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# Can urinary biomarkers be used in the outcome assessment of pyeloplasty in children?

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## Abstract

**Background:** To compare the urinary biomarkers—beta 2-microglobulin ( $\beta$ 2M), monocyte chemotactic peptide-1 (MCP-1), and transforming growth factor-beta (TGF- $\beta$ 1)—in the outcome assessment of children with pelviureteric junction obstruction (PUJO) undergoing pyeloplasty.

**Methods:** A prospective study was conducted on children with PUJO who had pyeloplasty in a tertiary care center from July 2016 to March 2018. Urine samples were obtained from freshly voided urine samples before surgery and after 6 months of pyeloplasty. Ratio between the levels of biomarkers and urinary creatinine before and after surgery were compared.

**Results:** A total of 72 patients had pyeloplasty during this period. The mean levels of standardized urinary  $\beta$ 2M, MCP-1 and TGF- $\beta$ 1 before surgery were  $3.94 \pm 4.06$ ,  $96.63 \pm 117.68$  and  $310.65 \pm 423.87$ , respectively, which was significantly higher than the corresponding values in the postoperative period, obtained after 6 months of surgery; postoperative mean values were  $3.12 \pm 3.95$ ,  $25.28 \pm 32.06$ ,  $109.95 \pm 118.72$  ( $P < 0.001$ ), respectively. Using Wilcoxon signed-rank test, fall of MCP-1 and TGF- $\beta$ 1 was more significant compared to  $\beta$ 2M.

**Conclusion:** Urinary biomarkers ( $\beta$ 2M, MCP-1 and TGF- $\beta$ ) offer an effective way of outcome assessment of pyeloplasty for PUJO in children, especially MCP-1 and TGF- $\beta$ 1.

**Keywords:** Biomarkers, Beta 2-microglobulin, Monocyte Chemotactic Peptide, Transforming growth factor-beta, Pelviureteric junction obstruction, Hydronephrosis

## 1 Background

Pelviureteric junction obstruction (PUJO) is the most common cause of hydronephrosis in children [1]. PUJO refers to obstruction to flow of urine across the PUJ. Gold standard for postoperative assessment after pyeloplasty is diuretic renal scintigraphy (DRS). Although drainage curve pattern and split function improvement on DRS is in line with predicting successful surgery, it has certain

disadvantages. Requirement of intravenous line, long image acquisition time in children are often cumbersome, especially with infants. Equivocal drainage curve pattern during interpretation of result, adds to the difficulty of the treating surgeon in monitoring the affected kidney, in addition to increasing the anxiety of the parents.

A viable alternative in the form of urinary biomarkers has been suggested in the past decade, but none of these has yet been advocated in daily clinical practice due to their limitations and the need for further validation in clinical studies. It is unlikely that a single protein will meet the required criteria for a urinary biomarker in PUJO, and given the multifactorial nature of PUJO,

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including the unpredictable course of the condition, more than one marker evaluation may be required [2]. Urinary biomarkers reflect the normal physiological status and can be used to diagnose the disease earlier and in making a plan for treatment and monitoring [3]. Being of simple and non-invasive nature, urinary biomarkers merit further evaluation to obtain sufficient evidence to be used as a diagnostic tool for PUJO.

## 2 Methods

A prospective study was conducted in the Department of Pediatric Surgery at our Institution from July 2016 to March 2018 after approval from the Institutional Ethics Committee.

All study participants/parents provided written consent for inclusion in the study.

### 2.1 Inclusion criteria

All consecutive children up to 15 years of age, admitted for elective unilateral pyeloplasty after informed consent were included in the study.

### 2.2 Exclusion criteria

Bilateral PUJO, nephrotic syndrome, glomerulonephritis, diabetes mellitus, secondary PUJO due to stone disease, children with associated renal problems in addition to PUJO such as vesicoureteric reflux (VUR), posterior urethral valve (PUV), vesicoureteric junction obstruction (VUJO), and multi-cystic dysplastic kidney (MCDK) were excluded from the study.

Baseline investigations for surgery were done in all the children in addition to urinary creatinine, sonography and nuclear scanning with differential function. Ultrasound (abdomen and pelvis) and DRS were done not only in the preoperative period but also 6 months after surgery to see the status of the involved kidney. The level of urinary biomarkers ( $\beta$ 2-M, MCP-1, and TGF- $\beta$ 1) was obtained at admission before surgery and during outpatient follow-up, after 6 months of surgery.

Two groups of data were thus obtained one with the level of biomarkers before surgery and other after surgery. Collected data were statistically analyzed using appropriate statistical methods. Data for individual parameters were presented as mean values and standard deviation. Paired T test and Wilcoxon signed-rank tests were used for data analysis. All statistical analyses were carried out at 5% level of significance. All the data analyses were carried out in IBM SPSS 19 software.

### 2.3 Methods to estimate urinary biomarkers level

After obtaining a midstream clean catch of urine sample, it was collected and stored frozen at  $-20^{\circ}\text{C}$ , and just before starting the estimation procedure, the sample was

thawed by keeping in a container with water at room temperature.

Estimation of all three urinary biomarkers was done by using enzyme-linked immunosorbent assay (ELISA) kit based on the biotin double antibody sandwich technology. Levels were expressed in nanograms per liter for MCP-1 and TGF- $\beta$ 1 and milligram per deciliter for  $\beta$ 2-M. All specimens were diluted to obtain concentration for optimal density according to instructions; the enzymatic reaction was quantified in an automatic microplate photometer. Linear regression equation of the standard curve was calculated according to standard concentrations and the corresponding optical density (OD) values. Then according to the OD value of samples, concentrations of the corresponding sample were calculated. Use of the ratio of biomarker value to urinary creatinine (standardized value) was obtained to correct for the intraindividual coefficients of variation.

## 3 Results

A total of 72 patients underwent pyeloplasty for PUJO involving unilateral kidney with age varying from 1 to 156 months, males outnumbered females by a ratio of 4.53.

A significant number of patients (52/72) had antenatal detection of PUJO and only a small number (20/72) presented late in childhood, without antenatal diagnosis. In most of the patients who had antenatal detection, it was the antenatal sonogram done between 24 and 30 weeks which detected PUJO (71%); around 17% had antenatal detection done on a sonogram after 30 weeks while 12% antenatally diagnosed cases were detected before 24 weeks of gestation.

In this study, the majority of PUJO was seen on the left side (72.2%). Only 24/72 PUJO kidneys were palpable and most of the cases did not have a palpable lump (48/72). Most of the cases were asymptomatic with only chance detection on a sonogram. Symptomatic patients (15/72) presented with flank pain, distension, and urinary tract infection in different combinations.

The mean level of urinary  $\beta$ 2M/Ucr before surgery was higher ( $3.94 \pm 4.06$ ) than the same after surgery ( $3.12 \pm 3.95$ ). Similar decrease in urinary MCP-1/Ucr and TGF- $\beta$ 1/Ucr was observed from  $96.63 \pm 117.68$  and  $310.65 \pm 423.87$  preoperatively to  $25.28 \pm 32.06$ ,  $109.95 \pm 118.72$  postoperatively, respectively. All the above biomarker reductions were statistically significant ( $P < 0.001$ ) (Table 1).

Negative rank in the table suggests that in the postoperative urine sample, in how many number of patients level of a particular biomarker was found to decrease from its preoperative value and positive rank gives the number of patients in whom postoperative value was

**Table 1** Comparison of  $\beta$  2 M, MCP-1 & TGF-  $\beta$  1 before and after pyeloplasty

Parameters	Preoperative mean N = 72	Postoperative mean N = 72	Statistical significance
$\beta$ 2M/Ucr	3.94 $\pm$ 4.06	3.12 $\pm$ 3.95	$P = < 0.003^*$
MCP-1/Ucr	96.63 $\pm$ 117.68	25.29 $\pm$ 32.07	$P = < 0.001^*$
TGF- $\beta$ 1/Ucr	310.65 $\pm$ 423.87	109.96 $\pm$ 118.73	$P = < 0.001^*$

$\beta$ 2M beta 2-microglobulin, MCP-1 monocyte chemotactic peptide-1, TGF- $\beta$ 1 transforming growth factor-beta-1, Ucr urinary creatinine

\*Paired t test

**Table 2** Distribution of positive and negative ranks

	Negative rank	Positive rank	Ties	Total
$\beta$ 2M/Ucr	48	24	0	72
MCP-1/Ucr	58	14	0	72
TGF- $\beta$ 1/Ucr	57	15	0	72

$\beta$ 2M beta 2-microglobulin, MCP-1 monocyte chemotactic peptide-1, TGF- $\beta$ 1 transforming growth factor-beta-1, Ucr urinary creatinine

not found not to decrease in comparison to preoperative value. Here, among all three urinary biomarkers, MCP -1 level decreased in 58 patients out of 72 patients followed by TGF- $\beta$ 1 had decreased in 57 patients out of 72 patients and  $\beta$ 2M had decreased in 48 patients out of 72 patients. Therefore, among all three urinary biomarkers, MCP -1 had the highest number of patients with a decrease in the urinary level of the biomarker in the post-operative period (Table 2).

#### 4 Discussion

Considering the improvement in techniques of antenatal sonograms, there has been a change in trend toward finding more antenatally diagnosed PUJO cases in the outpatient clinic. This is now a normally observed phenomenon in most centers [4]. In our study, 72.22% of children were antenatally diagnosed, out of which 71.34% were diagnosed in 24–34 weeks of the antenatal scan. Several investigators have reported sonographic diagnosis at an earlier age of gestation (18–20 weeks). This may be both due to the late presentation of the pregnant mother and expertise of the sonography [4].

Most of the patients in this study were boys and the left side was more involved, comparable to the documented literature [5].

As is the case with most other series, the majority of patients in our study were asymptomatic, and others followed with symptoms like an abdominal lump, flank pain, or infection [6, 7].

TGF- $\beta$ 1 is a multifunctional cytokine that plays an important role in wound healing and it regulates the deposition of collagen through modulation of collagen production and degradation. [8]. It has been found that

in a majority of PUJ obstruction, excessive renal TGF- $\beta$ 1 production is responsible for the dysregulation of extracellular matrix production and the development of progressive renal fibrosis [9–12]. Isaka et al. suggested that interstitial fibrosis in unilateral ureteral obstruction could be blocked with TGF- $\beta$ 1 antisense oligodeoxynucleotides [13].

Seremetis and Maizels found that increased levels of renal tissue TGF- $\beta$ 1 in response to urinary tract obstruction were associated with an increase in the urinary level of TGF- $\beta$ 1 [9]. It was further shown that significant differences in renal pelvic TGF- $\beta$ 1 concentrations occurred in children with PUJO when compared to bladder urine [14, 15]. It was also found that bladder urinary TGF- $\beta$ 1 concentrations were significantly higher in children with PUJO compared to normal controls and those with dilated non-obstructed kidneys. Moreover, bladder urinary TGF- $\beta$ 1 concentrations were seen to decrease following corrective surgery [15, 16].

Taha et al. described an interesting trend in the urinary TGF- $\beta$ 1 level; this comprised of an initial increase in the level during the first month after surgery, followed by a persistent but gradual decrease thereafter [16]. Follow-up after pyeloplasty at 1 year showed a statistically significant decrease in urinary TGF- $\beta$ 1 level. The initial increase might be attributable to the requirement of this growth factor in the successful healing of the pelviureteric junction [17].

Our study clearly shows that mean levels of urinary TGF  $\beta$ -1 were significantly ( $P < 0.05$ ) higher in the patients before surgery in comparison to levels 6 months after pyeloplasty.

A study conducted by Madsen et al. confirmed increased concentrations of neutrophil gelatinase-associated lipocalin (NGAL) and  $\beta$ 2-M in urine from obstructed kidneys when compared with the contralateral kidneys and controls. They observed consistent fall in urinary  $\beta$ 2-M concentrations following surgery in their series of 24 patients [18].

Mean levels of urinary  $\beta$ 2 microglobulin were significantly ( $P < 0.05$ ) higher in our patients before surgery in

comparison to levels 6 months after pyeloplasty. However, around 66.66% of patients showed a decrease in urinary  $\beta 2$  microglobulin in the postoperative period after 6 months of pyeloplasty.

Taranta-Janusz et al. [19] showed increased levels of urinary MCP-1 in children who developed obstructed kidney before undergoing pyeloplasty. Patients from the study group 1 (surgically managed cases) revealed a significant difference in urinary MCP-1 levels in comparison with study group 2 (managed conservatively) and control groups ( $P < 0.05$ ). Grandaliano and colleagues analyzed both MCP-1 expression on renal biopsies and urinary MCP-1 concentrations in severe PUJO and found a fourfold higher urinary MCP-1 concentration in studied children than in healthy controls [20]. By demonstrating a significant decrease in urinary MCP-1 after Pyeloplasty, our study is in line with the studies made by Taranta-Janusz et al. and Grandaliano et al. In our study, around 80.55% showed a decrease in urinary MCP-1 in the postoperative period after 6 months of pyeloplasty. Almodhen et al. found a significant linear correlation between initial hydronephrosis grade and initial bladder urinary TGF- $\beta 1$  concentration [21].

Although several urinary biomarkers have been identified to be useful in PUJO, use of the number and type of biomarkers appear to be limited by several factors such as the cost, availability and feasibility of processing the biomarker kits [4, 18, 19].

## 5 Limitations of the study

As discussed above, use of other biomarkers was limited due to the availability of the kits and cost. Urine from the renal pelvis would be ideal but would make the study invasive. The periodic levels of urinary biomarkers after longer follow-up and also their levels in failed cases would further strengthen the evidence of using urinary biomarkers to diagnose and follow obstructed kidneys after a corrective operative procedure. A cost comparison of the nuclear scintigraphy and biomarker could have established the actual working cost, but was beyond the scope of the present study.

## 6 Conclusion

Utilization of select urinary biomarkers ( $\beta 2$ -M, MCP-1, and TGF- $\beta 1$ ), especially MCP-1 and TGF- $\beta 1$ , in children with PUJ obstruction can adequately identify postoperative relief of obstruction offering a simple, efficacious and non-invasive way of follow-up.

## 7 Key message

- The mean urinary biomarker levels in 72 patients were  $\beta 2\text{M/Ucr}$ — $3.94 \pm 4.06$ ,  $\text{MCP-1/Ucr}$ — $96.63 \pm 117.68$  and  $\text{TGF-}\beta 1/\text{Ucr}$ — $310.65 \pm 423.87$  before pyeloplasty and fell to  $\beta 2\text{M/Ucr}$ — $3.12 \pm 3.95$ ,  $\text{MCP-1/Ucr}$ — $25.28 \pm 32.06$ ,  $\text{TGF-}\beta 1/\text{Ucr}$ — $109.95 \pm 118.72$  postoperatively, reaching statistical significance.
- Urinary biomarkers MCP-1 and TGF- $\beta 1$  provide an accurate, non-invasive and reliable method of follow-up in pyeloplasty in children.

### Abbreviations

$\beta 2\text{M}$ : Beta 2-microglobulin; MCP-1: Monocyte chemotactic peptide-1; TGF- $\beta 1$ : Transforming growth factor-beta; PUJO: Pelviureteric junction obstruction; DRS: Diuretic renal scintigraphy; NGAL: Neutrophil gelatinase-associated lipocalin; UCr: Urine creatinine; VUR: Vesicoureteric reflux; PUV: Posterior urethral valve; VUJO: Vesicoureteric junction obstruction; MCDK: Multi-cystic dysplastic kidney; OD: Optical density; ELISA: Enzyme-linked immunosorbent assay.

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### Authors' contributions

RK prepared the manuscript and analyzed the data. KK designed the study, supervised the overall conduct of the study and obtained intramural funding for the study. MR provided the results of biomarker analysis. KS collected and analyzed data. BK collected and analyzed data. BJ collected and analyzed data. All authors read and approved the final manuscript.

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### Availability of data and materials

Data collected are available as master chart.

### Declarations

#### Ethics approval and consent to participate

The study was approved by the Institute Ethics committee, Jawaharlal Institute of Postgraduate Medical Education & Research with approval no JIP/IEC/2016/27/912. All study participants/parents provided written consent for inclusion in the study.

#### Consent for publication

Not applicable as data are anonymized.

#### Competing interests

Authors declare no competing interest in the study/manuscript.

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### References

1. Thomas D (1990) Fetal uropathy. *Br J Urol* 66:225–231

2. Madsen MG, Norregaard R, Frokiaer J, Jorgensen TM (2011) Urinary biomarkers in prenatally diagnosed unilateral hydronephrosis. *J Pediatr Urol* 7:105–112
3. Mesrobian HG, Mitchell ME, See WA, Halligan BD, Carlson BE, Greene AS et al (2010) Candidate urinary biomarker discovery in ureteropelvic junction obstruction: a proteomic approach. *J Urol* 184:709–714
4. Hashim H, Christopher RJ (2012) Ureteropelvic junction obstruction. *Eur Urol Suppl* 11:25–32
5. Lee H, Han SW (2009) Uretero-pelvic junction obstruction: what we know and what we don't know. *Korean J Urol* 50(5):423–431
6. Thomas DFM (2010) Prenatal diagnosis: what do we know of the long term outcomes? *J Pediatr Urol* 6:204–211
7. Chertin B, Fridmans A, Knizhnik M, Hadas-Halpern I, Hain D, Farkas A (1999) Does early detection of ureteropelvic junction obstruction improve surgical outcome in terms of renal function? *J Urol* 162(3 Pt 2):1037–1040
8. Basile DP (1999) The transforming growth factor beta system in kidney disease and repair: recent progress and future directions. *Curr Opin Nephrol Hypertens* 8:21
9. Seremetis GM, Maizels M (1996) TGF-beta mRNA expression in the renal pelvis after experimental and clinical ureteropelvic junction obstruction. *J Urol* 156:261–267
10. Yamamoto T, Noble NA, Miller DE, Border WA (1994) Sustained expression of TGF-beta 1 underlies development of progressive kidney fibrosis. *Kidney Int* 45:916
11. Chung KH, Chevalier RL (1996) Arrested development of the neonatal kidney following chronic ureteral obstruction. *J Urol* 155:1139–1147
12. Seseke F, Thelen P, Heuser M, Zoller G, Ringert RH (2001) Impaired nephrogenesis in rats with congenital obstructive uropathy. *J Urol* 165:289–301
13. Isaka Y, Tsujie M, Ando Y, Nakamura H, Kaneda Y, Imai E et al (2000) Transforming growth factor-beta 1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction. *Kidney Int* 58:1885–1890
14. Furness PD III, Maizels M, Han SW, Cohn RA, Cheng EY (1999) Elevated bladder urine concentration of transforming growth factor-beta 1 correlates with upper urinary tract obstruction in children. *J Urol* 162:1033–1038
15. El-Sherbiny MT, Mousa OM, Shokeir AA, Ghoneim MA (2002) Role of urinary transforming growth factor-beta 1 concentration in the diagnosis of upper urinary tract obstruction in children. *J Urol* 168:1798–1805
16. Taha MA, Shokeir AA, Osman HG, Abd El-Aziz-Ael A, Farahat SE (2007) Obstructed versus dilated nonobstructed kidneys in children with congenital ureteropelvic junction narrowing: role of urinary tubular enzymes. *J Urol* 178:640–646
17. Liatsikos EN, Dinlenc CZ, Bernardo NO, Kapoor R, Jabbour ME, Smith AD et al (2001) Endopyelotomy failure is associated with reduced urinary transforming growth factor- $\beta$ 1 levels in patients with upper urinary tract obstruction. *J Endourol* 15:567–570
18. Madsen M, Norregaard R, Palmfeldt J, Oslen L, Frokiaer J, Jorgensen T (2012) Urinary NGAL, cystatin C,  $\beta$ 2-microglobulin, and osteopontin significance in hydronephrotic children. *Pediatr Nephrol* 27:2099–2106
19. Taranta-Janusz K, Wasilewska A, Dębek W, Waszkiewicz-Stojda M (2012) Urinary cytokine profiles in unilateral congenital hydronephrosis. *Pediatr Nephrol* 27:2107–2113
20. Grandaliano G, Gesualdo L, Bartoli F, Ranieri E, Monno R, Leggio A (2000) MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. *Kidney Int* 58:182–192
21. Almodhen F, Loutochin O, Capolicchio JP, Jednak R, El-Sherbiny M (2009) The role of bladder urine transforming growth factor-beta 1 concentrations in diagnosis and management of unilateral prenatal hydronephrosis. *J Urol* 182:292–298

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