

Detection of Extended - Spectrum Beta - Lactamases in *Escherichia coli* Isolates and it's Correlation with Vesicoureteral Reflux Nephropathy

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Abstract

Background: Vesicourethral reflux is the reverse flow of urine from the bladder to the ureter and then to kidneys. 30% to 40% of children are infected with urinary tract infections disease, and recently, one of the most common urinary tract infections has become Extended - Spectrum Beta - Lactamases (ESBL) *Escherichia coli* isolates.

Objectives: The aim of this was to investigate the correlation between extended Extended Spectrum Beta Lactamase *Escherichia coli* isolates and reflux nephropathy disease in children.

Methods: This was a case - cotrol study conducted on 200 children with or without reflux nephropathy referred to the pediatric clinic. The researchers selected two groups of children; 100 children with reflux nephropathy and 100 healthy children. Urinary tract infection was tested by urine culture and antibiogram. Correlation of reflux nephropathy and ESBL *Escherichia coli* infection were analyzed by the SPSS program.

Results: Infected children with Extended Spectrum Beta Lactamase *E. coli* infection compared with healthy children, were more influenced by reflux nephropathy. Overall, 53% of case and 9% of control group had infection of ESBL *Escherichia coli*.

Conclusions: Reflux nephropathy can be controlled by prevention of these types of infections.

Keywords: Reflux Nephropathy, Extended Spectrum Beta Lactamase, *E. coli*, Children

1. Background

Vesicoureteral reflux refers to reverse flow of the urine from the bladder to the ureter and then to kidneys (1, 2). In a normal position, the ureteral attachment to the bladder is oblique, and creates a flap - valve mechanism and prevents reflux (3-5). However, reflux occurs when the sub mucosal tunnel between the mucosa and detrusor muscle is short or absent; in other words VUR mostly results from congenital inefficiency of the Ureterovesical (UV) junction (1, 2).

Reflux predisposes the person to infection of the kidneys, by facilitating the transport of bacteria from the bladder and lower urinary tract to the kidneys and upper urinary tract (6). When infection ascend the kidneys, pyelonephritis and inflammatory reaction could occur that result in kidney injury and scarring, which is also called reflux nephropathy or reflux - related renal injury. In addition, reflux nephropathy is a great global public health problem, the incidence of which in the world is steadily increasing amongst children (7, 8). Although

febrile UTI at any age leads to pyelonephritis and then renal scarring, yet risk of UTI is highest in children younger than two years of age (9-11). Patients with febrile urinary tract infection and VUR were three times more likely to develop kidney injury compared to those without reflux (1, 7).

Urinary tract infection is one of the most common childhood infections that may be associated with vesicoureteral reflux (12). The infection may be symptomatic or asymptomatic; asymptomatic is colonization without any symptoms while symptomatic infection is concurrent with microbial colonization symptoms (3, 13). Infection prevalence is greater in children and females (7, 14). In addition, gram - negative bacilli are the most common infections that lead to urinary tract infections; *E. coli* has caused 80% of these infections, and ESBL - positive lead to more mortality and morbidity in children (15). Therefore, reflux accompanied with urinary tract infection lead to increased risk of renal injury and scarring (16).

2. Objectives

Urinary Tract Infections, as a high prevalence disorder, especially among females, can cause nephropathy. The aim of this study was to investigate children with kidney reflux nephropathy and its correlation with infection of ESBL *E. coli* bacteria in Arak city.

3. Methods

3.1. Inclusion and Exclusion Criteria

Inclusion criteria were absence of congenital kidney disease and consent to participate in the research project, age of one month to 12 years old, male and female gender. The exclusion criteria were presence of congenital renal abnormality, incomplete study process, and patient's lack of satisfaction with using their personal data in the study.

3.2. Study Setting

This was a case control and hospital - based study, which was conducted in Amir Kabir Hospital of Arak city.

3.3. Study Population

Samples was taken based on other studies, and the researchers considered 200 children for study conduction and patients were followed for 12 months (4). From 200 children, 100 children with reflux nephropathy (grade IV and V of VUR) as the case group, and 100 healthy children, without any disease, as the control group (control group selected from outpatients that were not affected by severe or systemic disease) were considered.

3.4. Measurements

At first, Voiding Cystourethrogram (VCUG) was performed on all children of case and control groups, while dimercaptosuccinic acid (DMSA) scan was performed on patients with positive VUR diagnosis, four to six months after acute UTI. Infection of ESBL *E. coli* bacteria was detected by urine culture, with standard loop in hospital admission, and resistance to various antibiotics was determined based on anti - biogram test, Disk Agar Diffusion approach, and patients were treated with usual antibiotics (Ceftriaxone at a dose of 50 to 75 mg/kg/day/iv in hospitalized patients and cefixime 8 mg/kg/day/po in outpatients). Moreover, demographic characteristics of patients including age, gender, education of parents, place of residence and maternal age in pregnancy was reviewed by interviews of doctors with patients.

3.5. Ethical Considerations

Ethical issues (including plagiarism, data fabrication, and double publication) were completely observed by the authors. In addition, the ethical committee of Arak University of Medical Sciences approved the study protocol with the ethical code of IR.ARAKMU.REC.1394.344.

3.6. Statistical Analysis

Data analysis was conducted by the chi-square test and significance level was considered as $P < 0.05$. The correlation of VUR nephropathy and infection of ESBL *E. coli* was evaluated.

4. Results

Table 1 shows the demographic information, and based on this demographic information, there were statistically significant differences regarding age, gender, parents education, living area, and maternal age of pregnancy ($P = 0.001$). In addition, as shown in Table 2, ESBL *E. coli* infection in the case group was significantly higher than the control group ($P = 0.001$).

Table 1. Comparison of Demographic Information in Case (n: 100) and Control (n: 100) Groups

Variables ^a	Case	Control	P Value
Age ^b	12 ± 3	48 ± 2	0.0001
Gender			0.0001
Male	13	7	
Female	87	93	
Parents education			0.0001
Diploma and lower	4	20	
Bachelor	89	67	
Master's degree and higher	7	13	
Living place			0.0001
Urban	68	91	
Rural	32	9	
Pregnancy maternal age^c			0.0001
< 20	15	10	
21 - 30	15	81	
31 - 40	61	4	
> 41	9	5	

^aPresented as percentage

^bPresented as mean ± SD and month

^cYears old

In Table 3 resistance to various antibiotics in VUR nephropathy was determined, and it was observed that

Table 2. ESBL *E. coli* Infection Information's in Case (n: 100) and Control (n: 100) Groups

ESBL <i>E. coli</i> Infection ^a	Case	Control	P Value
Positive	53	9	0.0001
Negative	47	91	

^aPresented as percentage

there was resistance to multiple types of antibiotics so that the greatest resistance was for cefixime, ampicillin and cephalothin (100%) and ofloxacin (29%) and nitrofurantoin resistance was lowest.

Table 3. Antibiotics Resistance in VUR Nephropathy Children

Antibiotics	Resistance Percentage
Cefotaxime	98
Cefixime	100
Ceftriaxone	99
Gentamicin	73
Amikacin	69
Ampicillin	100
Cotrimoxazole	89
Amoxiclav	69
Nitrofurantoin	39
Ciprofloxacin	41
Ofloxacin	29
Cephalothin	100
Nalidixic acid	57
Ceftazidime	95

5. Discussion

Infection can influence VUR and lead to increased risk of nephropathy. Furthermore, ESBL *E. coli* bacteria, as an important microorganism, has multiple drug resistance. In the current study, the correlation of ESBL *E. coli* infection and reflux nephropathy was proved, yet this correlation was not investigated in numerous other studies.

Sweeney et al. in a study about reflux nephropathy in children with UTIs, evaluated 81 male and 46 female infants. They concluded that incidence of VUR remained lower than cases with urinary tract infection (17). Chih et al., in a study about nephropathy in infants with urinary tract infection, evaluated 152 young infants (127 males and 25 females) and concluded that *E. coli* was the most common pathogen in urinary tract infection and had high ampicillin resistance, and leukocytosis indicated the risk

of VUR while fever indicated a risk of pyelonephritis (18). Koshesh et al., in their study, evaluated ESBL *E. coli* infections and they found that these isolates were higher in communities rather than inpatients (19). Rahman et al. in a case - control study reported male gender and high grade reflux as risk factors of nephropathy among children with UTI; also, the current study demonstrated that reflux was seen as a risk factor for urinary tract infections (7). In another study, Ramalingam et al. found that voiding dysfunction was considerably higher in females compared to males and voiding dysfunction was higher among those with first episode of UTI (3). Mishra et al. concluded that UTI in geriatric patients could result in significant complications; they found UTI in 28.5% of geriatric patients and *E. coli* was the most frequent pathogen (16). Yousefichaijan et al. showed a certain association between hyponatremia and reflux nephropathy (20). Ranjbar et al., in their study, evaluated molecular epidemiology of *Klebsiella pneumoniae* and found that blaCTX in *K. pneumoniae* isolates was at an alerting rate (21). In another study on risk factors of infantile reflux expressed in subgroup analysis by CKD subtype, low birth weight and maternal pregestational DM were considerable along with an increased risk of renal dysplasia/aplasia while low birth weight, maternal gestational DM, and maternal overweight/obesity were significantly associated with obstructive uropathy (22). Fallah et al. in their study found that Enteropathogenic *Escherichia coli* was a major cause of diarrhea in Iranian children (23). However, patients with congenital disorders were not included in the current study.

Furthermore, in a study by Yousefichaijan et al. in 2014, they concluded that there is an obvious solidarity between reflux nephropathy and mean platelet volume (24). Also, in another study by the same authors, it was concluded that percentage of sacral ratio abnormality in children with VUR was more than children without this (10). Therefore, it could be suggested that it is essential to identify and treat voiding dysfunction in children with UTI and the current study confirms these findings. The limitation of the current study was the small sample size in the two groups and lack of cooperation by some patients and their parents. Accordingly, further studies, especially prospective ones with greater number of patients, will be needed in the future to evaluate infection of ESBL *E. coli* in children with vesicoureteral reflux nephropathy.

5.1. Conclusion

Correlation between ESBL *E. coli* infection and VUR (grade IV and V) nephropathy was approved. In addition, the importance of drug resistance of UTI in VUR nephropathy has been approved. The best antibiotic for this infection was ofloxacin and nitrofurantoin. Therefore, early di-

agnosis and treatment of UTI, especially of ESBL *E. coli* infection can prevent serious complications and lead to healthy outcomes.

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