

Bioelectric Impedance Analysis in the Diagnosis of Vesicoureteral Reflux

Meral Torun Bayram^{1*}; Demet Alaygut¹; Mehmet Turkmen¹; Alper Soylu¹; Salih Kavukcu¹

¹Department of Pediatrics, Medical Faculty, Dokuz Eylul University, Izmir, Turkey

*Corresponding author: Meral Torun Bayram, Department of Pediatrics, Medical Faculty, Dokuz Eylul University, Izmir, Turkey. Tel: +90-2324123624, E-mail: meralt.bayram@yahoo.com

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Background: Vesicoureteral reflux (VUR) is a common abnormality of the urinary tract in childhood.

Objectives: As urine enters the ureters and renal pelvis during voiding in vesicoureteral reflux (VUR), we hypothesized that change in body water composition before and after voiding may be less different in children with VUR.

Patients and Methods: Patients were grouped as those with VUR (Group 1) and without VUR (Group 2). Bioelectric impedance analysis was performed before and after voiding, and third space fluid (TSF) (L), percent of total body fluid (TBF%), extracellular fluid (ECF%), and intracellular fluid (ICF%) were recorded. After change of TSF, TBF, ECF, ICF (Δ TSF, Δ TBF%, Δ ECF%, Δ ICF%), urine volume (mL), and urine volume/body weight (mL/kg) were calculated. Groups 1 and 2 were compared for these parameters. In addition, pre- and post-voiding body fluid values were compared in each group.

Results: TBF%, ECF%, ICF%, and TSF in both pre- and post-voiding states and Δ TBF%, Δ ECF%, Δ ICF%, and Δ TSF after voiding were not different between groups. However, while post-voiding TBF%, ECF% was significantly decreased in Group 1 (64.5 ± 8.1 vs 63.7 ± 7.2 , $P = 0.013$ for TBF%), there was not post-voiding change in TSF in the same group. On the other hand, there was also a significant TSF decrease in Group 2.

Conclusions: Bladder and ureter can be considered as the third space. Thus, we think that BIA has been useful in discriminating children with VUR as there was no decreased in patients with VUR, although there was decreased TSF in patients without VUR. However, further studies are needed to increase the accuracy of this hypothesis.

Keywords: Impedance; Vesicoureteral Reflux; Children

1. Background

Vesicoureteral reflux (VUR) is a common abnormality of the urinary tract in childhood. Diagnostic imaging of VUR is one of the most frequently used procedures in pediatric radiology departments all over the world. Voiding cystourethrography (VCUG) and radionuclide voiding cystography (RNC) are most commonly two methods used to diagnose VUR (1). However, there are three main problems associated with VCUG and RNC, including sedation, urethral catheterization, and ionizing radiation (2). Sedation issues come up regularly and some institutions use sedation in most of their bladder-imaged patients. Another problem is that urethral catheter is not well tolerated by children or their parents. Dysuria for a day or two almost always follows the catheterization. Stricture disease, UTI, and hematuria may result from catheter (2). Third problem is radiation exposure associated with VCUG and RNC. There is significant concern about radiation exposure in children in long term and cancer development. In children, the risk is not only associated with the amount of radiation exposure but how much time has to pass till it has to bring about effects. Because infants and children have a full life expectancy ahead of them, the overall risk is greater than in adults (2). Because of the unpleasant nature of the radiographic imaging and radiation exposure, signifi-

cant efforts have been undertaken to utilize non-invasive techniques for diagnosis of VUR.

Bioelectric impedance analysis (BIA) is an accurate, non-invasive, and inexpensive technique for quantitative estimating of body water compartments in both healthy individuals and those affected by pathologic conditions (3, 4). The technique is based on the measurement of the resistance generated in the body against a low voltage electrical current. It is possible to estimate the TBF and to separate the ECF and ICF volumes by using various frequencies (5). Previously BIA has been used to assess body water changes in patients with renal failure, ascites, acute dehydration, in the postoperative period, and congestive heart failure (6-10). However, the role of BIA in diagnosis of VUR is not yet defined. Because VUR is the retrograde flow of urine from bladder to kidneys (11), body water in children with VUR after voiding may show small change.

2. Objectives

Therefore, we hypothesized that change in body water composition before and after voiding may be different in children with VUR compared to those without VUR using BIA. Thus, in this study, we have investigated whether BIA can be used as a method in diagnosis of VUR.

3. Patients and Methods

3.1. Patients and Control Cases

This study was conducted in our institution between March 2011 and September 2012. Children with continence who had VCUG (in the last three months) to diagnose VUR were included in the study. Any patients who had a history of heart disease, chronic kidney disease, anatomic obstruction (e.g. urethral valves or ectopic ureterocele), post-void residual urine volume (PVR) greater than 10 mL, urinary incontinence, or active urinary tract infection were excluded from study. The patients and control cases were evaluated with respect to present age, gender, VCUG, 99 mTc-DMSA renal scintigraphy and multifrequency bioelectrical impedance analysis (MF-BIA).

The patients were grouped as VUR (+) (Group 1) and VUR (-) (Group 2 = control group) with respect to the presence of VUR in VCUG. VUR has been graded from grade 1 to 5 according to the grading system of international reflux study committee (12). Therefore, Group 2 was grouped as low-grade and high-grade VUR. First- and second-grade reflux was considered as low grade and third to fifth grades as high-grade reflux. The study was approved by the local ethical committee of our institution. All patients and controls signed the informed consent for participation in the study.

3.2. Anthropometric Measurements

Body weight was determined on a SECA balance scale (Hamburg, Germany) to the nearest 0.1 kg, with subjects dressed in a light t-shirt and shorts. Body height of the patients was recorded on a stadiometer with a standing subject to the nearest 0.1 cm with a Harpenden fixed stadiometer (Holtain Ltd., Crymych, Dyfed, Britain). Body mass index was calculated as weight/height² (kg/m²).

3.3. Multifrequency Bioelectric Impedance Analysis

Body composition and body water compartment analysis were carried out with the subject lying in a supine position on a flat, nonconductive bed with MF-BIA. MF-BIA was done between 8 and 10 in the morning to avoid bias due to diurnal variation with a Bodystat Qudscan 4000 bioimpedance analyzer (Bodystat Limited, British Isles). All patients were evaluated two times after an overnight fasting, firstly with full bladder and secondly after voiding. Current-detecting electrodes were placed between the styloid processes of the right radius and ulna and between the medial and lateral malleoli of the right ankle. Current-introducing electrodes were then placed on the respective dorsal surfaces of the metacarpals and metatarsals, 5 cm distal to the proximal electrodes (standard tetrapolar placement). A minimal inter-electrode distance of 5 cm has been recommended to prevent interaction between electrodes (13, 14). Later, body weight and height of patient were put into the analyzer. By the

MF-BIA, an alternating electrical current of constant frequency with low intensity was applied to the body.

Body mass index (BMI), fat mass index (FMI), fat-free mass index (FFMI), percent of total body fluid (TBF%), percent of extracellular fluid (ECF%), percent of intracellular fluid (ICF%), third space fluid (TSF) (L), impedance (a combination of resistance and reactance), resistance (R; i.e. opposition to the flow of an alternating current through intra- and extracellular ionic solution), reactance (Xc; i.e. the capacitive component of tissue interfaces, cell membranes and organelles), and phase angle (PA; Xc/R) were calculated by using the manufacturer's software. These measurements were performed before and after voiding.

3.4. Calculations

Urine volume (mL) of all cases was calculated after voiding. Later, urine volume was divided by body weight (kg) and so volume/body weight (mL/kg) was determined. Therefore, the changes (liter or percent) in TSF (ΔTSF), TBF (ΔTBF%), ECF (ΔECF%), ICF (ΔICF%) were calculated by the formula as TSF, TBF, ECF, and ICF after voiding minus TSF, TBF, ECF, ICF before voiding, then divided by the before voiding TSF, TBF, ECF, and ICF in each patient. For example, one case had his before voiding ECF as 48%, and after voiding ECF as 45%, then the DECF% is $45 - 48/48 = 1.95\%$. All these parameters were compared between Group 1 and 2.

In addition, impedance (ohm) of a cylindrical conductor, in fact, is proportional to its specific impedivity and to its length. Therefore, resistance/height (R/H) and reactance/height (Xc/H) were also calculated.

3.5. Statistical Analysis

All results are expressed as mean ± standard deviation unless otherwise specified. The data between groups were compared with Mann-Whitney U, Chi-Square test, paired sample t test, and Wilcoxon signed-ranks tests. Correlation between the grade of VUR and changes of body fluid was assessed using Pearson correlation. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using the scientific package for social science 16.0.1 (SPSS Inc., Chicago, IL, USA).

4. Results

A total of 78 (25 males) patients were included in the study. There are 52 patients in Group 1 (with VUR) and 26 patients in Group 2 (without VUR). In Group 1, 25 (48%) patients had severe VUR (grade ≥ 3) and 23 (44%) patients had bilateral VUR. Table 1 shows the demographic characteristics and body composition: age, sex, height, weight, BMI, FMI, and FFMI are similar in Groups 1 and 2.

Data on body fluid composition obtained by MF-BIA pre- and post-voiding are shown in Table 2. In Group 1, differences in pre- and post-voiding values of TBF%, ECF%, impedance, R, and R/H, showed statistically significant differences. In Group 2, differences in pre- and

post-voiding values of ECF%, TSF in L, PA, Xc, and Xc/H, showed statistically significant differences.

Pre- and post-voiding TSF in L, TBF%, ECF%, and ICF% values by MF-BIA and the Δ TSF%, Δ TBF%, Δ ECF%, and Δ ICF% were not different between Groups 1 and 2 (Table 3). Also, there

was no difference of same parameters between patients with high-grade VUR and other patients (data not shown).

By analyzing the relationship of VUR grade with changes of body fluid after voiding, there was only a significant positive correlation between the VUR grade and R/H (Table 4).

Table 1. Demographic Characteristics and Body Composition in Group 1 and Group 2^a

General Characteristics	Group 1, (With VUR), n = 52	Group 2, (Without VUR), n = 26	P Value
Age, mo	74.1 ± 35.6	76.6 ± 30.1	0.637
Gender			0.074
Male	13	12	
Female	39	14	
Renal scarring	37/12	4/12	0.001
Weight, kg	22.3 ± 10.3	24.6 ± 8.4	0.117
Height, cm	114.7 ± 19.5	119.4 ± 15.7	0.203
BMI, kg/m ²	15.9 ± 2.9	16.8 ± 2.2	0.071
FMI, kg/m ²	4.2 ± 3.5	4.5 ± 2.5	0.316
FFMI, kg/m ²	13.1 ± 1.7	13.7 ± 1.6	0.171

^a Abbreviations: BMI, body mass index; FFMI, fat-free mass index; FMI, fat mass index; VUR, vesicoureteral reflux.

Table 2. Pre- and Post-Voiding Distribution of Body Fluids and Body Weight in Group 1 and Group 2

	Group 1			Group 2		
	Pre-Voiding	Post-Voiding	P Value	Pre-voiding	Post-Voiding	P Value
Weight	22.3 ± 10.3	22.2 ± 10.2	0.067	24.6 ± 8.4	24.6 ± 8.3	0.414
TBF, %	64.5 ± 8.1	63.7 ± 7.2	0.013	63.8 ± 6.2	63.6 ± 6.2	0.125
ECF, %	42.6 ± 8.9	42.4 ± 8.8	0.001	39.6 ± 6.7	39.2 ± 6.4	0.015
ICF, %	40.5 ± 9.8	40.8 ± 10.2	0.415	40.8 ± 10.3	42.1 ± 10.7	0.479
TSF, L	-3.0 ± 2.0	-3.2 ± 2.1	0.077	-3.3 ± 2.2	-3.5 ± 2.2	0.013
Imp.50kHz	662.7 ± 90.2	670.4 ± 86.4	0.003	638.3 ± 65.4	641.6 ± 68.1	0.074
PA	4.7 ± 0.9	4.7 ± 0.7	0.620	4.9 ± 6.9	5.1 ± 0.8	0.010
R, Ω	660.4 ± 90.3	668.0 ± 86.5	0.004	635.9 ± 65.3	639.2 ± 68.2	0.080
Xc, Ω	45.3 ± 9.1	54.9 ± 9.3	0.053	54.3 ± 8.2	56.3 ± 8.1	0.001
R/H, Ω/m	592.1 ± 127.8	599.9 ± 129.5	0.006	542.7 ± 94.5	545.8 ± 98.5	0.065
Xc/H, Ω/m	48.2 ± 9.6	48.7 ± 9.4	0.126	45.9 ± 6.9	47.7 ± 7.8	0.002

^a Abbreviations: ECF, extracellular fluid; ICF, intracellular fluid; PA, phase angle; R, resistance at 50 kHz; R/H, resistance/height; TBF, total body fluid; TSF, third space fluid; Xc, reactance at 50 kHz; Xc/H, reactance/height.

Table 3. Pre-Voiding and Post-Voiding Distribution of Body Fluids and Urine Volume in Group 1 and Group 2

	Group 1	Group 2	P Value
Total Body Fluid (TBF), %			
Prevoiding TBF	64.5 ± 8.1	63.8 ± 6.2	0.845
Postvoiding TBF	63.7 ± 7.2	63.6 ± 6.2	0.890
Δ TBF	-0.011 ± 0.027	-0.004 ± 0.0124	0.450
Extracellular Fluid (ECF), %			
Prevoiding TBF	42.6 ± 8.9	39.6 ± 6.7	0.155
Postvoiding TBF	42.4 ± 8.8	39.2 ± 6.4	0.108
Δ TBF	-0.004 ± 0.007	-0.009 ± 0.037	0.928
Intracellular Fluid (ICF), %			
Prevoiding TBF	40.5 ± 9.8	40.8 ± 10.3	0.853
Postvoiding TBF	40.8 ± 10.2	42.1 ± 10.7	0.715
Δ TBF	-0.009 ± 0.077	0.039 ± 0.134	0.746
Third space Fluid (TSF), L			
Prevoiding TBF	-3.4 ± 2.0	-3.3 ± 2.2	0.393
Postvoiding TBF	-3.2 ± 2.1	-3.5 ± 2.2	0.370
Δ TBF	0.079 ± 0.296	0.095 ± 0.266	0.765
Urine Volume/Body Weight, mL	5.27 ± 4.33	5.59 ± 3.89	0.436

Table 4. Relationship Between Grade of VUR With Changes of Body Fluid After Voiding

Measure	Grade of VUR	
	r	P Value
Δ TBF, %	-0.097	0.402
Δ ECF, %	-0.114	0.323
Δ ICF, %	-0.093	0.419
Δ TSF, L	-0.037	0.750
Δ Imp, 50 kHz	0.043	0.712
Δ Phase Angle	-0.218	0.057
R/H, Ω /m	0.327	0.004
Xc/H, Ω /m	0.134	0.247

^a Abbreviations: Δ ECF, change in ECF after voiding; Δ ICF, change in ICF after voiding; Δ TBF, change in TBF after voiding; Δ TSF, change in TSF after voiding.

5. Discussion

Diagnostic methods used for the detection of VUR according to their characteristics can be categorized as direct/indirect, catheter-using/catheter-free, radiation-giving/radiation-free. Ideally, the diagnostic methods used for the diagnosis of VUR should be a safe, radiation-free, non-invasive, low-cost, high-sensitivity imaging method (2, 15). However, none of the current diagnostic methods include most of these criteria. Therefore, new diagnostic methods for the diagnosis of VUR must be investigated.

There are reference methods for measuring body fluid volumes such as isotope dilution techniques, total body nitrogen, and densitometry for TBF, bromide for ECF and radioactive potassium isotope for ICF (16-20). However, these methods are expensive and cumbersome, and expose children to radiation or invasive procedures (16-19, 21), and the use of these methods is limited in clinical practice (22). Approaches to overcome these restrictions are single-frequency BIA (SF-BIA), multi-frequency BIA (MF-BIA), and bioelectric impedance spectroscopy (BIS).

SF-BIA, MF-BIA, and BIS are used for estimating of TBF, ECF, ICF, and body composition on the basis of mathematical formulae using measurements of resistance, reactance, and impedance (23). These methods provide evaluation of body water compartments of healthy subjects and those affected by pathological situations (3, 4, 24). Among BIA techniques, MF-BIA seems to be a more accurate method for estimating the TBF compartment (24, 25). A meta-analysis showed that TBF in healthy individuals was significantly overestimated by SF-BIA or BIS in comparison with the reference values obtained using D₂O dilution. However, those studies that used MF-BIA only did not overestimate the TBF (24). The use of BIA as a bedside method has increased because the equipment is portable and safe, the procedure is simple and non-invasive, and the results are reproducible and rapidly obtained (25). BIA is also a painless method (26). The main

advantage of this method, in patients with alterations in water metabolism, is that it works independently of the body weight. Because VUR is the retrograde flow of urine from bladder to kidneys (11), change after voiding in body water composition, especially TBF and TSF, might be different between children with and without VUR. In this study, we wanted to confirm this hypothesis and chose MF-BIA due to above-mentioned assumptions. Thus, we investigated whether a BIA can be used as a method in diagnosis of VUR.

Body fluid compartments may be affected by body composition. For example, TBF is strongly related to FFMI, similarly, body cell mass, which is an important nutritional parameter, is also closely connected to ICF (27, 28). Therefore, the differences in body compartments prevent correct evaluation of body water compartments. In our study, body weight and height, BMI, FFM, and FFMI were similar between patients with and without VUR. In addition, the urine volume and urine volume/body weight did not differ between groups. Although there was no difference in body composition affecting body fluid between patients with and without VUR, pre-and post-voiding TSF (L), TBF%, ECF%, and ICF% were not different between these groups. In addition, the changes (% or L) in TSF, TBF, ECF, and ICF post-voiding were similar between same groups. However, when the groups were examined separately, post-voiding TBF% and ECF% was found to be lower than the pre-voiding TBF% and ECF% in Group 1. Nevertheless, TSF was not different

In addition to the above results, the difference in impedance, R, R/H, Xc, and Xc/H values after voiding were severally determined in both groups at the same time. However, these values are pitfalls of conventional BIA (29). In previous studies it was obs. On the other hand, post-voiding TSF value was determined lower than pre-voiding TSF value in Group 2. Urine in bladder and ureter is probably TSF and VUR is the retrograde flow of urine from the bladder to the kidneys (11). Therefore, change of TSF after voiding was likely found to be less in children with VUR.erved that R and R/H were strongly correlated with TBF, whereas Xc and Xc/H were more strongly related to ECF (30, 31). In this study, we also similarly determined decrease in TBF% and increase in R, after voiding among patients with VUR and decrease in ECF% and increase in Xc after voiding among patients without VUR.

VUR is associated with two related consequences: urinary tract infection and renal scarring. The management of VUR is based on preventing these sequelae. Renal scar causes secondary hypertension and chronic renal failure. One of the risk factors for renal scar formation is higher grade VUR (32). Also, prophylaxis in VUR is recommended for high-grade VUR. For these reasons, we also examined the availability of the BIA method in the diagnosis of high-grade VUR. However, there were no differences in TBF and TSF between patients with and without high-grade VUR in pre- and post-voiding states. In addition, there was no relationship between changes

in TBF and TSF after voiding. Moreover, when the relationship between body fluid changes after voiding with grade of VUR was examined, a significant correlation was not determined except for R/H.

The main disadvantages of BIA in diagnosis of VUR are limited visualization of the urethra, and inadequacy in diagnosis of patients with passive VUR. Despite these disadvantages, we think that BIA is a valid alternative to conventional VCUG or RC in a screening population of girls and in follow-up. However, for the present, we could not exactly determine that BIA is an alternative to conventional VCUG or RC in VUR diagnosis. On the other hand, we found that while post-voiding TSF value was lower than pre-voiding TSF value in patients without VUR, there was no difference between these values in patients with VUR and this result suggests having need for further studies with more patients.

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