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Article

Evaluation of Urinary L-FABP as a Tubular Damage Marker in Pediatric Neurogenic Bladder

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Abstract: The article aims to find potential biomarker for the detection of tubular damage in pediatric neurogenic bladder (NB) by investigating urinary levels of liver-type fatty acid-binding protein (uL-FABP). This prospective analysis was conducted on two groups: 42 children with NB and 18 healthy children. The uL-FABP concentrations were measured using ELISA methods. The children's medical charts were analyzed to determine age, sex, anthropometric measurements, activity assessment using Hoffer's scale and renal function parameters. The results revealed that the uL-FABP/creatinine ratio was higher in the studied group compared with the reference group. A significant positive correlation between uL-FABP and proteinuria was found. Children with a history of vesicoureteral reflux (VUR) tended to have elevated uL-FABP. In conclusion, uL-FABP may be considered a potential tubular damage biomarker in children with NB. Proteinuria and history of VUR may be the factors influencing uL-FABP.

Keywords: neurogenic bladder; L-FABP; proteinuria

1. Introduction

Neurogenic bladder (NB) is a heterogeneous entity that may result from a variety of conditions affecting the nervous systems at any level. In children the most frequent cause is myelomeningocele (MMC) with an estimated prevalence of 1/700 live births [1]. Although the incidence of MMC has decreased in recent years thanks to prenatal diagnosis and folic acid supplementation during pregnancy, there are still many children presenting with the signs of NB [2]. At birth the majority of patients has normal upper urinary tract, but approximately 60% of them will develop renal deterioration due to increased filling detrusor pressures, recurrent urinary tract infections (rUTIs) with or without vesicoureteral reflux (VUR) [3,4]. This process may occur silently and ultimately leads to chronic kidney disease [5]. There is an urgent need for biomarkers for renal deterioration in NB for its prompt diagnosis and treatment control. In this context, current studies have aimed at identifying novel biomarkers that can be used to monitor and predict renal impairment in children with NB [6,7].

Liver-type fatty acid-binding protein (L-FABP) is an intracellular protein that is expressed not only in the liver but also in the intestine, pancreas, stomach, lung, and the proximal tubule of the human kidney. Numerous studies have demonstrated that L-FABP is a promising biomarker in a wide variety of nephrological disorders, i.e. diabetic kidney disease and acute kidney injury [8–10]. Because of its small molecular size, L-FABP is freely filtered and can be easily detected in urine. Urinary L-FABP (uL-FABP) is released in response to tubular damage. Pediatric NB predisposes to upper tract damage, including renal tubules. The lack of proven biomarkers of tubular injury in children with NB pushed us to conduct this study.

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2. Materials and Methods

2.1. Patients

This prospective analysis was conducted on 60 children divided into two groups. The study group included 42 children with NB after MMC. All of them were under the care of the Department of Pediatrics and Nephrology at the University Children's Hospital in Bialystok, Poland. The presence of urinary tract infection was excluded on the basis of urinary testing and urine culture. A negative C-reactive protein excluded current infection. Additionally, a history of recurrent urinary tract infections (rUTIs) and the presence or history of vesicoureteral reflux (VUR) were recorded. We divided the children with NB into 3 groups: 1 - without VUR, 2 - with present VUR and 3 - with a history of VUR. The ambulatory function of MMC patients was defined according to Hoffer's scale (HS) using the 4 categories of community: 1HS – nonambulatory, 2HS - nonfunctional ambulator, 3HS - household walkers, and 4HS - community walkers [11]. The lesion level in MMC patients was reported intraoperatively and radiologically and scored from 1 to 3 (1- thoracolumbar, 2-lumbosacral, 3-sacral lesion).

The reference group consisted of 18 participants who visited a pediatrician for well-child visits and had no abnormalities in the urinary or nervous systems.

We assessed all participants' medical charts to determine age, sex, anthropometric measurements, standard deviation scores WHO (z-scores) and renal function parameters: urinary and serum creatinine. Glomerular filtration rate (GFR) was assessed according to the new Schwartz formula.

2.2. Biochemistry

First morning void spot urine samples were collected for measurement of L-FABP. The levels of biomarkers were measured using the L-FABP ELISA Kit (Cloud-Clone Corp.) according to the manufacturer's instructions. The urinary creatinine (Cr) concentration was used to normalize L-FABP measurements to account for the influence of urinary dilution on concentration. Urinary levels of the biomarker were expressed as the uL-FABP/Cr ratio as ng/mg creatinine.

2.3. Statistics

The data were collected in a Microsoft Excel database. Statistical analysis was performed using Statistica 13.0. (StatSoft Inc, Tulsa, OK, USA). All the studied parameters were analyzed using a nonparametric Mann-Whitney test. Correlations were assessed with the Spearman test. Values of $p<0.05$ were considered significant.

2.4. Ethical Issues

This study was approved by the Ethics Committee of the Medical University of Bialystok which complies with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. Patients and their caregivers were enrolled into the study after obtaining informed consent.

3. Results

The characteristics of the studied children are presented in Table 1. The median age of the enrolled patients was 11.5 (0.67 – 18) years. There were no differences in age, sex, z-scores weight to age and z-scores BMI to age excluding z-sores height to age. This resulted from a shorter vertebral dimension and malformations of the bone structure due to MMC in the NB group. We found statistically significant differences in urine, serum creatinine concentration and eGFR Schwartz between the studied groups.

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Table 1. Demographic characteristics of patients with NB and reference group. .

Variables	NB patients n=42	Reference group n=18	p value
Gender: Female/Male n(%)	24(57)/18(43)	15(83)/3(17)	0.06
Median (minimum – maximum)			
Age (years)	11 (2.75 – 18)	12 (0.67 – 18)	0.62
Z-score height-to-age	-1.85 (-7 – 1.5)	0.3 (-3.3 – 2.6)	<0.001*
Z-score weight-to-age	-0.55 (-5 – 1.5)	-0.05 (-1.5 – 2.7)	0.06
Z-score BMI (kg/m2)	0.45 (-4.2 – 2)	-0.1 (-3 – 2.9)	0.51
Serum creatinine (mg/dl)	0.37 (0.18 – 3.08)	0.52 (0.2 – 0.85)	0.03*
Urinary creatinine (mg/dl)	50.1 (16 – 200)	98.3 (23.2 – 244)	<0.001*
eGFR Schwartz (ml/min/1.73m²)	77.3 (17 – 167)	117 (110 – 200)	<0.001*

NB - neurogenic bladder, * p<0.05.

Comparison of the uL-FABP and uL-FABP/Cr ratio is presented in Table 2. As shown, children with NB had an elevated median uL-FABP/Cr ratio in comparison with the reference group but the differences were not statistically significant (p= 0.52, p>0.05). However, the range of the uL-FABP/Cr ratio was wider in contrast to the ratio range in the reference group.

Table 2. Biochemical parameters in patients with NB and reference group. .

Variables	NB patients n=42	Control group n=18	p value
Urinary L-FABP (ng/ml)			
a) Mean	7.56	5.38	
b) Median	1.96	4.15	
c) Min - max	0.25 – 49.5	0.53 – 12.7	0.14
Urinary L-FABP/Cr ratio (ng/mg)			
a) Mean	25.37	4.45	
b) Median	4.77	3.75	
c) Min - max	0.46 – 215	0.8 – 13.8	0.52

Cr - creatinine.

We divided the patients with NB into 3 groups due to uL-FABP/Cr ratio value: 1 – with ratio in a range between 0 to 10 ng/mg, 2 – between 11–100 ng/mg, 3 – ratio greater than 100 ng/mg to assess factors which influenced the increase of uL-FABP/Cr ratio. Significant differences between above-mentioned groups in terms of the history of VUR, proteinuria in 24 hour urine collection and urinary creatinine level were detected. More detailed data is presented in Table 3.

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Table 3. Comparison between L-FABP / Cr ratio groups in NB patients.

	I	II	III	Chi²	p value
	L-FABP/Cr ratio (ng/mg)				
	0 – 10	11 – 100	>100		
NB patients n (%)	27(64)	11(26)	4(10)		
Proteinuria in 24 hour urine collection (mg/24h)	55 (0 – 192)	4.9 (0 – 101)	149.6 (105 – 194)	6.72	0.047*
VUR n (%)					
a) without VUR	27 (100)	6 (54)	2 (50)	8.43	0.01*
b) with VUR	0	3 (28)	0		
c) VUR in the past	0	2 (18)	2 (50)		

NB - neurogenic bladder, VUR - vesicoureteral reflux, Cr - creatinine, * p<0.05.

27/42 (64%) of children with NB had no history either active or in the past and their median uL-FABP/Cr ratio equaled 3.01 ng/ml (0.46 - 181.6 ng/mg). Only 3/42 (7%) of patients with NB had active VUR during our investigation with a median uL-FABP/Cr ratio of 14.37 ng/mg (13.63–15.83 ng/mg) and in the group of children with VUR diagnosed in the past that resolved the median uL-FABP/Cr ratio was 34.87 ng/mg (13.4 –214 ng/mg). Interestingly, all VURs either active or present in the patient's medical history were unilateral and left-sided, but they differed in the grading according to the International Reflux Study classification. In children with NB and active VUR, 2/3 (66%) of VUR were low graded (I°) and one was high – grade (IV°) whereas in children with NB and VUR in the past all VURs were high-grade (IV° and V°).

In our study, a statistically significant positive correlation between uL-FABP/Cr ratio and proteinuria was revealed (r=0.377, p<0.05).

The majority of patients with NB - 30/42 (%), had a lumbosacral spinal lesion, 7/42 (%) had thoracolumbar, and 5/42 (%) sacral level lesion. From all the study subjects, 22/42 (52%) were classified as 1HS, 11/42 (26%) as 2HS, 6/42 (14%) as 3HS, and 10/42 (24%) as 4HS. We did not find statistically significant differences in the uL-FABP level between the above mentioned groups but we noticed statistically significant differences in the urinary creatinine (Chi2=11.59, p<0.001) and the number of rUTIs (Chi2=19.2, p<0.001) in Hoffer's scale groups. Children from the 1HS group described as wheelchair dependent, had the lowest urinary creatinine levels with a median of 42 mg/dl (16 – 102 mg/dl) and 77% (17/22) of them had a history of rUTIs in contrast to children with different stages of walking impairment from remaining HS groups. In 2HS the median urinary creatinine level was 109 mg/dl (45 – 200 mg/dl), in 3HS - 65.7 mg/dl (55.8 – 66 mg/dl) whereas in 4HS described as community walkers - 64.5 mg/dl (25 – 137 mg/dl).

4. Discussion

This paper is a modest contribution to the ongoing discussions about renal biomarkers in NB population. The main purpose of the paper was to draw attention to the uL-FABP. This marker is located in the proximal tubular cells that may be the first localisation of upper urinary tract injury in NB. The role of L-FABP excreted in the urine as a biomarker of tubular damage in children with NB has not been described.

Renal deterioration in the NB is usually an asymptomatic process. Elevated serum creatinine and decreasing eGFR depend on several factors such as body muscle mass. In children with MMC muscle wasting due to denervation and impaired linear growth are observed [12]. There may be substantial inaccuracy when we compare eGFR between patients with NB and the general population

[13]. In our study no correlations between anthropometric parameters and the uL-FABP were observed. It seems that the uL-FABP might be more reliable and does not depend on weight and growth by contrast with traditional kidney function markers, which is a precious quality of a renal deterioration biomarker in the NB population.

Our observations showed that the uL-FABP/Cr ratio was higher in patients with NB but the differences between the studied groups did not reach statistical significance. The most likely explanation of the negative result might be a small sample size. However, the range of ratio values was wider in NB in contrast to healthy participants. There are plenty of factors that determine the uL-FABP concentration. In our analysis children with NB who had significantly elevated uL-FABP/Cr ratio (> 100 ng/mg) tended to have lower urinary creatinine concentration, proteinuria in the 24 hour urine collection and more frequent VUR diagnosis. Decreased urinary creatinine excretion and presence of proteinuria are well-known factors of progressive renal deterioration and predict greater risk of renal failure [14]. The increase of uL-FABP concentration may reflect the fact that the damage to the tubular cells has been done.

Our study revealed that proteinuria was positively correlated with the uL-FABP/Cr ratio. Under normal circumstances, proteinuria should not be detected in the final urine.

Pathological proteinuria may develop after dysfunction of the glomerular filtration barrier, impaired reabsorption of protein in the proximal tubule or both [15]. The uL-FABP is classified as a low-molecular weight protein (14 kDa) that is detectable in the urine after the proximal tubule damage. The measurement of the uL-FABP may play a useful role in distinguishing glomerular from tubulointerstitial damage. However, higher concentrations of uL-FABP in NB patients from our research cannot be explained by proteinuria. Only 15/42 (36%) of children with NB had proteinuria. No statistically significant differences in urinary biomarkers in patients with and without proteinuria were observed ($p=0.56$, $p>0.05$).

The history of VUR is another factor affecting the uL-FABP concentrations. Very few publications are available in the literature that address the issue of VUR and the uL-FABP [16,17]. Benzer et al. [16] reported that the uL-FABP levels had risen significantly in patients with active VUR when compared to a reference group. Additionally, the uL-FABP levels were positively correlated with urinary protein excretion in children diagnosed with primary VUR. In our study, only 3/42 (7%) children from the NB group had active VUR and they all had elevated the uL-FABP/Cr ratio in a range between 13.62–15.4 ng/mg. Parmaksız et al. [17] demonstrated that the uL-FABP/Cr level was higher in patients with renal parenchymal scarring due to VUR. However, studies on an association between the uL-FABP and VUR that had been resolved are still lacking. In our investigation, we have also considered the consequences of VUR in the past. The significant differences between the uL-FABP concentrations in children with and without a history of VUR were found. In our study, all of VUR in the medical history of the enrolled patients with NB were high-graded and required surgical procedures. We assumed that increased the uL-FABP/Cr in these cases was associated with tubulointerstitial damage and fibrosis secondary to high grade VUR in the past.

Children with NB require clean intermittent catheterization (CIC) which predisposes to rUTIs [18]. This is a frequent complication that remains a challenge for specialists taking care of these patients. In our study, we did not observe higher L-FABP in children with NB with a rUTIs history. This is a special features and in contrast to other biomarkers i.e. NGAL [19,20].

5. Conclusions

To conclude, we would like to highlight that:

1) L-FABP may be considered a potential biomarker of tubular injury in children with NB due to MMC.
2) Proteinuria and history of VUR may be the factors influencing the uL-FABP concentrations.

However, further studies in the NB population are still warranted to gain further insights on uL-FABP in the NB population.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Medical University of Bialystok.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available for ethical and privacy reasons.

Conflicts of Interest: The authors declare no conflict of interest.

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