciou-se a busca por novos biomarcadores [5,6]. No entanto, ainda há pouco material na literatura que aborda este tema nesta população específica.

No presente estudo, observamos que os pacientes com RSF congênito apresentaram queda da taxa de filtração glomerular (TFG) com o passar do tempo, o que sugere uma tendência a piora progressiva da função renal, a qual, possivelmente, vem acompanhada da perda gradual do mecanismo de hiperfiltração compensatória.

Além disso, foi visto que algumas moléculas se comportaram de forma diferente entre pacientes e controles. O fator de crescimento epidérmico (EGF) apresentou valores reduzidos nos pacientes com RSF, resultado consistente com os dados disponíveis na literatura [7,8]. Entretanto, nossos dados não nos permitem inferir o fator responsável por esse resultado, uma vez que os níveis urinários reduzidos de EGF podem ser reflexo tanto da massa renal reduzida dos pacientes avaliados, quanto da perda da capacidade de hipertrofia compensatória. A lipocalina associada a gelatinase e neutrófilos (NGAL), considerada um preditor de lesão renal aguda [5,9,10], foi encontrada em níveis aumentados nas crianças com RSF. Esse resultado nos chamou atenção pois o aumento de NGAL foi observado apesar da massa renal reduzida dos pacientes, o que indica um potencial promissor desta molécula neste subgrupo específico de pacientes. E, por fim, a observação de valores reduzidos de cistatina C no grupo dos pacientes nos sugere que a avaliação urinária dessa molécula seja menos acurada que sua dosagem sérica.

O presente estudo apresenta algumas limitações, dentre as quais destacam-se o desenho transversal e o tamanho da amostra. Além disso, a disponibilidade de poucos estudos sobre o tema foi um fator que limitou, em alguns momentos, a comparação e interpretação dos dados.

Como perspectivas para o futuro, consideramos a possibilidade do uso de um painel de marcadores de função renal - ao invés da dosagem isolada de uma única molécula - como sendo uma estratégia promissora para a vigilância desses pacientes. Além disso, acreditamos que protocolos de

seguimento mais rigorosos e prolongados devam ser instituídos, pois entendemos que essa é a melhor maneira de identificar as alterações precocemente e intervir em momento oportuno.

Sendo assim, esperamos que as mensurações de biomarcadores urinários realizadas no presente estudo estimulem a realização de investigações prospectivas e com maior tempo de acompanhamento, que proporcionem validação dos dados encontrados para que sejam futuramente incorporados na prática clínica.

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7. ANEXOS

ANEXO 1 – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIDADE DE NEFROLOGIA PEDIÁTRICA
CENTRO DE MEDICINA FETAL
HOSPITAL DAS CLÍNICAS – UFMG

INFORMAÇÃO PARA CONSENTIMENTO

Nas últimas décadas, a maioria das anomalias do trato urinário tem sido diagnosticada antes do nascimento pela ultrassonografia durante a gestação. Esse diagnóstico precoce das doenças renais pode proporcionar muitos avanços na identificação e tratamento para as crianças que sofrem dessa doença. Nossa Unidade está fazendo um estudo analisando os fatores prognósticos e potenciais biomarcadores para a evolução desfavorável da doença, incluindo a análise dos exames no pré-natal e no pós-natal. Para que este estudo seja realizado, serão realizados exames laboratoriais, de imagem e clínicos fazem parte da rotina de atendimento no Ambulatório de Nefrologia Pediátrica, além de coleta de amostra de urina para dosar substância que podem estar relacionadas com o funcionamento do rim. Os exames de imagem a serem realizados são os seguintes: ultrassonografia dos rins; radiografia contrastada da uretra e bexiga; cintilografia renal. A realização desses exames tem como objetivo diagnosticar a doença e, assim, indicar o melhor tratamento. Além disso, serão realizados exames para avaliar a função renal (dosagem no sangue de ureia e creatinina), exames de urina para diagnosticar e tratar os casos que tenham infecção na urina e dosagens de substâncias na urina que podem se relacionar à função renal.

Você pode decidir agora por permitir a inclusão de seu filho no estudo. Se você decidir não participar, essa decisão não implicará qualquer alteração nos cuidados que seu filho já vem recebendo. Nós responderemos a qualquer questão relativa ao estudo, agora ou em qualquer momento que for necessário.

Consentimento

Declaro que fui informado sobre o estudo e concordo com a inclusão do meu filho(a) _____ . Eu entendo que os resultados deste estudo são de caráter sigiloso e somente serão usados para fins científicos. Estou ainda ciente de que esta pesquisa não acarretará aumento da permanência da criança no hospital e que caso eu me arrependa desta autorização tenho o direito de requerer a retirada do mesmo da pesquisa.

Belo Horizonte, ____ de _____ de 20__

Assinatura do responsável Assinatura do pesquisador Assinatura da testemunha Tel:

COEP/UFMG 3248-9364

ANEXO 2 – PARECER COEP/UFMG

UFMG — Universidade Federal de Minas Gerais
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 214/12

Interessado(a): Prof. Ana Cristina Simões e Silva
Departamento de Pediatria
Faculdade de Medicina-UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 15 de setembro de 2012, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Aspectos clínicos, laboratoriais e moleculares de crianças com rim único funcionante"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – CepJ l 270-901 – BH-MG
Telefone/Fax: (31)3409.4592 - e-mail: coep@prpg.ufmg.br

ANEXO 3 – COMPROVANTE DE SUBMISSÃO DO ARTIGO ORIGINAL NO JORNAL DE PEDIATRIA

Jornal de Pediatria

URINARY BIOMARKERS IN PEDIATRIC PATIENTS WITH SOLITARY FUNCTIONING KIDNEY: A PILOT STUDY

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original article
Keywords:	Solitary functioning kidney; renal function; urinary biomarkers; CAKUT
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	Eduardo Araújo Oliveira, MD, PhD
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Abstract:	Background: Solitary Functioning Kidney (SFK) is an important subgroup of the Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) spectrum. Serum urea and creatinine levels are still the most used markers to assess renal function, even though these measurements do not allow early detection of renal injury. In this sense, the aim of this pilot study was to measure urinary concentration of kidney biomarkers in pediatric patients with SFK and to compare with the same biomarkers measurements in healthy children and adolescents. Methods: This cross sectional study included 30 pediatric patients with congenital SFK (renal agenesis, primary renal hypodysplasia, and multicystic dysplastic kidney) and 20 healthy individuals paired for gender and age (controls). All the participants were submitted to a single urine collection to measure seventeen urinary biomarkers, known to be related to kidney function and/or lesion. Results:Cystatin C, Osteopontin, Calbindin, Osteoactivin, Tissue inhibitor of metalloproteinase 1, Kidney injury molecule-1,Protein induced by interferon, Renin, Epithelial Growth Factor and Clusterin were significantly reduced in urine samples of patients in comparison with controls. Lipocalin-2/NGAL was the only molecule with significantly increased levels in patients when compared with controls. The remaining molecules were similar in both groups. Serum levels of creatinine increased significantly from baseline to the time of biomarker collection, whereas estimated GFR reduced. Conclusion:In pediatric patients with congenital SFK, the behavior of some urinary molecules is different from healthy controls. Longitudinal studies should be performed to validate the clinical utility of these biomarkers.
Suggested Reviewers:	Simone Sanna-Cherchi ss2517@cumc.columbia.edu Great expertise in CAKUT and in solitary functioning kidney
Opposed Reviewers:	

ANEXO 4 – COMPROVANTE DE ACEITE DO ARTIGO DE REVISÃO NA *CURRENT MEDICINAL CHEMISTRY*

ENC: Manuscript Provisional Acceptance letter | BMS-CMC-2021-660

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Submission Title: CONGENITAL SOLITARY FUNCTIONING KIDNEY: A REVIEW

Dear Dr. Ana Cristina Simões e Silva,

I am pleased to inform you that your article Reference No. "BMS-CMC-2021-660" entitled **"CONGENITAL SOLITARY FUNCTIONING KIDNEY: A REVIEW"** has been provisionally approved for publication in "**Current Medicinal Chemistry**" journal.