Urinary Beta 2-Microglobulin as a Prognostic Marker in Children With Pyelonephritis

Mostafa Sharifian¹, Abdollah Karimi¹, Masoumeh Mohkam¹, Reza Dalirani¹, Nasrin Esfandiar¹, Sedigheh Rafiei Tabatabaei¹, Mahmood Nouri-Safa¹

¹Pediatric Infections Research Center (PIRC), Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

ABSTRACT

Background: Urinary tract infection (UTI) is common in children. UTI with or without vesico-ureteral reflux (VUR) may result in renal scarring. Severe renal scarring impairs renal function and may result in hypertension, renal insufficiency, and end stage renal disease requiring dialysis or transplantation. Beta 2 micro-globulin (β2MG) is a low molecular weight protein freely filtered by the glomeruli and then actively reabsorbed normally up to 99.9% in the proximal tubules; its urinary excretion is an indication of proximal tubular cell dysfunction.

Objectives: The current study aimed to determine whether urinary β2MG excretion would be elevated in patients with various grades of renal scar, and also its relationship with renal outcome in long term follow-up.

Materials and Methods: Urinary β2MG and Creatinine (Cr) were measured in 83 spot urine samples of patients that 53 of them did DMSA renal scan both at the time of admission to confirm pyelonephritis, and 6 month later to detect scars. β2MG was measured by radioimmunoassay method using β2MG 96-test kit (RADIM Company; Germany), and the creatinine was measured by spectrophotometry and was recorded as microgram per mg creatinine. Twenty children had various grades of renal scars. Results were compared with the ratios of 19 children with low uptake scanning, 14 children with normal scanning after recovery from pyelonephritis, and 30 normal children served as controls. EXCEL and SPSS softwares were employed to compare the mean urinary β2MG in groups by student t-test, ANOVA, and Unpaired t-test at P<0.05 significance level. Subsequently patients were followed up for 6 years.

Results: The mean urinary β2MG/Cr ratio was significantly higher in the scarring group (5.23 ± 10.6) than in the normal group (0.19 ± 0.2), and in low uptake group (0.49 ± 0.86) (P<0.05). When mean β2MG/Cr ratios were compared for each grade of scarring; patients with severe scar (grade III) had higher values (14.69 ± 15.82) than grades I (0.36 ± 0.35) and II (3.37 ± 5.20) (P<0.05). Patients without renal scar had a β2MG/Cr ratio below 0.46 microgram/mg Cr. The mean β2MG was also higher in the refluxing group (3.45 ± 7.97) than nonrefluxing group (0.23 ± 0.24) ug/mgCr (P = 0.01). Three patients who had the highest β2MG/Cr ratio values (33.3, 27, and 26.6 microgram/mg Cr) had severe scar that rapidly progressed to ESRD. They were transplanted 2 years later; after transplantation they still had recurrent UTIs. 2nd patient underwent native nephrectomy for renal abscess.

Conclusions: Results of the current study revealed that mean β2MG/Cr ratio was higher in patients with renal scar and poor outcome. Measurement of Urinary β2MG may be useful in the early detection of tubular damage in refluxing patients with patients with renal scars and has prognostic significance.

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*Corresponding author: Mostafa Sharifian, Pediatric Infections Research Center (PIRC), Department of Nephrology, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. Tel: +98-2122225941, Fax: +98-2122220254, E-mail: sharifian.dorche@gmail.com

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Urinary B2MG and Pyelonephritis
Sharifian M et al.

1. Background

Urinary tract infection (UTI) is one of the most common infectious diseases in children (1). The risk of UTI in children is up to 5% in girls and 1.6% in boys (2). Girls have a 30% recurrence within the first year and 50% within 5 years after the first UTI (2). Vesico-ureteral reflux (VUR), reflux of urine from bladder to ureters is considered as a risk factor for pyelonephritis. VUR is present in 1.8% of all children while its prevalence in children with UTI is as high as 34% (1). Sixteen to forty percent of the siblings of these children have VUR as well. It has been shown that VUR is inherited in an autosomal dominant pattern (2). UTI is the most common clue to VUR diagnosis. Since the combination of VUR and UTI leads to renal damage, and subsequently hypertension, renal insufficiency, and end stage renal disease requiring dialyses or transplantation, diagnosis of VUR in patients presenting with UTI is of great importance (1).

Voiding Cysto-Uretero-Graphy (VCUG) is carried out in children presenting with pyelonephritis and recurrent UTI to rule out VUR (3).

Dimercaptosuccinic acid scan (DMSA), which is commonly used to detect renal damages after pyelonephritis, has a great burden of radiation exposure. Thus, it is of utmost importance to find out a method to detect VUR and renal scars without the above-mentioned disadvantages.

There have been several efforts to diagnose VUR and renal injuries without radiation and catherization, and to evaluate prognosis in these children. In a study entitled "Clinical significance of urinary interleukin-6 in children with Reflux Nephropathy (RN)," Wang J et al. concluded that urinary interleukin-6 may be useful in monitoring progression of reflux nephropathy (4). A non-invasive test for VUR in children was introduced by Meveroch RA in the year 2000; he processed acoustic signals during urination and detected VUR in 35/37 of patients in VUR group and no VUR in 16 out of 17 non-refluxing children (5).

β2MG is a low molecular weight protein of HLA complex, which is freely filtered via glomerular membrane and nearly 99.9% is reabsorbed in proximal tubules. It has been introduced as an indicator of proximal tubular dysfunction (1).

β2MG is measured by enzyme linked immunosorbent assay (ELISA) or radioimmunoassay. Since it is a non-specific index of tubular damage, it can not differentiate different pathologies of urinary system, however it can be used to verify and follow up known cases. Sensitivity and specificity of this method in diagnosing renal scar is not investigated yet.

Evaluation of renal function in children with primary VUR was undertaken by Ohta S et al. in 1990, it was found that β2MG, Alpha micro-globulin, N-acetyl-B-D-Glucosaminidase (NAG) were elevated in 76%, 51% and 92% of patients respectively (6).

β2 Microglobulinuria in children with VUR and recurrent UTI was found to be abnormal in urine and serum of these patients by Kaminska et al. in 2000 (7). In a study entitled Urinary β2 Microglobulin as a marker for VUR, Dr Asadi F K found that urinary β2MG was increased in high grade VUR (8).

2. Objectives

The current study aimed to measure the urinary levels of β2MG and creatinine in children who were to undergo VCUG and DMSA scan in Mofid Children’s Hospital and to evaluate its relationship with VUR and renal scars and its grades; then to follow up these patients for 6 years.

3. Materials and Methods

All patients less than 12 years admitted for pyelonephritis in Mofid Children Hospital from December 2005 to December 2006 were included in the study. Patients with renal failure or on nephrotoxic drugs were excluded. β2MG and Creatinine were measured in 83 urine samples (47 sample from patients with VUR and 36 from non refluxing children) at the time of VCUG using 96 test β2MG kit (Radim Co. Germany).

β2MG was measured by enzyme immunoassay (EIA) method and Cr with Auto analyzer. Standard VCUG was performed using contrast medium and was reported by a radiologist based on international VUR classification. The urine samples were obtained during the catheterization phase of VCUG and were neutralized with 0.1% sodium hydroxide if pH < 6 and stored at 20 degrees centigrade until tested.

β2MG/Cr ratio was calculated to consider diuresis dependent excretion of this protein. VCUG and urine sample analysis were performed in Mofid Children Hospital and the operators were not aware of the results of the

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Implication for health policy/practice/research/medical education:
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other test. DMSA renal scan was performed in 43 patients with pyelonephritis at the time of infection and 6 months later to detect scar, and was classified in 4 grades as proposed by Goldraich et al. (9) (Box).

Urinary β2MG and Cr were measured in random urine samples in 83 cases, and 20 children with various grades of scar. The results were compared with ratios of 19 children with low uptake scanning, 14 normal scanning, and 30 normal healthy children. The goal and procedure of the study were fully explained to the families of the cases and considering the ease and time sparing nature of the procedure, their cooperation was excellent.

β2MG/Cr ± SD was calculated and recorded in cases under study and the control group with different grades of VUR and renal scars. Excel and SPSS softwares were employed to compare the mean β2MG/Cr ratio in the control group and those of patients with different VUR and renal scar grades by Student t-test, ANOVA, and Unpaired t-test assuming unequal variances.

4. Results

Of the 83 children under study, there were 47 cases (31 girls and 16 boys) with VUR and 36 cases without VUR (24 girls and 12 boys). Twenty one patients (13 girls and 8 boys) had bilateral VUR while unilateral VUR was seen in the remaining 26 patients (18 girls and 8 boys). The mean β2MG/Cr ratio was higher in patients with VUR grades 4 and 5 (8.93 ± 12.01) than those of patients with VUR grades 1, 2 and 3 and non-refluxing patients (0.20 ± 0.7, 1.21 ± 3.98, 0.21 ± 0.18 and 0.23 ± 0.24 respectively (P = 0.29).

No patient in the non-refluxing group had β2MG/Cr ratio higher than 0.85. There was no significant difference in β2MG/Cr ratios in patients with bilateral (4.94 ± 9.91) and unilateral (1.91 ± 5.9) reflux (P = 0.11). Three patients with high grade VUR and the highest levels of β2MG/Cr ratio (33.3, 27 and 26.6) progressed to renal failure very rapidly and in 2 years time the first patient was treated by hemodialysis and the second and third patients underwent preemptive renal transplantation (Figure 1). The first patient also subsequently underwent renal transplantation. After 6 years his plasma creatinine was around 1.7 mg/dL. He had repeated episodes of UTI which was treated as outpatient. After transplantation, the second patient also had repeated episodes of UTI and rising Cr so that she was treated parenterally as inpatient base. She underwent left side native nephrectomy because of persistent UTI and xanthogranulomatous pyelonephritis. Her last Cr was around 4-5 mg/dL waiting for her second transplant.

5. Discussion

Vesico-ureteral reflux is present in 1.8% of children and has been long known as a leading risk factor for UTI and renal scar in children. Evaluation for VUR is essential in children with high grade VUR and the highest levels of β2MG/Cr ratio (33.3, 27 and 26.6) progressed to renal failure very rapidly and in 2 years time the first patient was treated by hemodialysis and the second and third patients underwent preemptive renal transplantation (Figure 1). The first patient also subsequently underwent renal transplantation. After 6 years his plasma creatinine was around 1.7 mg/dL. He had repeated episodes of UTI which was treated as outpatient. After transplantation, the second patient also had repeated episodes of UTI and rising Cr so that she was treated parenterally as inpatient base. She underwent left side native nephrectomy because of persistent UTI and xanthogranulomatous pyelonephritis. Her last Cr was around 4-5 mg/dL waiting for her second transplant.
2MG/Cr ratio increased in 36% of the cases and β2M ratio was significantly higher in children with renal scar. Arch Pediatr Infect Dis. 2012;12, and 5% in adults (1).

Dimercaptosuccinic Acid Scan (DMSA) reveals that one third of the children with VUR have scars in their kidneys and reflux nephropathy accounts for up to 25% of pediatric cases of end stage renal disease (ESDR) (8,10). Even isotope VCUG, which is used to follow up the patients and to screen their siblings, is associated with exposure to a remarkable radiation. Recently, there have been tremendous efforts to establish a diagnostic module for VUR and renal scar without radiation (8,10-12).

Measurement of urinary level of β2M is one of these noninvasive modules. Ohta S. studied 62 under 2 year-old children with VUR for urinary level of β2M, α1-microglobulin and N-acetyl-β-D-glucosaminidase (NAG); 45% of the cases had VUR grades 4 and 5. Urinary levels of β2M, α1-microglobulin and N-acetyl-β-D-glucosaminidase were increased in 76%, 51% and 92% of these cases respectively (6).

Asadi FK in a study in 1996 showed that urinary β2M was higher in patients with VUR than the patients without VUR(8). The study included 56 cases of VUR of different grades and 39 children in control group. The mean β2M/Cr ratio was higher in the cases with VUR, and the cut off point for VUR was accounted as 0.72 µg/mgCr. This value was based on the fact that all cases without VUR had a β2M/Cr ratio of below this value. Comparing this ratio with those of patients with different grades of VUR and the patients without VUR in the current study, revealed that β2M/Cr ratio was significantly higher in patients with VUR. This increase was more remarkable in patients with VUR of grades 4 and 5 (8.93 µg/mgCr vs. 0.23). This ratio was significantly higher in children with renal scar as well. Another study on 204 patients has revealed that β2M/Cr ratio increased in 36% of the cases and remarkably decreased after corrective surgery of VUR (10). Another study investigated β2M/Cr ratio as an indicator of tubular damage in 82 patients with VUR; β2M/ Cr ratio increased in all of them (11).

The point in the current study and Asadi's study was lack of significance of the difference between the β2M/Cr ratios of patients with VUR grades 1-3 and those of patients without VUR. It suggests that β2M/Cr ratio cannot be a good indicator in patients of lower grades of VUR who do not have remarkable tubular damage. On the other hand, in patients with higher grades of VUR and more tubular damage, measurement of urinary level of β2M can be used as a reliable screening test. VUR grades 1-3 are most likely self limited (70-80%) and can be managed successfully by prophylactic antibiotic therapy and/or prompt follow-up and rarely need a corrective surgery; however, VUR of higher grades is self limited in 10 to 40% of the cases and mostly warrants anti-reflux surgery. Thus, it can be claimed that measuring urinary level of β2M is an effective module in diagnosing the patients who need a surgery (1-3). Measurement of β2M may be useful in the early detection of tubular damage in refluxing patients and patients with renal scars.

In the current study standard deviation was high; this was prominent in analysis of patients with VUR, this could be the result of the low number of patients and the fact that scar can occur in Pyelonephritis without reflux. Further studies are required with a higher number of patients.

This study revealed that the maximum β2M/Cr ratio value in non refluxing children and those without renal scar was 0.85 and 0.46 microgram/mg Cr respectively, higher values can be considered as a cut off point in screening children for high grade VUR and renal scar.

Measurement of urinary level of β2M is recommended prior to VCUG in patients with UTI and the siblings of the patients with VUR. Urinary β2M/Cr ratio Values can be used to evaluate the prognosis in patients with VUR and renal scars.

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