Research Article

Can Serum Galectin-3 Be used for Diagnosis of Childhood Urinary Tract Infections?

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Abstract

Background: Early diagnosis of Urinary Tract Infection (UTI) is important. Delayed diagnosis and management of UTI can result in renal damage. Specific biomarkers are needed to allow earlier diagnosis of UTI. Therefore, we aimed to test whether serum galectin-3 can be used as a biomarker in diagnosis of UTI in childhood.

Method: The study group consisted of 30 children with UTI and 30 controls. Serum galectin-3, NGAL, IL-6 and acute phase reactants were measured.

Results: Serum neutrophil (p=0.01), serum NGAL (p=0.03) and serum galectin-3 (147.5±62.1 vs. 111.3±56.8, p=0.022) were significantly higher in the patient group. Eight (26.6%) of the patients had upper UTI, and 22 (73.3%) of them had lower UTI. Serum neutrophil (p=0.001), CRP (p=0.001), IL-6 (p=0.01) and galectin-3 (185.3±60.7 vs. 133.9±58, p=0.02) were significantly higher in patients with upper UTI. There was a significant positive correlation between serum galectin-3 level and IL-6 (r=0.57, p=0.001), NGAL (r=0.68,p=0.001) and CRP (r=0.4, p=0.02). Serum galectin-3 positivity was influenced by CRP (r²=0.14, p=0.01), IL-6 (r²=0.5, p<0.001) and NGAL (r²=0.88, p<0.001) as well as fever (OR:7.7, p=0.04) and vomiting (OR:48.1, p=0.02). Serum galectin-3 can diagnose lower UTI at 130 pg/ml cut-off value (p=0.02).The sensitivity and negative predictive value of galectin-3 was higher in the upper UTI (87% and 93,7%, respectively).

Conclusions: Serum galectin-3 can be used an alternative method for diagnosis and discriminating type of UTI

Introduction

Urinary Tract Infections (UTI) are among the common diseases in the pediatric age group. The diagnosis of the disease is not always easy because of the variable clinical findings. Delays in diagnosis and treatment may lead to undesirable conditions such as renal scarring, hypertension, and chronic renal insufficiency. In order to minimize these problems, early diagnosis and effective treatment of the disease is necessary [1-3]. For this purpose, effective biomarkers are needed that will enable both early diagnosis of UTI and distinguish upper UTI from lower UTI. Therefore, levels of serum leukocytes (WBC), C-Reactive Protein (CRP), and Erythrocyte Sedimentation Rate (ESR) [4], and serum IL-6 [5] and NGAL [6,7] levels are currently being used.

Galectin-3 is a protein that plays a role in acute inflammation and is produced by macrophages, activated T cells, epithelial cells, and fibroblasts [8]. Galectin-3 activates NADPH-oxidase and increases the production of superoxide from neutrophils [9], increases macrophage chemotaxis and also increases the capacity for phagocytosis [10]. It also plays a role in the adhesion of neutrophils to the endothelium [11].

It has been shown in previous studies that high galectin-3 levels have protective effects against pneumonia caused by Gram (+) [11] and meningitis caused by Gram (-) [12] bacteria. However, there is no study about its role in the diagnosis of urinary tract infection. In this study, we aimed to test whether the galectin-3 molecule can be used in the diagnosis of UTI.

Material and Methods

The local Ethics Committee approved the study. Between September 2015 and September 2016, patients admitted to our pediatric clinic with findings suggesting a UTI diagnosis were included in the study. Included complaints at admission were fever, abdominal pain, vomiting, and side pain for upper UTI; and dysuria, suprapubic pain, pollakuria, and enuresis for lower UTI. Urine samples were taken from children under 2 years with sterile bags or probes, and from older children by mid-flow urine sample method. Blood was drawn for complete blood count, CRP, ESR, serum creatinine, IL-6, NGAL, and galectin-3 levels. Hemogram and other biochemical tests were

immediately performed, and the sera separated for the measurement of IL-6, NGAL, and galectin-3 levels were stored at -80 C. Those with positive urine culture (10^5 colonies) were included in the study. Those with negative urine culture, newborns, and those who had serum creatinine levels above normal levels were excluded from the study. The control group was selected from those applied to our hospital within the same period. They were comprised of age-and sex-matched healthy individuals who had normal urinalysis and acute phase reactants, no evidence of acute infection, and negative urine culture. Serum galectin-3 (Hangzhou Eastbiopharm Co. Ltd), serum IL-6 (Hangzhou Eastbiopharm Co. Ltd), and serum NGAL (Hangzhou Eastbiopharm Co. Ltd) were measured with micro-ELISA method.

Statistical analysis was performed using SPSS 18.0 for Windows. The distribution of the parametric variables was assessed using the one-sample Kolmogorov–Smirnov test. Independent variables were compared with Student's t-test for normally distributed data and Mann–Whitney U test for non-normally distributed data. Comparisons of categorical data were made using the Chi-square test. After correlation analysis was done, regression analyses were performed according to the categorical and nominal values of the groups, and ROC analysis was performed. Statistically significance was defined as p<0.05.

Results

The study included 30 patients with UTI and 30 healthy controls. Seventeen of the patients (56.6%) were female, and their mean age was 6.3 years (40 days-14 years). Of the control group, 12 (40%) were female, and their mean age was 7.8 years (3 months to 14 years). There were no significant differences in sex and age between the two groups (p=0.2, p=0.18, respectively).

Serum NGAL (131.9 ± 71.3 ng/ml versus 97.2±50.3 ng/ml, p=0.03), WBC (5618.7±3971.9 µL versus 7037.6 ± 1807.5 µL, p<0.01), total neutrophil count (5618.7± 3971.9 µL versus 3726.0 ± 1443.9 µL, p=0.01), total lymphocyte count (3269.3± 1786.8 µL versus 2359.7 ± 1083.4 µL, p=0.02), and ESR (17±13 mm/h versus 8.3 ± 3.9 mm/h, p=0.02) were found to be significantly higher in patient group compared to control group. There were no significant differences between the other parameters (p>0.05) (Table 1). Serum galectin-3 levels were significantly higher in the patient group compared to control group (147.5 ± 62.1 pg/ml vs111.3±56.8 pg/ml, p=0.022).

From those patients diagnosed with UTI, the following were grown in the urine culture: *Escherichia coli* in 19 (63%), *Enterococcus faecalis* in four (13%), *Klebsiellapneumoniae* in three (10%), and *Proteus vulgaris*, negative staphylococcus susceptible to methicillin-sensitive coagulase, negative staphylococcus methicillin-resistant coagulase, and non-*albicans Candida* in one case each (3%).

When the DMSA results of the patients are examined, scar was seen in two cases (20%) and hypoactive area in kidney was seen in one case (10%), while no pathology was detected in 7 cases (70%).
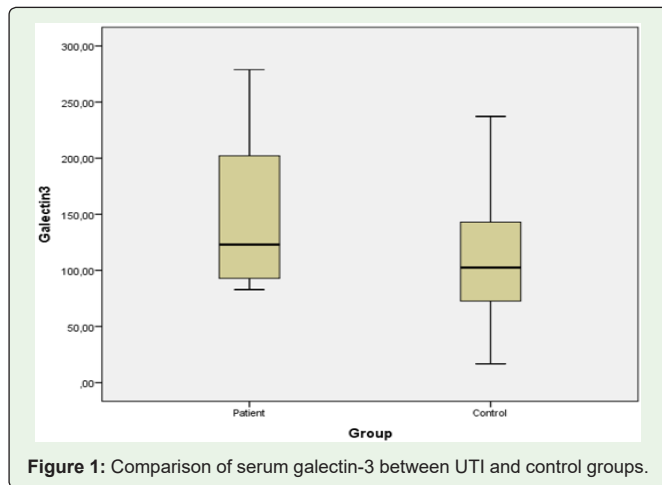
The ROC curve was made for serum galectin-3 level in order to predict the diagnosis of UTI. We determined that serum galectin-3 was almost found to be able to diagnose UTI at a cut-off value of 94.8 pg/ml (Sensitivity 57%, specificity 59%, p=0.053).

Eight (26.6%) of the patients were diagnosed with upper UTI, and 22 (73.3%) were diagnosed with lower UTI. Four of the patients (50%) diagnosed with upper UTI were female, and their mean age was 7.9 ± 3.4 years. There were no significant differences in sex and age between the upper and lower UTI groups (p=0.73, p=0.27, respectively). When the clinical characteristics of the patients were examined, it was

Table 1: Comparison of patients with UTI and control group.

	UTI (n=30)	Control (n=30)	p
Age, mean±SD (years)	6.3±4.5	7.8±4.1	0.18*
Sex (female, %)	56.6	40	0.2*
Galectin-3 (pg/ml)	147.5±62.1	111.3±56.8	0.022
NGAL (ng/ml)	131.9±71.3	97.2±50.3	0.03*
IL-6 (ng/l)	100.8±67.4	82.2±39	0.19*
CRP (mg/l)	28.2±51	1.9±2.8	0.24*
ESR (mm/hour)	17±13.3	8.3±3.9	0.03*
Total WBC count (/µl)	9905±3464.4	7037.6 ±1807.5	<0.001
ANC (/µl)	5618.7± 3971.9	3726.0±1443.9	0.01
ALC (/µl)	3269.3± 1786.8	2359.7±1083.4	0.02
NLR	2.7±2.8	1.8±1	0.84*
Platelet (/µl)	312233±81448	288700±105794	0.33
MPV (fl)	7.6±1.7	7.7±1.3	0.091
Urine pH	5.6±0.9	5.4±0.9	0.51
Urine density	1014.5±8.3	1018.8±6.5	0.07

*Mann-Whitney U; ALC: Absolute Lymphocyte Count; ANC: Absolute Neutrophil Count; CRP: C-Reactive Protein; ESR: Erithrocyte Sedimentation Rate; MPV: Mean Platelet Volume; NGAL: Neutrophil Gelatinase-Associated Lipocalin; NLR: Neutrophil Lymphocyte Ratio; UTI: Urinary Tract Infection).

Table 2: Comparison of patients with upper and lower UTI.

	Upper UTI (n=8)	Lower UTI (n=22)	p
Age, mean±SD (years)	7.9±3.4	5.8±4.8	0.27*
Sex (female, %)	50	59	0.73*
Galectin-3 (pg/ml)	185.3±60.7	133.9±58	0.02
NGAL (ng/ml)	171.9±77.3	117.4±64.8	0.05
IL-6 (ng/l)	145.1±59	84.8±64	0.01
CRP (mg/l)	95.8±58.8	3.7±8.8	<0.001
ESR (mm/hour)	32.9±15.5	11.3±6	<0.001
Total WBC count (/µl)	12941.3±4735.8	8801.8±2076.4	0.05
ANC (/µl)	9865± 4653.3	4074.5±2280.6	0.001
ALC (/µl)	1955± 799.1	3747.3±1816.2	0.003
NLR	6±3.2	1.5±1.6	0.002
Platelet (/µl)	262250±70609	330409±78748	0.055
MPV (fl)	7.1±1.1	7.8±1.9	0.45
Urine pH	5.4±0.7	5.8±1	0.31
Urine density	1017.5±6.5	1013.4±8.8	0.18

(*: Mann-Whitney U, İL-6: İnterlökin-6, NGAL: Neutrophil Gelatinase-Associated Lipocalin, WBC: White Blood Cell, CRP: C-Reactive Protein, MPV: Mean Platelet Volume, RDW: Red Cell Distribution Width, NLR: Neutrophil to Lymphocyte Ratio).

Figure 1: Comparison of serum galectin-3 between UTI and control groups.

Figure 2: ROC curve analysis of serum galectin-3 in diagnosis of upper UTI.

determined that fever (75% versus 9%, p=0.005) and costo-vertebral angle sensitivity (100% versus 0%, p=0.003) were significantly higher in the patients with upper UTI. No statistically significant differences were found for other clinical findings (p> 0.05). When laboratory findings of the patients were examined, IL-6 (145.1±59 ng/L versus 84.8±64 ng/L, p=0.01), total lymphocyte count (3747.3±1816.2 µL versus 1955.0±799.1 µL, p=0.003), total neutrophil count (9865.0 ± 4653.3 µL versus 4074.5 ± 2280.6 µL, p=0.001), CRP (95.8±58.8 mg/L versus 3.7±8.8 mg/L, p=0.001), ESR (32.9±15.5 mm/h versus 11.3±6.0 mm/h, p<0.001) and NLR (6.0±3.2 versus 1.5±1.6, p=0.02) were significantly higher in the patients with upper UTI; there were no significant differences between other parameters (p <0.05) (Table 2). Serum galectin-3 levels were significantly higher in the upper UTI group compared to lower UTI group (185.3±60.7 pg/ml versus 133.9±58 pg/ml, p=0.02) (Figure 1).

A significant positive correlation was found in the patient group between serum galectin-3 level and IL-6 (r=0.57, p=0.001), serum galectin-3 level and NGAL (r=0.68, p=0.001),serum galectin-3 level and CRP (r=0.4, p=0.02), and serum galectin-3 level and ESR (r=0.38, p=0.03).

We performed regression analyses for the factors affecting serum galectin-3 level in the study group. In the logistic regression analysis we performed, we found that fever (OR: 7.7 (95% CI: 1.1-52.1), p=0.04) and vomiting (OR: 48.1, 95% CI: 1.8-1235.4, p=0.02) have effects on serum galectin-3 levels. In the linear regression analysis, CRP (r²=0.14, p=0.01), ESR (r²=0.18, p<0.001), IL-6 (r²=0.5, p<0.001) and NGAL (r²=0.88, p<0.001) have effects on serum levels of galectin-3.

The *Candida*-growing case was excluded from the patient group, and the remaining microorganisms in the urine cultures of the remaining cases were classified as Gram (+) and Gram (-). According to this, while the galectin-3 level was 161.5 ± 61.4 pg/ml in Gram (-) cases, this value was 99.5 ± 22.3 pg/ml in Gram (+) cases. But the difference was not statistically significant (p=0.057).

DMSA was performed in only 5 (62.5%) of the patients diagnosed with upper UTI, and renal scars were detected in only 2 (40%). While the level of galectin-3 was 217.7 ± 30.9 pg/ml in those with renal scar, this level was 122.9 ± 38.2 pg/ml in those without scar. The difference between these two groups was not statistically significant (p=0.054).
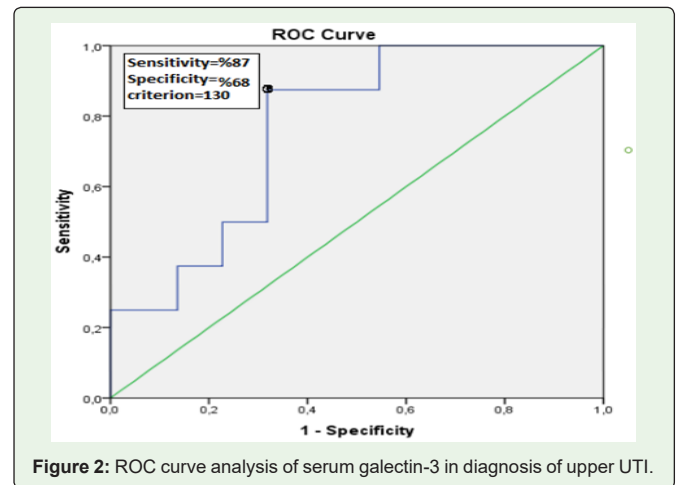
ROC curves were made for serum galectin-3 levels on estimating upper UTI diagnosis. Serum galectin-3 was found to be able to diagnose upper UTI at a cut-off value of 130 pg/mL (p=0.02) (Figure 2). Seven patients were diagnosed with upper UTI in this value resulting in an 87% sensitivity (specificity 68%) and a 93,7% negative predictive value (positive predictive value is 50%).

Discussion

UTI is among the most common diseases in the pediatric age group. Upper UTI patients often present with fever and tenderness on costavertebral angle as well as with abdominal pain and nausea/vomiting, whereas lower UTI patients have complaints of dysuria, enuresis, impingement sensation, and suprapubic tenderness [13]. Gram (-) bacteria are the most common causes of UTI; E. coli is found in most of these patients, and other genera include *Klebsiella*, *Proteus*, *Enterobacter* and *Enterococci* [13].

The levels of CRP in serum are increased in patients with UTI [14]. Cytokines such as IL-6 play an important role in this increase. IL-6, a pro-inflammatory cytokine produced during early inflammation, stimulates hepatocytes to increase fibrinogen and CRP production, resulting in increased fibrinogen and ESR [15-18].

NLR is a cheap and easily calculated index used to diagnose systemic inflammatory diseases [19,20]. It is accepted that NLR shows the severity of inflammation due to increased neutrophil production in bone marrow [21]. In our study, NLR was not beneficial in diagnosing UTI, but it was significantly higher in patients with upper UTI compared to lower UTI.

Serum levels of IL-6 [5] and NGAL [6,7] are also found to be increased in patients with UTI. In our study, we found that only NGAL is effective in diagnosing UTI. When we compared the patients with upper and lower UTI, we determined that serum levels of NGAL and IL-6 were significantly higher in the upper UTI patients compared to lower UTI patients, which is compatible with the literature [7,22].

Galectins play a key role immunocyte homeostasis and inflammation [23,24]. Galectin-3, a member of this family, is an important proinflammatory cytokine. It has been shown that these cytokines secreted from monocytes and macrophages contribute to the regulation of natural and acquired immunologic responses by activating neutrophils and lymphocytes [10,11,23-25]. In our study,

we found that serum galectin-3 levels were significantly higher in patients with UTI compared to healthy subjects. Serum galectin-3 level is also found to be associated with the activity and severity of diseases with infectious and inflammatory presentations [26,27]. In our study, we also found that serum galectin-3 levels were significantly higher in upper UTI patients, which is a more severe presentation compared to the lower UTI. In addition, we found that serum galectin-3 alone could diagnose the upper UTI at a cut-off value of 130 (Sensitivity 87%, Specificity 68%, p=0.02). The sensitivity and negative predictive value of galectin-3 was higher in the upper UTI. These findings suggest that serum galectin level is a marker that can be used in the diagnosis of UTI infection and in the discrimination of upper and lower UTI, but additional work is needed in this regard.

Scar is a chronic inflammation that leads to tissue damage and eventual organ failure. Studies have shown that in the case of fibrosis, increased expression of galectin-3 induces collagen synthesis in fibroblasts [28,29]. Therefore, we expected serum galectin-3 levels to be higher in cases with renal scarring, but, although galectin-3 levels were high in the patients with renal scar, the difference was not statistically significant (p=0.054), which could be the result of the limited number of patients with scar in our study.

The microorganisms cultured in urine cultures were classified as Gram (+) and Gram (-). Galectin-3 level was found to be almost significantly higher in the Gram (-) group compared to the Gram (+) group (p=0.057); the main reason for this is the low number of UTI patients with Gram (+) origin. Larger scale studies are needed to determine the role of Galectin-3 in predicting the microorganism responsible for UTI.

There are studies showing a correlation between serum galectin-3 and CRP [30]. In our study, when the relationships between serum galectin-3 levels and other inflammatory markers were examined, we found a strong correlation with NGAL, weak correlations with IL-6 and CRP, and a weak correlation with ESR. We also found that CRP contributes 14%, ESR contributes 18%, IL-6 contributes 50%, and NGAL contributes 88% to increasing serum galectin-3 levels. These results indicate that serum galectin-3 may be a good marker for diagnosis in inflammation and infection.

The most important limitations of our study are that our work was done in a single center and with a small study population.

Conclusions

Serum galectin-3 may be an alternative to traditional serum inflammation markers both in diagnosing UTI and in the discrimination of upper and lower UTI. Serum galectin-3 discriminates the upper and lower UTI at a cut-off value of 130 and it has a positive correlation with markers such as CRP, ESR, IL-6 and NGAL, which are accepted as valid for upper UTI, strengthening our opinion that galectin-3 can be used to make the diagnosis of upper UTI.

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