Urinary Adrenomedullin Level in Children With Acute Pyelonephritis Before and After Treatment

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Introduction. Adrenomedullin (AM) is a 52-amino acid peptide that causes vasodilatation by increased synthesis of nitric oxide. Its production by different cells such as cardiac myocytes, smooth muscle, endothelial, and oncogenic cells is stimulated by inflammatory processes. It has been shown that in the presence of inflammation in the urinary system, concentration of AM increases. In this study, we measured urinary AM in children with acute pyelonephritis before and after treatment and compared its level with that in healthy children.

Materials and Methods. In a case-control study, 31 children with clinical and paraclinical documentation of pyelonephritis (case group) and 30 healthy children without pyelonephritis or other infections (control group) were studied. Urinary AM were measured on spot urine samples by high-performance liquid chromatography, and creatinine was measured by spectrophotometry to report the AM-creatinine ratio.

Results. Urinary AM- creatinine ratios were 61.3 ± 119.4 pg/mg and 4.26 ± 11.4 pg/mg, respectively, in the case and control groups (P = .01). After treatment of pyelonephritis in the patients of the case group, this ratio decreased to 13.1 ± 21.9 (P = .048). The coefficient correlation between urinary AM and leukocytes count was 0.252 (P = .17). Urinary AM levels were 1896 ± 1748 pg/dL and 391 ± 477 pg/dL in the patients with 4+ versus negative C-reactive protein levels, respectively (P = .008).

Conclusions. Urinary AM increases in the course of pyelonephritis and decreases significantly after treatment.

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INTRODUCTION

Urinary tract infection (UTI) is one of the common diseases in children with a prevalence of 3% to 5% in girls and 1% to 2% in boys.[1,2] Although mortality rate of UTI is not significant, morbidity of this disease such as the injury of renal parenchyma and renal insufficiency is significant.[3] Investigations show that some degrees of renal parenchymal scar can develop in about 40% of patients with acute pyelonephritis. This can result in hypertension and renal insufficiency in the future.[4-8]

Delay in diagnosis and treatment of pyelonephritis, is an independent risk factor for developing scar and renal insufficiency. Using fast and easy diagnostic methods have considerable importance for patients and physicians.[9] At present, technetium Tc 99m dimercaptosuccinic acid (^{99m}Tc-DMSA) renal scintigraphy, in addition to clinical and

laboratory criteria, is used for definite diagnosis of acute pyelonephritis. This diagnostic method has a high sensitivity for detection of renal scars and to predict prognosis.[10] However, DMSA scintigraphy needs equipment and time and it imposes radiation exposure.[11] For this reason, investigators are trying to find safer diagnostic methods.

One of the alternative methods includes measurement of urinary adrenomedullin (AM), which is a peptide with 52 amino acids. Adrenomedullin shows a vasodilator effect via nitric oxide and cyclic adenosine mono phosphate.[12] Recent studies have shown that the kidney is one of the major tissues for producing AM,[13] and inflammatory processes and urinary infections can stimulate the tissue of the kidney to secrete it in the urine.[14-16] These investigations indicate that urinary AM levels can be a marker for acute urinary infections. In this study, we measured urinary AM levels in children with pyelonephritis and compared results with a control group.

MATERIALS AND METHODS

This case-control study was performed in Mofid Pediatric Hospital during a period from 2009 to 2010. Our study population was children aged 1 month to 10 years old who had documented pyelonephritis with (case group) and healthy children who had been referred for vaccination or growth monitoring (control group). Pyelonephritis was confirmed based on clinical manifestation and ^{99m}Tc-DMSA renal scintigraphy. These patients had no other infections other than UTI, and also, they had no underlying disorders. Moreover, physical examination was done in the control group to confirm absence of any underlying diseases and one urine sample for urinalysis and culture for confirming lack of urinary infection.

According to error type 1 (α) that was considered to be 0.05 and error type 2 (β) as 0.2, the sample size was calculated to be 30 individuals in each group. After approval of the study protocol by the ethics committee of the university and written consent from parents before any therapeutic intervention, a sterile urine sample were obtained, as well as, a blood sample from the patients of the case group for measuring erythrocyte sedimentation rate (ESR), leukocyte count, and C-reactive protein (CRP).

Urine samples were stored in at -20°C for maximum 8 weeks before measurement of urinary AM. Urinary AM concentrations were measured using high-performance liquid chromatography and recorded as pg/dL and creatinine of the same urine sample by auto-analyzer. To eliminate the effect of dilution and concentration of urine in assessing results, urinary AM-creatinine ratio was considered for analyses. Also, urine AM and creatinine levels were measured after treatment with antibiotic to compare the results of both groups as well as before and after treatment.

For statistical analysis, the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA) was used. The Student *t* test, 1-way analysis of variance, and Pearson correlation coefficient tests were for comparisons and evaluation of correlations. A *P* value less than .05 was considered significant.

RESULTS

The mean age of the children was 48.8 ± 30.7 months and 36.0 ± 30.0 months in the case and control groups, respectively ($P = .10$). The mean urinary AM and urinary AM-creatinine ratio in the case and control groups are shown in Table 1. The effect of treatment on the mean of urinary AM is summarized in Table 2.

The urine AM-creatinine ratio was significantly correlated with ESR levels (r = 0.660; $P < .001$; Figure 1), but not with leukocyte count (r = 0.271; $P = .14$; Figure 2) and not with polymorphonuclear cells (r = 0.232; $P = .21$; Figure 2). The associations between CRP and urinary AM and AM-creatinine ratio are shown in Table 3.

Considering the cutoff point of urinary AM for

Table 1. Comparison of the Mean Urinary Adrenomedullin (AM) and Urinary AM-Creatinine Ratio in Children With Urinary Tract Infections and the Control Group

Parameter	Case	Control	*P*
Urinary AM, pg/dL	546.6 ± 770.4	251.2 ± 674.3	.12
Urinary AM-creatinine ratio, pg/mg	61.3 ± 119.4	4.26 ± 11.4	.01

Table 2. Comparison of the Baseline Mean Urinary Adrenomedullin (AM) and Urinary AM-Creatinine Ratio With Posttreatment Levels in Children With Urinary Tract Infections

Parameter	Before Treatment	After Treatment	*P*
Urinary AM, pg/dL	546.6 ± 770.4	316.1 ± 567.7	.13
Urinary AM-creatinine ratio, pg/mg	61.3 ± 119.4	13.1 ± 21.9	.02

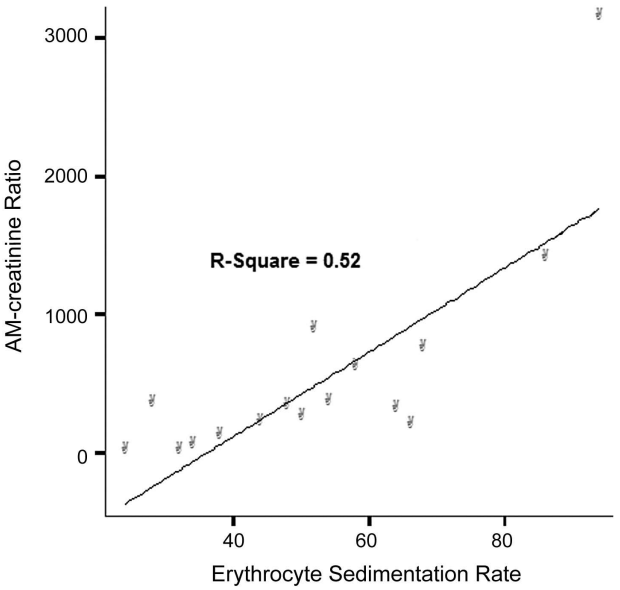

Figure 1. The relationship between Adrenomedullin (AM)-creatinine ratio and erythrocyte sedimentation rate.

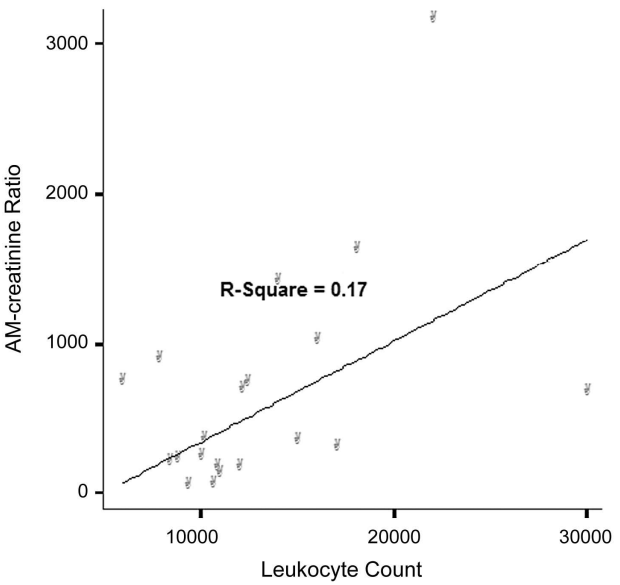

Figure 2. The relationship between Adrenomedullin (AM)-creatinine ratio and leukocyte count.

diagnosis of pyelonephritis equal to 100 pg/dL, the sensitivity of AM for diagnosis of pyelonephritis was 67.7%, the specificity was 70%, and the positive

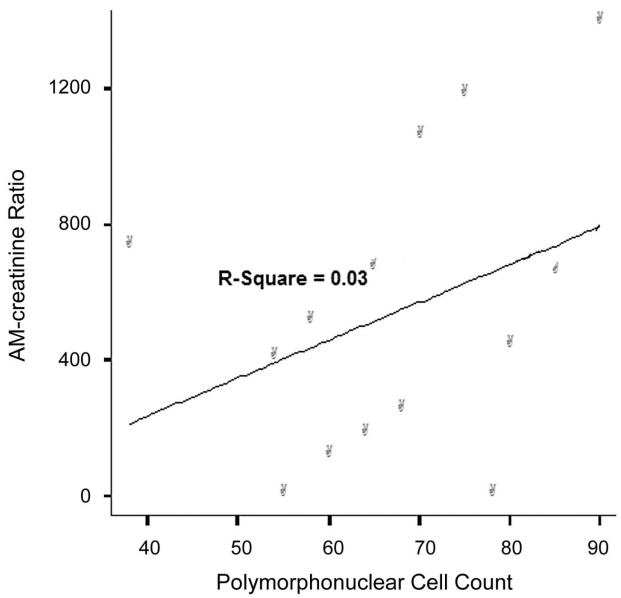

Figure 3. The relationship between Adrenomedullin (AM)-creatinine ratio and polymorphonuclear cell count.

and negative predictive values were 70% and 67.7%, respectively (P = .003).

DISCUSSION

Adrenomedullin is a peptide with multiple effects; it is a dilator of smooth muscle cells,[15] as well as a vasoconstrictor.[16] It has been isolated from various tissues such as the kidneys, adrenals, lung, and heart.[14] It has been shown in recent years that cytokines and bacterial endotoxins stimulate its production and secretion. Regarding its potential role in the inflammatory processes, this study was designed to evaluate the association between pyelonephritis and urinary adrenomedullin. In addition, the association between AM and other inflammatory markers such as ESR, CRP, and leukocytosis were evaluated. Also, the effect of treatment on adrenomedullin level was investigated, which is not included in other studies.

Our findings showed that age had no potential confounding role in this study. According to our results, urinary AM-creatinine ratio in case (pyelonephritis) group is significantly higher

Table 3. Baseline Mean Urinary Adrenomedullin (AM) and Urinary AM-Creatinine Ratio by C-reactive Protein in Children With Urinary Tract Infections

Parameter	C-reactive Protein					*P*
	Negative	1+	2+	3+	4+	
Urinary AM, pg/dL	391 ± 477	248 ± 288	265 ± 385	1037 ± 955	1896 ± 1748	.008
Urinary AM-creatinine ratio, pg/mg	92 ± 127	18.7 ± 41.9	9.68 ± 14.4	161.5 ± 197	143 ± 165	.048

than control group. This finding is similar to findings of Kalman and colleagues[14] and Dotsch and colleagues.[15] However, in another study done by Kalman and colleagues,[16] the results showed that urinary AM levels in the patients with renal parenchymal scar due to urinary reflux was lower in comparison to the control group. It seems that urinary AM levels increases in the patients with urinary infection and renal parenchymal involvement, because other studies in which renal parenchymal injuries was due to noninfectious inflammatory processes did not show elevation in urinary AM levels.[17-22] Confirming the results of the first part of our study, our study revealed that pyelonephritis treatment significantly decreases urinary AM-creatinine ratio.

Results of our study have showed that ESR has a strong direct association with urinary AM (r = 0.63). Moreover, a significant association was shown between CRP and urinary AM, as such with increasing semiquantitative CRP, particularly 3+ and 4+, the levels of urinary AM elevated significantly, while in CRP 1+ and 2+ or CRP-negative patients, urinary AM level was very low and negligible. We did not have qualitative CRP in our hospital at the time of study and this was one of our limitations in this study.

Our findings show that adrenomedullin may be considered as an inflammatory marker and with increasing degree of inflammation, AM levels elevate accordingly. In spite of finding a significant relationship between ESR and semiquantitative CRP with urinary AM, we could not find a significant correlation between circulatory leukocytes or polymorphonuclear cells and urinary AM level. Designing studies and evaluating association between plasma AM and immune system cells is recommended in this regard.

Our study showed that urinary AM level was undetectable in 25% of all studied individuals (including cases and controls) and less than 20% of them had an AM level greater than 1000 pg/dL. Regarding the distribution of urinary AM concentrations in the case and control groups, a cutoff point equal to 100 pg/dL is more suitable for diagnosis of UTI as with this cutoff, its sensitivity will be 67.7% and its specificity will be 70%. With rising cutoff point up to 200 pg/dL or 300 pg/dL, the sensitivity decreases and specificity increases. With decreasing cutoff point, decreasing specificity of this test and increasing false negatives has been expected. Therefore, we recommend considering a cutoff point equal 100 pg/dL based on high-performance liquid chromatography method. However, changing method of assessment would change the cutoff point and sensitivity and specificity of the test.

CONCLUSIONS

Urinary AM increases in the course of UTI and decreases significantly after treatment. This marker can be used for confirmation of diagnosis and evaluation of response to treatment adjunct to other markers. However, further studies are required with higher numbers of patients for achieving more definite results.

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CONFLICT OF INTEREST

None declared.

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