European Review for Medical and Pharmacological Sciences 2013; 17: 2598-2604

High sensitive C-reactive protein: a new marker for urinary tract infection, VUR and renal scar

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Abstract. – BACKGROUND: Accurate diagnosis and early treatment of urinary tract infections (UTI) are important because of its association with renal scarring (RS).

AIMS: To investigate the serum levels of fibronectin, high sensitive CRP (Hs-CRP), urinary fibronectin, and beta-2 microglobulin (beta2MG) levels in patients with UTI and relationship of these parameters with VUR (vesicoureteral reflex) and RS.

PATIENTS AND METHODS: 72 patients were included in study and divided into three groups: Group I (20 patients with first UTI); Group II (16 patients with recurrent UTI with VUR); Group III (16 patients without UTI with VUR).

RESULTS: Serum and urine fibronectin levels were similar in all study groups and controls. Urinary beta2MG levels were higher in Group II (302±179 ng/ml) than in the Group I (134±90 ng/ml) ($p < 0.001$). Moreover, beta2MG levels were similar in Group II (302±179 ng/ml) and group III (218±147 ng/ml). By contrast, beta2MG levels were higher in Group III (218±147 ng/ml) than in the controls (64±32 ng/ml) ($p < 0.001$). Hs-CRP levels were higher in Group I (1.8±2.7 mg/L), Group II (23.1±32 mg/L), and III (0.4±0.1 mg/L) than the controls (0.2±0.08 mg/L) ($p < 0.001$). Hs-CRP levels were higher in Group II (23.1±31.9 mg/L) than in the Group I (1.8±2.7 mg/L) ($p < 0.001$). Hs-CRP levels were higher in Group I (1.8±2.7 mg/L) and Group II (23.1±31.9 mg/L) than in the Group III (0.4±0.1 mg/L) ($p < 0.001$). Hs-CRP levels were higher in group III (0.37±0.17 mg/L) than in the controls (0.2±0.08 mg/L) ($p < 0.001$). Hs-CRP (18.8±25 mg/L) and beta2MG levels (349.4±128.5 ng/ml) were different in UTI with RS from the controls (0.2±0.08 mg/L and 64±32 ng/ml respectively, $p < 0.001$). Fibronectin levels were similar in patients with and without RS.

CONCLUSIONS: Increased urinary beta2MG and Hs-CRP were observed in initial UTI and recurrent UTI with VUR. Fibronectin levels were not useful for detection of first and recurrent UTI with VUR and RS. Elevated Hs-CRP levels can help us predetermine the patients with VUR prone to proceed to clinical chronic renal failure.

Key Words:

Hs-CRP, Fibronectin, Beta-2 microglobulin, Urinary taract infection, VUR, Renal scars.

Introduction

Urinary tract infection (UTI) is one of the most frequent bacterial infections in children. Possible complications of UTI may include renal scarring, hypertension, and chronic renal disease. North American Pediatric Renal Transplant Collaborative Studies (NAPRTCS) confirm that renal injury from reflux nephropathy and chronic pyelonephritis continue to be a major concern and the most common etiologic factor of potentially preventable long-term renal disease in children. More than 8% of children enrolled in NAPRTCS with chronic renal disease carried the diagnosis of reflux nephropathy. Several risk factors for UTIs have been identified and discussed in the past. The risk of UTI is significantly increased in the presence of recurrent UTI and associated nephro-urologic abnormalities such as vesicoureteral reflux (VUR). However, a reliable method for determining the relationship between UTI, VUR and renal scars is not available currently. The aim of the present study was to elucidate the high sensitive CRP (Hs-CRP) levels and their potential relationship to renal scars and VUR in UTI patients compared with the fibronectin (FN) and beta-2-microglobulin (β2MG) levels.

Pataients and Methods

UTI was defined as growth of single pathogen of 10^5 (bag), 10^4 (transurethral) and 1 (suprapubic aspiration) colony forming units/ml in properly collected urine specimens in children with urinary symptoms including fever, chills, flank pain, dysuria, urgency and pyuria (defined > 10 leukocyte/high power field). Tecnetium 99m dimercaptosuccinic acid scintigraphy (DMSA) was performed in all patients; therefore, renal injury was determined with DMSA. When renal injuries were detected, DMSA was also used for

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the follow up of patients for detection of persistent change in the same injury site. Renal scars were defined as focal decreased uptake associated with contracted and loss of volume in the involved cortex. VUR was detected with voiding.

Patients and Controls

In this study, 52 patients were included. The patients were divided into three groups. In Group I, there were 20 patients with first UTI; in Group II, there were 16 patients with recurrent UTI with VUR; in Group III, there were 16 patients with VUR but without UTI. The control grouped contained 16 healthy children.

Study Protocol

The serum levels of FN, β2MG, CRP and Hs-CRP were measured in all subjects and controls using commercial kit. Blood samples for FN were kept at –80ºC until the use. The urine and serum levels of FN assays were carried out using ELISA (Bender medSystem, Vienna, Austria). The urine β2MG levels were determined using chemiluminescent method. The serum CRP and high-sensitiving-CRP (Hs-CRP) levels were measured by immunochemitric method.

Statistical Analysis

Data were analyzed using the SPSS for Windows package (SPSS Inc., Chicago, IL, USA). All ranges quoted represent the standard error or deviation. Student's *t* test and Mann Whitney U test, ANOVAx^2 test and Pearson and Spearman correlation tests were used. $p < 0.05$ was considered statistically significant.

Ethics

The study was approved by the Research Ethics Committee of Eskisehir Osmangazi Medical Faculty, Eskisehir Osmangazi University. Informed consent was obtained from the parents or guardians for all the study patients and control subjects.

Results

The levels of serum FN were decreased in all study groups with respect to the controls but it was not statistically significant ($p > 0.05$) (Figure 1). The urine FN levels were higher in Group III than in Group I and II, but lower than in controls but it was not statistically significant as well (Figure 1B). Levels of urinary β2MG were higher in Group I (134±90 ng/ml), Group II (302±179 ng/ml), and III (218±147 ng/ml) than in the controls (64±32 ng/ml) ($p < 0.001$, Figure 2). Furthermore, levels of urinary β2MG were higher in Group II (302±179 ng/ml) than in the Group I (134±90 ng/ml) ($p < 0.001$, Figure 2). Levels of urinary β2MG were similar in Group II (302±179 ng/ml) and Group III (218±147 ng/ml) ($p > 0.05$). Levels of urinary β2MG were higher in Group III (218±147 ng/ml) than in the controls (64±32 ng/ml) ($p < 0.001$, Figure 2). Serum Hs-CRP levels were elevated in Group I (1.78±2.72 mg/L), Group II (23.1±31.9 mg/L), and III (0.37±0.17 mg/L) with regard to the controls (0.2±0.08 mg/L) ($p < 0,001$, Figure 3). In addition, serum Hs-CRP levels were higher in Group II (23.1±31.9 mg/L) than in the Group I (1.78±2.72 mg/L) ($p < 0.001$). Serum Hs-CRP levels were higher in Group I (1.78±2.72 mg/L) and Group II (23.1±31.9 mg/L) than in the Group III (0.37±0.17 mg/L) ($p < 0.001$, Figure 3). Hs-CRP levels were higher in Group III (0.37±0.17 mg/L) than in the controls (0.2±0.08 mg/L) ($p < 0.001$).

Renal scars were detected in 13 patients (1 in group I; 7 in Group II and 5 in Group III). Serum Hs-CRP (18.8±25 mg/L) and urinary β2MG levels (349.42±128.46 ng/ml) were different in UTI with renal scars than in those of the control group (0.2±0.08 mg/L and 64±32 ng/ml respectively, $p < 0.001$, Figure 4). However, serum and urinary FN levels were similar in patients with and without renal scars and controls (546.3±173/573.8±55.3/mcg/ml and 71.2±18.1/ 69.4±20.4/ng/ml respectively, $p > 0.05$, Figure 4).

Discussion

Fibronectins belong to a family of large (440 kDa) dimeric glycoproteins implicated in a variety of biological processes, including tissue remodeling during wound healing and embryonic development (1). They exist in two forms, serum and cellular (2). Serum FN is generated by hepatocytes and circulated in the blood; cellular FN is produced by epithelial and connective tissue cells including renal interstitial tissue, mesangium and basement membrane of glomeruli[2,3]. FN levels are shown to be decreased in renal disease such as renal ischemia, tubulointerstitial fibrosis, glomerulopaties[4-6]. Although FN has been studied in various infection states; the numbers of the studies investigating the role of FN in the urinary tract infections in children are limited. Riordan et

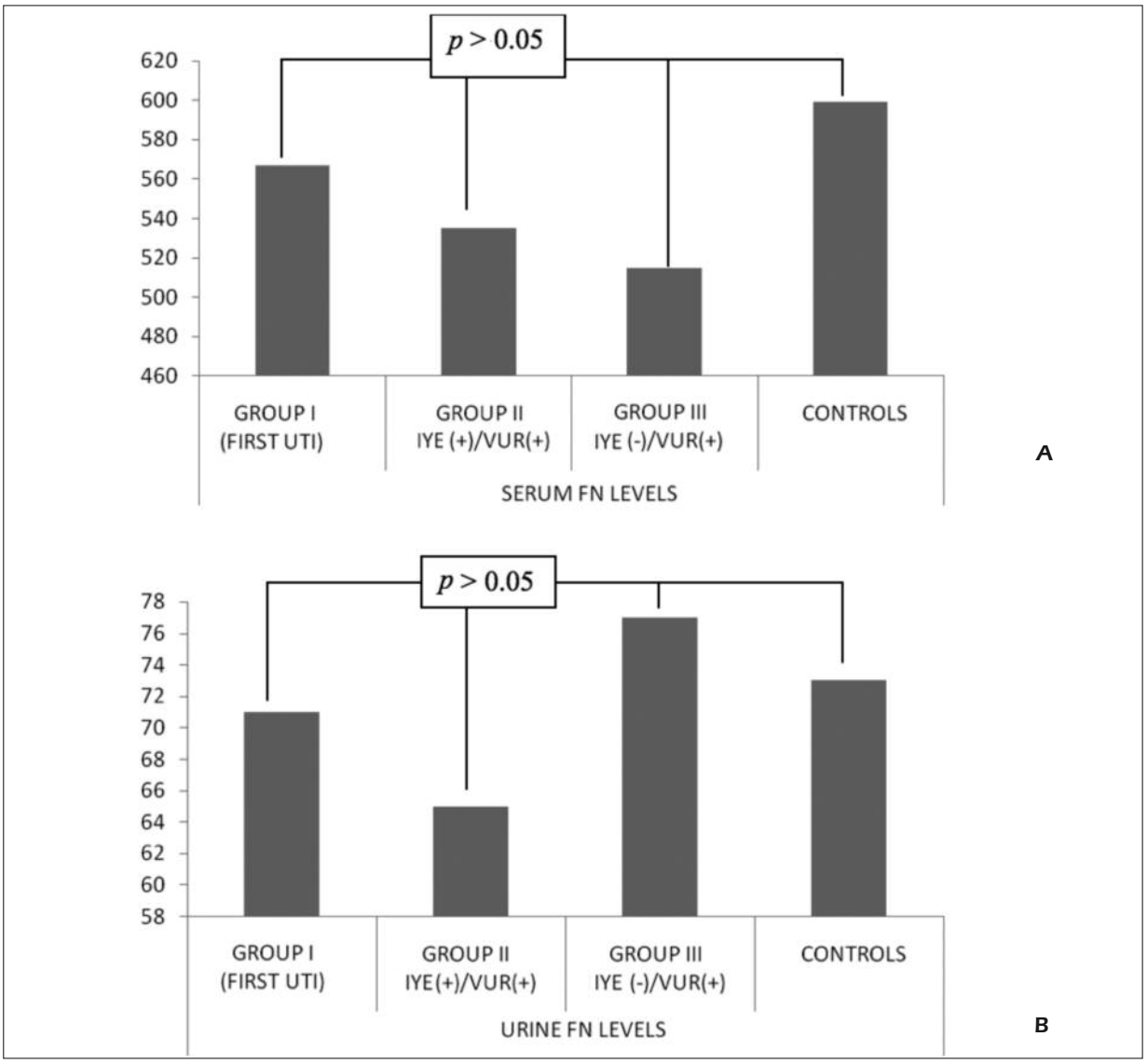

Figure 1. The levels of serum ***(A)*** and urine ***(B)*** fibronectin (FN) levels were similar in all study groups and controls ($p > 0.05$).

al[7] showed that plasma FN levels decreased in meningococcal disease in children. In peritoneal dialysis patients, plasma fibronectin concentration and *in vitro* fibronectin secretion by cultured peripheral blood monocytes were shown to be similar between high infection rate patients and low infection rate patients even though the level was less than normal[8]. Similarly, in sepsis syndrome, patients with the lowest blood fibronectin levels generally have the worst prognosis[9]. The plasma FN level is reported to be significantly decreased in patients with various viral hepatitis infections[10]. Present study determined that serum and urinary FN levels were similar in patients with first and recurrent UTI with VUR and renal scars ($p > 0.05$). There are several potential explanations for these results. One is that, the serum FN level was decreased due to the consumption of FN because of inflammation. Another explanation may be the decrease in FN in infection due to decreased hepatic synthesis via cytokines[11]. In fact, FN production is regulated by IL-6[11]. Last explanation may be over expression of matrix metalloproteinase which cleaves extracellular matrix component (EMC) including FN. Several expression analyses have shown that matrix metallo proteinases (MMPs) are indeed up regulated during infection with different Gram-negative bacteria. For example, serine proteinases derived from Escherichia coli are specific acti-

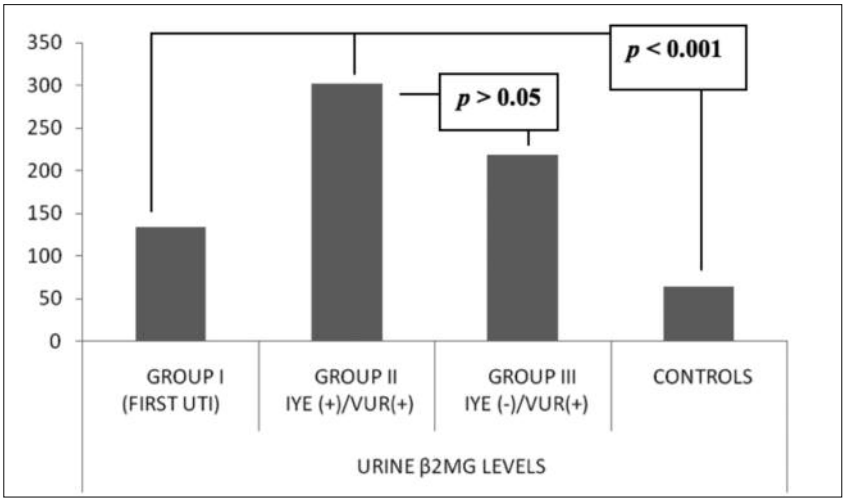

Figure 2. Urine β2MG levels in study groups and controls.

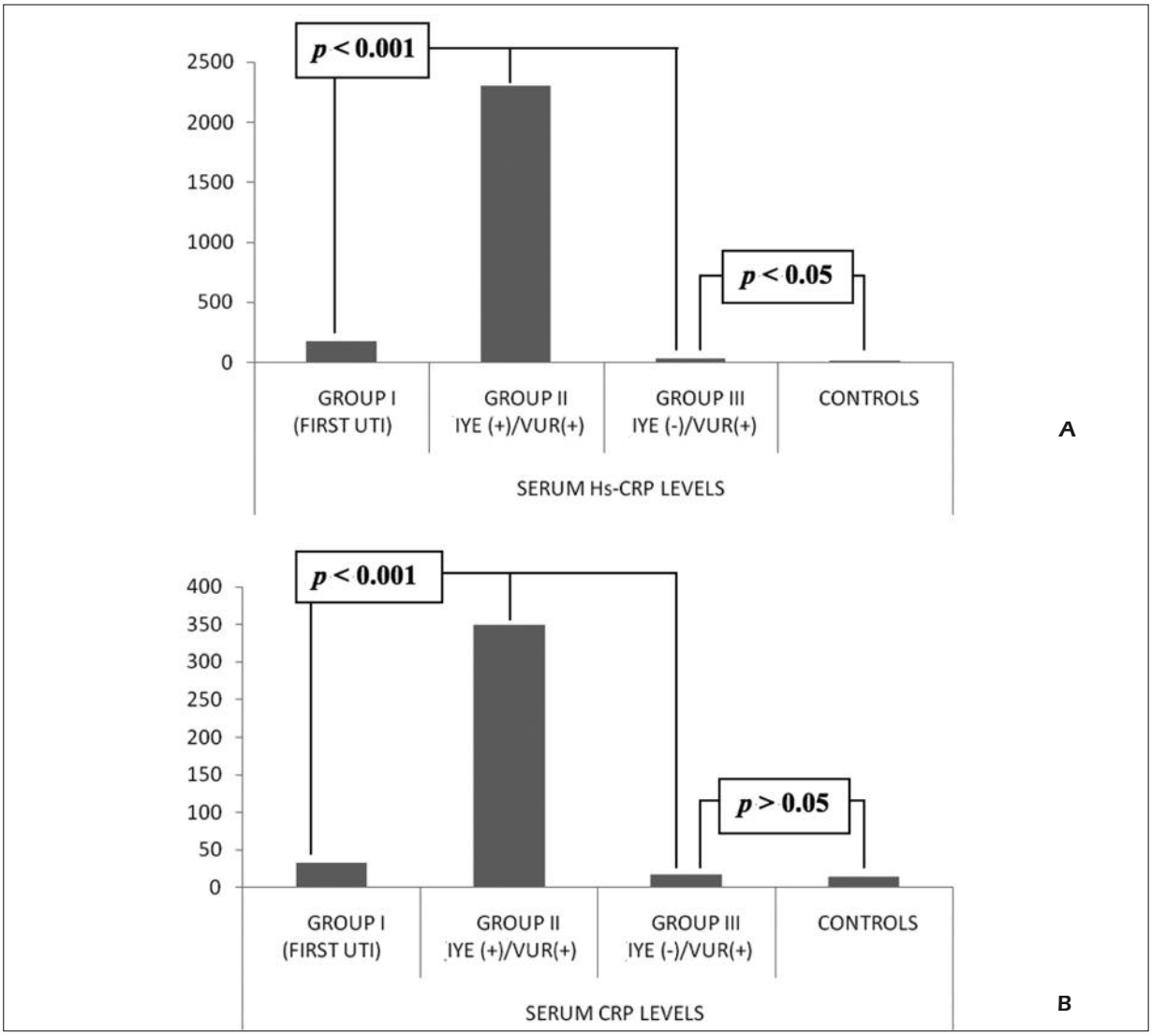

Figure 3. Serum Hs-CRP ***(A)*** and CRP ***(B)*** levels in study groups and controls.

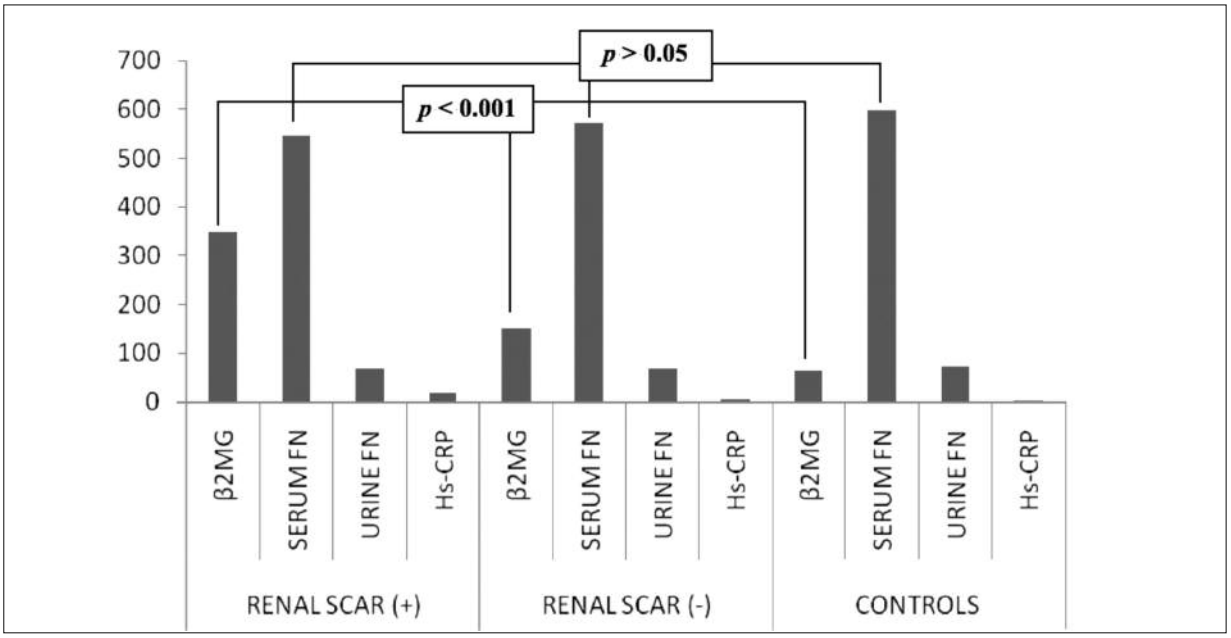

Figure 4. Serum Hs-CRP, fibronectin and urine fibronectin and β2MG levels in patients with and without renal scars and controls.

vators of pro-MMP-2[12,13]. Inconclusion, based on these results neither serum nor urine FN concentration is useful predictive tools for first UTI, recurrent UTI with VUR or renal scars in children. FN levels did not distinguish between first UTI from recurrent UTI in our patients.

VUR and recurrent UTI are believed to predispose children to renal scarring. The pathogenesis of renal scarring/fibrosis following UTI is not well understood. The renal fibrosis can progress even after VUR owing to hyperfiltration, autologous tubular antigens, reaction to Tamm-Horsfall protein, superoxide production, and hypertension[14]. Numerous non-invasive biomarkers such as epidermal growth factor (EGF), interleukin (IL)-8, IL-6, endothelin-1 have been investigated for monitoring of renal scarring[15]. Present study demonstrated for the first time that inflammation continued even without UTI period and Hs-CRP levels were higher in VUR patients without UTI than in the controls whereas standard CRP levels did not increase in non-infectious period (Figure 3 A and B). Additionally, Hs-CRP levels were higher in patients with renal scars than the patients without renal scars and controls ($p < 0.001$). In fact, increased levels of serum Hs-CRP have been suggested to be an indicator for low-grade systemic inflammation in several disorders, such as cardiovascular disease and diabetes mellitus[16-18]. Although it was not statically significant, we found urine FN levels higher in VUR patients without UTI than the patients with first UTI and recurrent UTI as well (Figure 1B). This result indicates that extracellular matrix component expansion continues even in the absence of infection in VUR. These findings suggest that increased Hs-CRP levels may reflect chronic inflammation in VUR patients without UTI. Elevated Hs-CRP levels could be a new non-invasive candidate biomarker for presence of renal scars. Consequently, elevated Hs-CRP levels can help us predetermine the patients with VUR prone to proceed to chronic renal failure in clinic. Hs-CRP and standard CRP levels did not distinguish between first UTI from recurrent UTI in our patients.

Low molecular weight proteins, shown to serve as a risk marker for tubular injury, are metabolized and reabsorbed by the proximal tubules[19]. This occurs when there is an increased re-absorptive load (increased transglomerular passage of proteins reaching the tubular lumen), or functional or structural damage (toxic effect of substances reaching the tubular lumen)[20]. Therefore, several studies have attempted to differentiate between upper and lower UTIs in children on the basis of tubular proteinuria; in particular in urinary β2MG excretion. Jantausch et al[21] showed that increased urinary β2MG were not associated with renal inflammation. Moreover, Chiou et al[22] measured urinary albumin and

β2MG using immunoassay in 61 pediatric patients with febrile UTI and noted increased urinary albumin but not β2MG. They also reported statistically significant increase in urinary β2MG/Cr ratio correlated with the presentation of a high grade of vesicoureteral reflux. Ginevri et al[23] reported that retinol-binding protein, β2MG, and brush border antigens increased in children with VUR but these increases were not related to renal scarring. In contrast, according to our findings; (1) UTI appears to be associated with elevated urine B2MG levels in children, (2) β2MG levels were associated with renal inflammation where CRP and Hs-CRP levels were increased in all the study groups (first UTI, recurrent UTI with VUR and VUR without UTI), (3) urinary β2MG levels were correlated with renal scars ($p = 0.002$), and (4) β2MG levels were increased in VUR patients without UTI. Increased β2MG levels in our patients seemed to be associated with the immune response of tubular cells. They are capable of processing and presenting foreign antigen, various cytokines, producing different chemokines, a group of low-molecular-weight cytokines with chemotactic functions[24-26]. Furthermore, IL-8 causes tubulo-interstitial lesions by recruiting target cells, including macrophages/monocytes, T cells, neutrophils, eosinophils and basophils, into the tubulo interstitium, where the target cells secrete cytokines, including TNF-α. Therefore, similar to Hs-CRP, we think that urine β2MG levels are useful marker for tubulo-interstitial inflammation in VUR patients with or without UTI.

Conclusions

Urine β2MG and serum Hs-CRP levels were increased in first and recurrent UTI. Serum and urine fibronectin levels were not related with UTI and VUR. The present study provides evidences that tubulo interstitial inflammation can continue in infection free period in VUR patients. We suggest that due to instability of β2MG molecules in alkali urine, increased serum Hs-CRP levels may be beneficial marker for follow up of VUR patients without UTI for chronic renal inflammation or progression of chronic renal failure.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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