Original Article

Role of Urinary Transforming Growth Factor Beta-B1 and Monocyte Chemotactic Protein-1 as Prognostic Biomarkers in Posterior Urethral Valve

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Background: Posterior Urethral Valves (PUV) are the most common cause of congenital LUT obstruction in males. Biomarkers of glomerular or tubular injury may be of particular value in predicting the need for surgical intervention or in tracing progression of chronic kidney disease. Measurement of biomarker in urine is relatively easy.

Aim: To evaluate the changes in values of urinary Transforming Growth Factor Beta 1(TGF-B1) and Monocyte Chemotactic Protein (MCP-1) before and after valve ablation and its prognostic value in Posterior urethral valve.

Material and Method: This prospective study was conducted from September 2016 to August 2018. The study group included 20 consecutive male babies with the diagnosis of PUV treated and followed up versus equal numbers of age matched control without any renal or urinary tract disease. Pre-operative urine samples were collected in Operative room. Cystoscopy and valve ablation was done. Follow up was done clinically by urinary stream and radiologically with VCUG. Follow-up was planned at 1 month, 3 months and 6 months following cystoscopic valve ablation. All collected urine samples were centrifuged at 10,000 rpm for 20 minutes. Supernatant was collected and two divided aliquots were stored at -20°C to be thawed on the day of assay. Optical density of each well was recorded at 450 nm and 540 nm. A p-value of <0.05 was considered to be statistically significant.

Result and Discussion: Out of 20 cases of PUV, 14 (70%) cases were 1st born males of their family. The median age at the time of valve ablation in PUV cases was 2.5 (1.20-3.87) years. Most common symptoms are fever and UTI. The preoperative median serum creatinine level was 1.65 mg/dl (1.22-2.42) pre-ablation, and fall significantly after ablation. Median eGFR level (calculated) was 25.635 (16.38-35.40) and after 6 months was 71.490 (45.44-96.93). Preoperative median MCP1 in PUV cases was 147.2 (82.8-512.5) and significant difference was also found in 1st, 3rd and 6th months after surgery (p<0.001, p=0.004 and p=0.002). Preoperative median TGF-B1 level was 197.8 pg/ml (79.9-386.4). There was no statistically significant change in TGF-B1 level at preoperative to 1 month and preop to 3 months after surgery but at 6 months after surgery the median TGF-B1 level significantly decreased as compared with preoperative TGF-B1 level.

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INTRODUCTION

Posterior urethral valve (PUV) is a congenital obstructing membrane of the male urethra being the most common cause of bladder outlet obstruction in male children. PUV as a cause of obstructive uropathy is an important etiology behind end-stage renal disease (ESRD) in children.

A biomarker has been defined as a characteristic that is objectively deliberate and evaluated as a marker of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Urinary biomarkers are attractive considering they can be collected noninvasively and reveal the condition of renal tubular and bladder outlet obstruction. The principal concerns with lower tract obstruction as in PUVs are the timing of surgical intervention and prediction of the rate of progression of chronic kidney disease.

Unfortunately, renal function and outcome are not dependent on the severity of hydronephrosis and markers of glomerular filtration rate (GFR) which are poorer measures of functioning nephron number. Oligohydramnios, urinary ascites, and perinephric urinoma are cardinal signs of severe bladder outlet obstruction. Attempts to gauge fetal renal function is based on the measurement of urine β2-microglobulin, sodium, chloride, and calcium concentrations and urine osmolality whose sensitivity is enhanced by sampling sequential fetal bladder aspirates. This intervention is not feasible in various centers. The evaluation of the severity of kidney injury is more complex. Poor prognostic factors include prenatal detection at <24 weeks’ gestation, respiratory distress at birth, urinary sepsis, dyselectrolytemia, nadir serum creatinine >0.8 mg/dL, bilateral vesicoureteric reflux (VUR), hyperechoic kidneys, and absence of the pop-off mechanism. It is speculated, but it is unclear that later presentations have a better outcome. No available tests can predict which patients will progress to ESRD. However, the severity of functional renal impairment is directly related to structural damage of the developing kidneys.

Regardless of surgical correction (ablation of urethral valves), persistent low-grade obstruction in the setting of renal dysplasia may contribute to ongoing injury to nephrons and the urinary tract. Such injury is not detected by current imaging techniques or by measures of whole-kidney GFR by radionuclide scan or creatinine clearance. The primary objective throughout the life cycle of valve patients should be the preservation of nephrons by protecting them from insidious obstructive injury that could be revealed by age-appropriate biomarkers. Transforming growth factor-beta 1 (TGF-B1) is a multifunctional cytokine that is thought to play a critical role in fibrosis. It is released from parenchymal cells and inflammatory cells and can result in the production of extracellular matrix proteins and differentiation of tubular epithelial cells into myofibroblasts. Urinary TGF-B1 levels are correlated with renal parenchymal TGF-B1 and the degree of interstitial fibrosis in humans. Monocyte chemotactic protein (MCP-1) is another chemokine that is expressed at sites of injury or inflammation that functions to recruit inflammatory monocytes. Elevated concentrations of MCP-1 have been associated with the degree of inflammatory infiltrate, proteinuria, and severity of disease in humans. MCP-1 is particularly involved in the initiation and progression of tubulointerstitial damage.

Therefore, this study focuses to assess the role of urinary TGF-B1 and MCP-1 in PUV patients before valve ablation and after 1 month, 3 months’, and 6 months’ postablation. Furthermore, the correlation of these urinary biomarkers with other traditional serum markers is done.

METHODS

This prospective study was conducted in the department of pediatric surgery from September 2016 to August 2018. The study was approved by the institute’s ethical committee. The study group included 20 consecutive male babies with the diagnosis of PUV treated and followed up versus equal numbers of age-matched control without any renal or urinary tract disease admitted for other elective procedures such as inguinal hernia, undescended testis, thyroglossal cyst, and branchial sinus. Informed consent was obtained from all the cases for endoscopic treatment of PUV, collection of urinary samples, and use of medical records for scientific purposes in both groups.

Conclusion: TGF β1 and MCP1 can be considered as prognostic urinary biomarkers in patients of PUV and can be used to specify and counsel patient’s attendant regarding possibility of ESRD and need for further intervention.

Keywords: Monocyte chemotactic protein, posterior urethral valve, receiver operating characteristic curve, transforming growth factor-beta 1
Clinical history with relevant investigations was done. Antibiotics and fluid management was prerequisite in all cases. A 5- or 7-Fr feeding tube was inserted per urethra. Foley’s or balloon filled catheter was avoided. After adequate and intense initial management, patients were planned for cystoscopy under general anesthesia. Preoperative urine samples were collected in operative room. Cystoscopy and valve ablation were done. The follow-up was done clinically by urinary stream and radiologically with voiding cystourethrography (VCUG). The follow-up was planned at 1 month, 3 months, and 6 months following cystoscopic valve ablation. Fresh mid-stream urine samples in sterile containers were collected at room temperature. Furthermore, serum urea and creatinine, electrolytes, anthropometric data were recorded. Ultrasonography (on each followup) to assess postvoid urine residue (PVUR) in addition to VCUG (6 months followup) for ascertaining ratio of the posterior urethra to anterior urethra diameter were performed. All collected urine samples were centrifuged at 10,000 rpm for 20 min. The supernatant was collected and two divided aliquots were stored at −20°C to be thawed on the day of assay. Human CCL2/MCP-1 Immunoassay kit (Quantikine® ELISA, R and D Systems, Inc., USA) and human TGF-β1 (Quantikine® ELISA, R and D Systems, Inc., USA) was used for this purpose. Steps for assay were carried out as an instruction manual provided with each ELISA kit. The optical density of each well was recorded at 450 nm and 540 nm. Values were calculated using software in the unit of pg/ml. A P < 0.05 was considered to be statistically significant. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and Graph Pad Prism 5.0 (Graph Pad Software, Inc., La Jolla, CA) software were used for the statistical analysis.

**Result and Discussion**

Of 20 cases of PUV, 14 (70%) cases were 1st born males of their family. The median age at the time of valve ablation in PUV cases was 2.5 (1.20–3.87) years. Delayed presentation is due to patients are from low socially economic status and lack of awareness among population. Mandelis et al.[7] found the median age at the time of valve ablation was 5 months (range 3 days to 9 years). A study by MacRae Dell et al., age of the patient range was 3.2 years to 14.5 years.[8] The most common symptoms are fever and urinary tract infection (UTI) in 13 (65%) cases followed by abdominal distention and dribbling in 12 (60.0%), acute retention and sepsis in 11 (55%), and hematuria in 9 (45%) cases. The most common presenting symptoms were poor urinary stream

**Table 1:** The comparison of serum urea, creatinine, e-GFR, MCP-1, and TGF-β1 preoperative, after 1 month, 3 months, and 6 months of surgery between cases and controls group

| Variables         | Group | Pre-Op   | After 1 month | 3 months | 6 months | p-value (Pre-Op) | p-value (After 1 month) | p-value (3 months) | p-value (6 months) |
|-------------------|-------|----------|---------------|----------|----------|-----------------|------------------------|---------------------|--------------------|
| Serum urea (mg/dl) | Case  | 73.15    | 65.30         | 49.00    | 33.50    | <0.001          | <0.001                 | 0.001               | 0.024              |
|                   | Control | 28.50    | 28.50         | 28.50    | 28.50    | (24.25-31.75)   | (24.25-31.75)         | (24.25-31.75)       | (24.25-31.75)       |
| Serum creatinine (mg/dl) | Case  | 1.65     | 1.10          | 0.90     | 0.65     | <0.001          | <0.001                 | 0.001               | 0.023              |
|                   | Control | 0.45     | 0.45          | 0.45     | 0.45     | (0.37-60)       | (0.37-60)             | (0.37-60)           | (0.37-60)           |
| eGFR (ml/min/1.73m²) | Case  | 25.635   | 32.545        | 48.650   | 71.490   | <0.001          | <0.001                 | 0.001               | 0.472              |
|                   | Control | 72.55    | 72.55         | 72.55    | 72.55    | (57.71-107.63)  | (57.71-107.63)        | (57.71-107.63)      | (57.71-107.63)      |
| MCP-1 (pg/ml)     | Case  | 147.170  | 156.290       | 112.450  | 88.650   | <0.001          | <0.001                 | 0.004               | 0.002              |
|                   | Control | 6.250    | 6.250         | 6.250    | 6.250    | (0.000-22.950)  | (0.000-22.950)        | (0.000-22.950)      | (0.000-22.950)      |
| TGF-β1 (pg/ml)    | Case  | 197.85   | 150.10        | 144.000  | 73.650   | <0.001          | <0.001                 | 0.028               | 0.632              |
|                   | Control | 80.050   | 80.050        | 80.050   | 80.050   | (79.025-81.425) | (79.025-81.425)       | (79.025-81.425)     | (79.025-81.425)     |

Values are median (inter quartile range)
and straining at micturition in 17 patients of 30 patients, 13 patients had a history of repeated UTI.

On VCUG, trabeculation, contracted bladder, and posterior urethral dilatation were detected in 15 (75%) cases and VUR was detected in 25% cases. PVUR was significant in 16 cases (80%) preablation and persisted in only 35% of cases at 6 months’ postablation of valve.

Fifteen out of 20 cases (75%) had posterior: anterior urethra diameter ratio >2:1 which got significantly low (5%) after 6 months’ surgery. Babu et al.[9] assessed the value of the posterior urethral: anterior urethra ratio in predicting successful PUV ablation. Monitoring PVUR, urethral ratio followed by check cystoscopy if needed, is preeminent for good prognosis. Another consensus study by Sharma et al.[3] clearly states indicated check VCUG at 3 months’ postablation if symptoms persist, before labeling valve bladder or any other bladder sequelae.[3,10]

The comparison of serum urea, creatinine, estimated GFR (eGFR), MCP-1, and TGF-β1 preoperative, after 1 month, 3 months, and 6 months of surgery between cases and controls is shown in Table 1.

In PUV cases, the preoperative median serum creatinine level was 1.65 (1.22–2.42), after 1 month of ablation was 1.10 (1.10–1.92), after 3 months was 0.90 (0.65–1.80) and after 6 months was 0.65 (0.50–1.25), this showed the serum creatinine level was significantly decreasing from preoperative level to different follow-up intervals. Similar results in a study conducted by Menon et al.,[10] prefulguration mean serum creatinine level was 1.37, and postfulguration mean serum creatinine was 0.79 which was statistically significant. Serum creatinine showed a decreasing trend in our study as expected and highlighted in the past by many researchers as a useful prognostic indicator.[11,12]

In terms of calculated values, the preoperative median eGFR in PUV cases in our study was 25.635 (16.38–35.40) and in the control group
was 72.55 (57.71–107.63) which was statistically significant ($P < 0.001$). Significant difference was also found in 1st, 3rd, and 6th months after surgery ($P < 0.001$, $P = 0.022$, and $P = 0.472$). On comparing preoperative eGFR level with eGFR level at 1, 3, and 6 months after surgery in PUV cases, the preoperative median eGFR level was 25.6 (16.4–35.4), after 1 month was 32.5 (19.2–44.6), after 3 months was 48.6 (21.2–70.9), and after 6 months was 71.5 (45.4–96.9). This showed the median eGFR level was significantly increased at different follow-up intervals. Reason for preserved eGFR was probably compensatory hypertrophy and hyperfiltration of the remaining nephrons which signifies ongoing inflammatory process without change in eGFR as stated by Dagli and Ramchandani.[13]

In our study, the preoperative median MCP1 in PUV cases was 147.2 (82.8–512.5) and in the control group was 6.2 (0.0–22.9) which was statistically significant ($P < 0.001$). Significant difference was also found in 1st, 3rd, and 6th months after surgery ($P < 0.001$, $P = 0.004$, and $P = 0.002$). There was no statistically significant change in MCP1 level at preoperative to 1 month (156.3 [40.6–486.1]) and preoperative to 3 months (112.4 [9.0–265.8]) after surgery but significant decrease in MCP1 level was observed at preoperative to 6 months (88.6 [22.1–199.7]) after surgery ($P = 0.014$). Similar assay results were found in other studies having higher levels of MCP1 in pediatric obstructive uropathy and reflux nephropathy.[14–16]

In addition, Madsen et al.[17] and Bartoli et al.[18] used this biomarker with other biomarkers in ureteropelvic junction obstruction. A cohort study concluded that there is a significant increase of urinary MCP-1 in children with severe hydronephrosis.[19]

The pivotal role of the profibrotic cytokine TGF-B1 in the pathogenesis of progressive renal failure is proved by many studies.[20–23] On comparing preoperative TGF-B1 level with TGF-B1 level at 1st, 3rd, and 6th months after surgery in PUV cases, the preoperative median TGF-B1 level was 197.8 pg/ml (79.9–386.4), after 1 month was 150.1 pg/ml (102.9–286.1), after 3 months was 144.0 pg/ml (100.4–297.4), and after 6 months was 73.6 pg/ml (49.9–209.1); this showed that there was no statistically significant change in TGF-B1 level at preoperative to 1 month and preoperative to 3 months after surgery but at 6 months after surgery, the median TGF-B1 level significantly decreased as compared with preoperative TGF-B1 level. MacRae Dell et al.[8] used urinary TGF-B1 excretion in 14 children with PUV and reported that urinary TGF-B1 excretion was significantly greater in patients with PUV than in healthy controls. Similar result was also reported by Mandelia et al.[7] in 30 PUV patients than in healthy controls depicting gradual downregulation of RAS following removal of the bladder outlet obstruction.

The change in the level of MCP-1 and TGF-B1 is shown in Figures 1 and 2. On correlating eGFR, MCP-1, and TGF-B1 values after 6 months of follow-up in PUV cases, there was statistically significant correlation between all the above variables. The previous study[8] had concluded that there was no correlation between urinary TGF-B1 excretion and eGFR, past urinary diversion surgery, or bladder wall thickening in patients of PUV. It can be concluded from the above correlation that probably before valve ablation, the obstructive urinary tract had an ongoing independent inflammatory mechanism without any relation to kidney function. Moreover, following ablation having obstructive relief to urinary tract, both inflammatory markers and eGFR are changing as expected in a significant correlation at 6 months of follow-up. Thus, eGFR or serum creatinine cannot be flagged as an early prognostic marker as per our study.

Plotting receiver operating characteristic curve graph using software, the preoperative urinary TGF-B1 and MCP1 level of the patients and controls which gave the highest sensitivity and specificity was 81.20 pg/ml (sensitivity 75% and specificity 70%) and 32.50 pg/ml (sensitivity 95% and specificity 90%), respectively [Figure 3]. After 6 months of follow-up, urinary TGF-B1 and MCP1 level of the patients and controls which gave the highest sensitivity and specificity was 79.60 pg/ml (sensitivity 44.4% and specificity 40.0%) and 21.62 pg/ml (sensitivity 77.8% and specificity 80.0%), respectively [Figure 4]. MCP-1 stands out as a better predictor of both preoperative and short-term 6 months’ follow-up biomarker with 90% and 80% specificity and 95% and 77.8% sensitivity, respectively. Sharif-Afsar et al.[24] and Howard et al.[25] established the role of TGF-B1 in hypertrophy and matrix expression of human bladder smooth muscle cells explaining less specificity and sensitivity of TGF-B1 in predicting exclusively renal inflammatory parameters.

**Conclusion**

This study substantiates that there is ongoing renal damage in a subgroup of PUV patients even after valve ablation. Routine serum kidney function markers are deranged late in the pathologic process being unsuitable as prognostic biomarker. Thus, new diagnostic approaches and alternative therapies for pediatric end-stage renal diseases are clearly necessary. TGF-B1
and MCP-1 might contribute to quantify progressive renal insufficiency in PUV patients.

Limitation of study
Further long-term follow-up studies are indicated to determine if agents that affect TGF-β1 expression, such as angiotensin converting enzyme (ACE) inhibitors, can halt the progress of renal disease in PUV if used early in patients.

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Conflicts of interest
There are no conflicts of interest.

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