



Pyuria Associated Pyocolpos in the Neonates with Rare manifestations of Distal Vaginal Obstruction: Clinical, and Biomarkers Profiles in the Management Strategies

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ABSTRACT

Background: *The diagnosis of distal vaginal atresia is rare in the neonate unless the hydromucocolpos was developed. Pyuria and pyometocolpos may complicate hydromucocolpos, it may present with severe obstructive uropathy and septicemia.*

Aim: *to evaluate the role of combinations of clinical, microbial and immunological parameters in enhancing and to discuss the management strategies.*

Patients and Methods: *Twenty-eight patients presented to the pediatrics surgery ward with clinical signs of congenital distal vaginal obstruction were prospectively studied and compared with 20 healthy control group. The clinical records and radiological findings together with laboratory findings were reviewed. Urine sample collected for enzyme-linked immunosorbent assay to measure the urinary levels of interleukin IL-4, IL-6, IL-8 and IL-10 and for macroscopically examination and culture.*

Results: *There was a significant rising in the immunological biomarkers in all patients group as compared with control group regardless they have associated anomalies or not, and significant correlation between IL-6, IL-8 and TNF- α rising scores with patients already presented with obstructive uropathies.*

Conclusion: *Pyuria associated pyocolpos in congenital vaginal obstruction is a marker for underlining urinary tract abnormalities. This should rise management strategies with early picking up the pathology promoting optimal outcome.*

Keyword: *Pyuria, Pyocolpos. Obstructive uropathies, Pro-inflammatory cytokine.*

INTRODUCTION

These are spectrum of female reproductive tract anomalies present with varying degrees of Müllerian fusion defects and agenesis during embryogenesis leading to a broad spectrum of phenotypes. These anomalies are generally encountered in 2- 3% of female reproductive tract abnormalities^[1]. Various combinations have been described, the most common one is imperforate

hymen. Incidence of isolated vaginal agenesis is 1:5000 women^[2]. The diagnosis of distal vaginal atresia is rare in the neonate unless the hydromucocolpos was developed (HMC) as a result of obstruction. Rarely, in some cases, copious amounts of fluid may collect in the vagina proximal to the obstructing segment causing a mass effect with compression on the surrounding organs. Compression may cause and progress for

serious sequels if not promptly diagnosed and treated [3]. Hydromucocolpos may be diagnosed prenatally by sonography (U/S) during third-trimester. In these cases, an abdominal or pelvic mass can cause fetal abdominal distension [4]. Failure of the distal part of the vagina to develop (agenesis), transverse vaginal membrane, or an imperforate hymen are considered as the usual causes [5]. Usually these anomalies encountered in the spectrum of syndromes with associated others defect, but often remain undiagnosed for years in children with normal renal function if the radiological assessment (U/S or Intravenous urography IVU) is not performed [6-8]. The usual presentation of the neonates with distal vaginal atresia can include abdominal mass, findings of obstructive uropathy and recurrent urinary tract infections, sometimes for to late presentation with neonatal sepsis due to severe secondary infection. Pyocolpos is a result of an excess of secondary-infected cervical secretion secondary to a congenital atresia of the vaginal orifice. Children with pyometocolpos due to distal vaginal atresia may present with acute illness, with severe obstructive uropathy and septicemia [9]. However, there are very few reports of pyocolpos in children. Imaging assessments following pyuria demonstrated a high incidence of abnormalities in the renal tract, with obstructive uropathies in 1-4% [10]. Using its diversity and by qualified and sophisticated mechanisms, mucosal pathogens have own roles to access the tissues at their preferred site of infection [11]. Cytokines have a key role in the innate immunity and present the early biomarkers of the epithelial response to infection [12]. Interleukin-6 may cause fever triggering the acute-phase response, while IL-8 recruit inflammatory cells to the site of infection. Patients with urinary tract Infection (UTI) might carry high urine cytokine levels [13,14], in the murine UTI model the epithelial cells have been identified as early producers of cytokines [15]. The epithelial cytokine response of the human mucosa in situ has not been widely studied and evaluated. TNF- α behave as a pathogenic role in the

maintenance and induction of glomerular barrier dysfunction [16].

Objective: Evaluation the role of combinations of clinical, microbial and immunological parameters in enhancing and to discuss the management strategies, if this information will be useful in determining the optimal intervention, follow up, outcome and to roll out infection complication of these rare congenital anomalies.

PATIENTS AND METHODS

The sample of this study was conducted in the Pediatric surgery unit at The Maternity and Child Teaching Hospital, Al-Qadisiya, from the 1st of august 2014 to the end of July 2016, all patients at the age < 30 days and presented with abdominal mass, vaginal obstruction to the outpatient clinic or to the surgical ward were investigated prospectively. The clinical course with manifestations, laboratory findings and underlying genitourinary (GU) tract anomalies were reviewed. With aseptic work up, all patients had renal function tests, urinalysis, urine culture and sensitivity (any patient showing any number of white blood cells per high-power field on a centrifuged specimen of urine microscopy was included, urine samples were taken either by catheterization or sticking of a sterile bag according to age and 5 patients were obtained from nephrostomy specimens. After informed consent and on all eligible subjects, a detailed history and examination was conducted immediately after admission. Information about age, antenatal history, birth weight, clinical examination, laboratory findings and outcome were recorded on a designed questionnaire for this study. Plain radiograph, ultraasonography (US) and computerized scan (CTS) were performed in all patients after family consent based on legal acceptance, 20 neonates were enrolled in this study as a control group during their attendance for usual routine assessment, all considered healthy and none of them carrying associated anomalies, particularly urological anomalies according their initial evaluations. The diagnosis

of pyuria was picked up according to the signs and symptoms of UTI in the newborn, fever, screaming, dysuria, frequency and a bacterial colony of at least 10^5 organisms/ milliter in a midstream, clean voided specimen, 10^3 or more organisms/ milliter in a catheterized urine or any growth in urine specimen aspirated through a nephrostomy tube. Urine sample from all patients and control groups were inoculated into the MacConkey and blood agar for culture and sensitivity. The enzyme-linked immunosorbent assay was used to measure the levels of urinary interleukins including IL-4, IL-6, IL-8 and IL-10. All were measured by using a commercially available ELISA kits from (Cusabio. Germany; KOMABIOTECH INC. Korea). Urine specimens were centrifuged at $1,000 \times g$ for 10 min, and the supernatants were assayed directly or after dilution with the dilution buffer. All assays were done at least in duplicate. The detection limits of this assay were: IL-4 (1 pg/ mL), IL-6 (0.7pg/mL), IL-8 (3.5pg/mL), and IL-10 (3.9 pg/mL) respectively. By using commercially available ELISA kits from (Medgenix Diagnostics), urine TNF- α levels in normal (control) subjects and patients were measured, the detection limit was 10 pg/ml for the kit. Structural anatomy was determined by clinical examination, ultrasonography, and computerized tomography and reassessed for associated anomalies. Data were translated into a computerized database. Expert statistical advice was sought and the values are given as means and ranges, statistical analysis were done by using excel 2016 and SPSS version 22 software. Mann Whitney U test used to analyze the differences between healthy controls and patients.

RESULTS

Twenty-eight patients presented to the pediatrics surgery ward with clinical signs of congenital distal vaginal obstruction were prospectively studied. Their mean gestational age was 34-37 weeks. Prenatal, postnatal sonography with initial radiological assessment including intravenous

pyelogram (IVP). The study reported associated congenital urinary tract malformations in 20 of them, figure (1). These included bilateral hydronephrosis in 50% of anomalies, right ureter duplication (15%), left ureteropelvic junction stenosis (15%), bilateral multiple cystic dysplastic kidney (10%) and right ureterocele (10%), figure (2). Evidence of obstructive uropathies encountered in 19 of patients group, 10 of them developed sever bilateral hydronephrosis, figure (3). Twenty out of 28 patient had urinary tract infections (UTI), as evidenced by pyuria and positive urine bacterial cultures with a colony count greater than 10^5 /ml, figure (4). The remarkable causative agent was Escherichia coli in 50% of these cultures, and there was significant correlation between obstructive uropathy, anomalies and urine culture among patients with pyocolpos as OR=90 and (P value < 0.0005), table (1&2).

Significant rising in the immunological biomarkers in all patients group as compared with control group regardless they have associated anomalies or not, especially IL-6, (mean 125.3pg/ml), (SE 9.13) and (P value < 0.001), IL-8 (mean 119.98pg/ml), (SE 9.8) and (P value < 0.001) and TNF- α (mean 118 pg/ml), (SE 9.6) and (P value < 0.001), To have a signaling about the predictive value of the anti-inflammatory biomarkers that may reflect any risk of renal damage associated vaginal obstruction and obstructive uropathies, we recorded statistically significant role or changing in scores for IL-4 between patients and control, (P value < 0.001), with no significant correlation in IL-10 concentration between the two groups, table (3).

There was a significant elevations in symptom scores in the patients (with associated urological anomalies with culture proven urine) and IL-6 (mean143.69 pg/ml), (SE 12.7) and (P value < 0.001), IL-8 (mean133.22 pg/ml),(SE 11.86) and (P value < 0.001), and TNF- α (mean 128.44), (SE 11.42) and (P value < 0.001).But still there is a remarkable associated high level score in IL-6,IL-8,and TNF in patients with associated urological

anomalies with negative urine culture (These findings indicate that during urosepsis, the pro-inflammatory cytokine response is generated predominantly during this insult), table (4). Significant correlation between IL-6, IL-8 and TNF- α rising scores with patients already

presented with obstructive uropathies, (mean 146.06 pg/ml, 136.58 pg/ml and 137.95 pg/ml) respectively (P value < 0.001), and patients without obstructive uropathies, table (5).

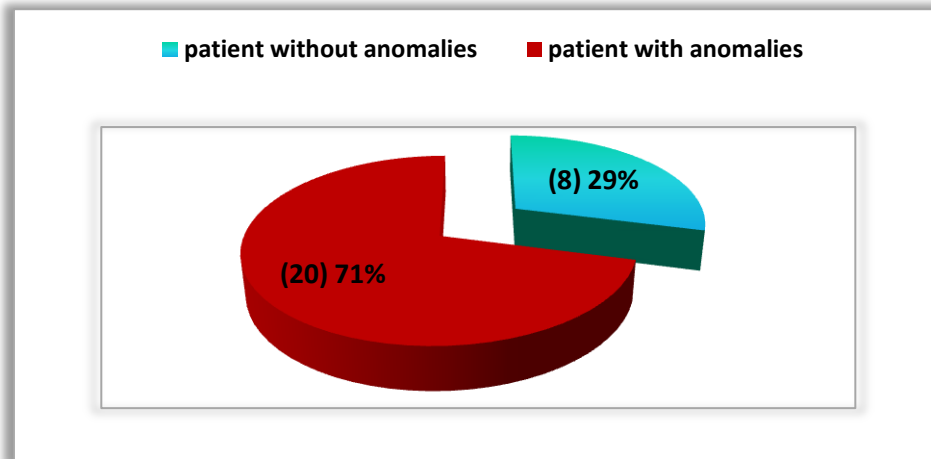


Figure (1): Pie chart show frequency of anomalies (congenital urinary tract malformations) among patients with vaginal atresia (P < 0.05).

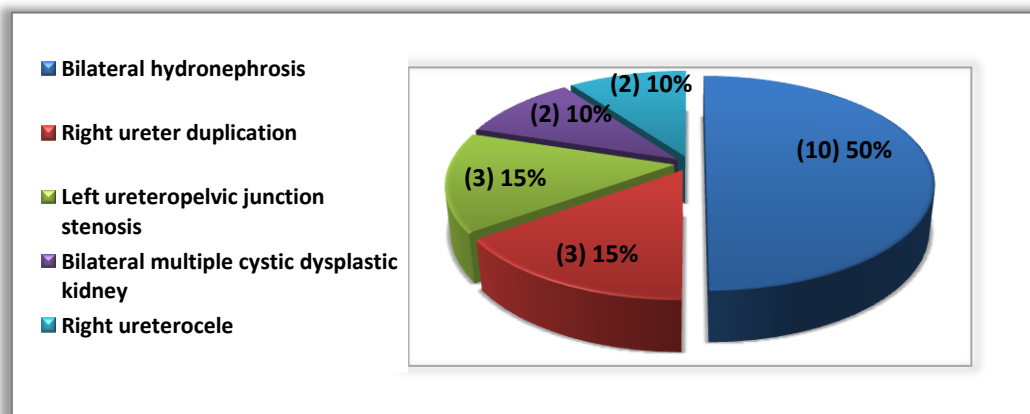


Figure (2): Pie Chart show distribution the types of congenital urinary tract malformations in patients with vaginal atresia.

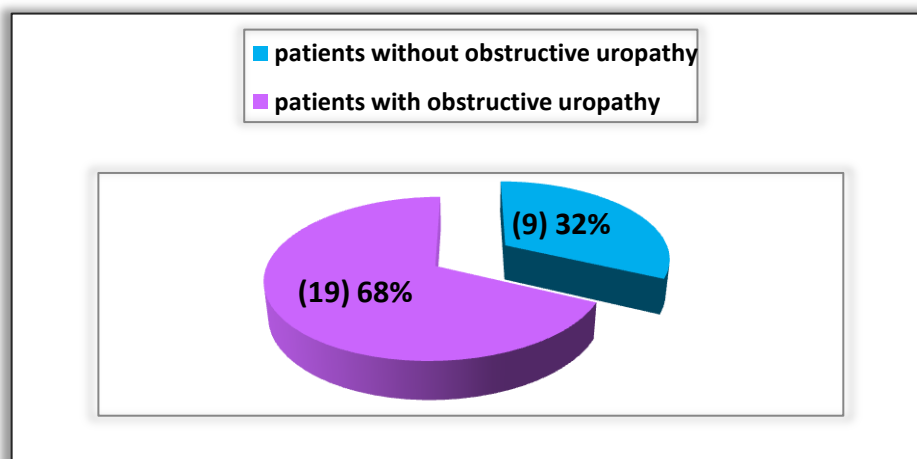


Figure (3): Pie Chart show frequency of obstructive uropathy among patients with pyocolpos (P < 0.05).

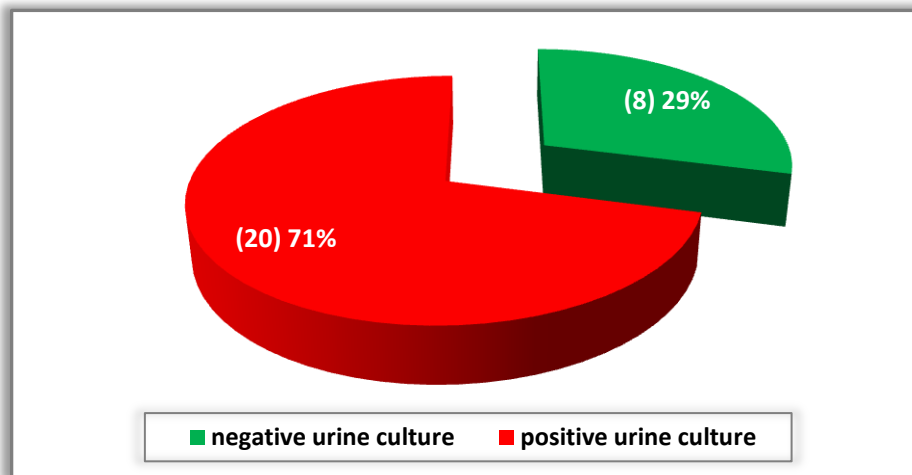


Figure (4): Pie chart show profile of urine culture among patients with pyocolpos (P< 0.05).

Table (1): Patients with pyocolpos correlated with each anomaly and urine culture.

Anomalies	Patients With anomalies		Urine culture	Patients with positive urine culture	
	N	%		N	%
Bilateral hydronephrosis	10	50	<i>Pseudomonas aeruginosa</i>	4	20
Right ureter duplication	3	15	<i>Klebsiella pneumoniae</i>	2	10
Left ureteropelvic junction stenosis	3	15	<i>Proteus mirabilis</i>	1	5
Bilateral multiple cystic dysplastic kidney	2	10	<i>Escherichia coli</i>	10	50
Right ureterocele	2	10	<i>staphylococcus</i>	3	15
Total	20	100	Total	20	100

Table (2): Correlation between obstructive uropathy, anomalies and urine culture among patients with pyocolpos.

	Patient with obstructive uropathy (N=19)	Patient without obstructive uropathy (N=9)	OR* (95%CI**)	P value
	N (%)	N (%)		
Anomalies	19 (100)	1 (11)	90 (7.197 - 1125.45)	0.0005
Positive urine culture	19 (100)	1(11)	90 (7.197 - 1125.45)	0.0005

*OR= Odds ratio. **CI= confidence interval.

Table (3): Compared Concentration of Immunological Markers between Cases and Controls.

Immunological markers	Case	Control	P value
TNF α pg/ml			< 0.001
Range	66.7-218.3	2.8-13.7	
Mean	118	8.6	
SD*	51.03	2.96	
SE**	9.6	0.66	
N***	28	20	
IL-4 pg/ml			< 0.001
Range	14.9-63.5	0.7-13.7	
Mean	39.42	7.21	
SD	42.7	3.73	
SE	8.7	0.83	
N	28	20	
IL-6 pg/ml			
range	60.7-220.7	0.7-12.3	
mean	125.03	6.545	
SD	48.3	3.64	
SE	9.13	0.81	
N	28	20	
IL-8 pg/ml			< 0.001
Range	66.4-232.8	2.8-18.5	
Mean	119.98	9.365	
SD	51.8	4.27	
SE	9.8	0.95	
N	28	20	
IL-10 pg/ml			NS****
Range	11.5-25.1	0.7-14.6	
Mean	16.59	7.485	
SD	4.06	3.86	
SE	0.77	0.863	
N	28	20	

*SD= standard deviation. **SE= standard error. *** N= Number. ****NS=non-significant.

Table (4): Compared concentration of some immunological markers between positive urine culture and negative urine culture of cases with urological anomalies.

Immunological markers	Positive urine culture	Negative urine culture	P value
TNF α pg/ml			< 0.001
Range	67.5-218.3	66.7-197.3	
Mean	128.44	91.93	
SD*	51.07	43.33	
SE**	11.42	15.32	
N***	20	8	
IL-4 pg/ml			NS****
Range	14.9-63.5	23.5-50.4	
Mean	42.42	31.93	
SD	15.64	8.6	
SE	3.479	3.04	
N	20	8	
IL-6 pg/ml			< 0.001
Range	66.7-220.6	60.7-94.1	
Mean	143.69	78.39	
SD	56.8	10.87	
SE	12.7	3.843	
N	20	8	
IL-8 pg/ml			< 0.001
Range	69.3-232.8	66.4-140.9	
Mean	133.22	86.875	
SD	53.03	22.90	
SE	11.86	8.096	
N	20	8	
IL-10 pg/ml			NS
Range	11.5-25.1	12.5-23.2	
Mean	15.89	18.338	
SD	3.87	4.256	
SE	0.865	1.505	
N	20	8	

*SD= standard deviation. **SE= standard error. *** N= Number. ****NS=non-significant.

Table (5): Compared concentration of some immunological markers between patients with and without obstructive uropathy.

Immunological markers	Patient with obstructive uropathy	Patient without obstructive uropathy	P value
TNF α pg/ml			< 0.001
Range	70.7-218.3	66.7-87.1	
Mean	137.95	75.91	
SD*	50.71	8.17	
SE**	11.63	2.72	
N***	19	9	
IL-4 pg/ml			NS****
Range	14.9-63.5	23.5-50.4	
Mean	43.19	31.456	
SD	15.68	8.16	
SE	3.597	2.72	
N	19	9	
IL-6 pg/ml			< 0.001
Range	66.7-220.6	60.7-98.6	
Mean	146.06	80.63	
SD	57.33	12.19	
SE	13.15	4.06	
N	19	9	
IL-8 pg/ml			< 0.001
Range	70.2-232.8	69.3-140.9	
Mean	136.58	84.922	
SD	52.25	22.21	
SE	11.99	7.40	
N	19	9	
IL-10 pg/ml			NS
Range	11.5-25.1	12.5-25.1	
Mean	16.06	17.7	
SD	3.89	4.41	
SE	0.892	1.47	
N	19	9	

*SD= standard deviation. **SE= standard error. *** N= Number. ****NS=non-significant

DISCUSSION

large series of patients with congenital uterovaginal atresia and obstruction were reported [17]. The diagnosis can be made during or between the perinatal period and adolescence. The commonest presentations were related to genitourinary obstruction (54.4%) followed equally by abdominal pain and pelviabdominal

mass (45.5% for each) [18]. A fundamental component of the workup is usually approached by the physical examination but often was not adequate to establish a definitive diagnosis [19]. Pyocolpos usually presented as a mass in the lower abdomen; however, in rare cases, it was reported with infected urinary tract, acute gastrointestinal obstruction, and urinary retention [20,21].

A high incidence of urological anomalies, e.g. vesicoureteric reflux (VUR) in about 30-50% and obstructive uropathies in about 1-4% were reported in 43% of infants and children with symptomatic UTI followed by Imaging assessment ^[22,23]. In our series 19 cases with obstructive uropathies due to sever urethral outlet compression and bilateral VUR. Regardless that the distal vaginal atresia studied here carrying these series of uropathies, again many literates announced risk of UTI or urosepsis associated anomalies e.g. In Williams' series ^[24], the UTI incidence with pelviureteric junction (PUJ) obstruction was 45.8% and Bauer et al ^[25] demonstrated that the UTI is the most common presentation in children with bladder diverticula. In our series *Escherichia coli* presented as a causative agent in more than 50% of our culture proven urine in patients group. Despite there was a little dispute that *E. coli* is the most common pathogen found in pediatric age group, many epidemiological studies reported that Uropathogenic *Escherichia coli* (UPEC) is the most common etiological agent responsible for uncomplicated UTI ^[26]. Recently the serum and urine levels of pro-inflammatory cytokines became important biomarkers in the assessment of urological disorders ^[27]. Focusing on our results the high inflammatory biomarkers correlating with anomalies or without, with obstructive uropathies or without, proved the localizations of renal system were stained in favor of evidence for biomarkers particularly the role of IL-6, IL-8 and TNF- α in such insults and pathology. Many articles highlighted e.g. the usefulness of IL-6 as an early marker for renal pathology and even anomalies, proven its implications and differences in the staining between the both disorders with and without pathology of the epithelium in the urinary tract ^[28,29]. This reflect the enrollment of IL-6 high scores in our patient's series due to the anatomical defect (obstruction and compression) made the epithelial cells of renal system (bladder and kidney) producing IL-6 as a response to external stimuli ^[30]. It is also a product of

inflammatory cells such as mononuclear phagocytes, mast cells and lymphocytes. On the other hand, IL-6 expressed in the cell of the smooth muscle, epithelial cell, endothelium and fibroblasts of the normal bladder, as well. Due to its widespread cellular sources (an inducer of activation and differentiation of B and T cells during inflammation) ^[31]. The highest scoring IL-6, IL-8 and TNF- α in sever obstructive uropathies and bilateral hydronephrosis in our patients group reflected the sever renal damage might happen by these anomalies with particular and clear correlation with the excretion of IL-8 (neutrophil-activation protein excreted from the renal tubules due to acute inflammatory insults) proven its role with such higher initial urine levels. many researchers reported such evidence in children with acute pyelonephritis and the significant of cytokines markers in renal disorder despite no significant differentiate between upper or lower urinary pathology or infection ^[32,33]. In our study the similar score might indicate the early renal insult and flare up the pictures of inflammation regardless presence of infection or not. Galanakis et al, hypothesized same pathway correlating the IL-8 and renal pathology ^[34]. With the same pattern TNF- α recorded itself with such high scores in our patients with obstructive, associated anomalies and culture proven urine, indicating that such pathology may potentiate the production of pro-inflammatory biomarker TNF- α by monocytes in human upon stimulation of different types of cells, including renal fibroblasts which is able to produce IL-8 in response to TNF- α ^[35]. Despite the known sequel and complication of UTI, our critical anomalies with such critical age group should emphasized empirical surveillance and strict follow up to pick up the early sign of renal scaring reflected by these pro-inflammatory cytokines in the urine. Other researchers declared the correlation of elevated urinary biomarkers IL-6, IL-8 and TNF- α and renal parenchymal damage rather than VUR and they can early predict this pathology ^[36-39]. Our records with significant finding and the correlation of IL-4 (which

considered as anti-inflammatory interleukin that exerts immunosuppressive effects on macrophages and suppresses pro-inflammatory cytokine production) between the patients and control group may reflect the fact of its contribution in pathological changes in glomerular level by stimulating collagen synthesis in human mesangial cells^[40]. Many hypotheses pointed to the enrollment of IL-10 (a cytokine with anti-inflammatory and immunomodulatory functions, regulates the biology of B and T cells) in the induction of cellular and molecular pathways and their link to the progression of kidney damage^[41]. Our results with such low score in patients group (slightly more than control one) may hypothesized identical report by Madsen, he submitted significantly lower levels of IL-10 in renal parenchyma and urine of animals with acute unilateral obstruction, while renal levels of IL-1 β , IL-6, and TNF- α were increased to sham-operated animals (normal operated as a control)^[42]. Some studies may deny such associations and their conclusions were controversial, possibly due to the small sample sizes^[43]. Our results went beside many studies dealing with the pivotal role and the balance between pro- (IL-6, IL-8 and TNF- α) and anti-inflammatory cytokines (IL-4 and IL-10) which determines the inflammatory response and may be mediate the progression of renal disease^[44-46]. Our small size study for both patients and control groups made some limitations. Although we think that we have submitted a promising results with such sample studied in our local community, we agreed much more remain need to be investigated and evaluated dealing with such rare congenital anomalies.

Conclusion: Focusing in our results with such critical anomalies carrying serious sequel especially due to the associated urological anomalies, accordingly we should highlight the strict assessment and follow up for those patient proposing algorithm including non-invasive clinical and para clinical methods of investigation with complete imaging work up. Pyuria associated pycolpos in congenital vaginal obstruction is a

marker for underlining urinary tract abnormalities. This should rise management strategies with early picking up the pathology promoting optimal outcome.

COMPETING INTERESTS

The author declares that they have no competing interests.

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