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Interaction between *Escherichia coli* P fimbrae Gene and Host Innate Immune Response in Urinary Tract Infection of Children

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

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List of Abbreviations

Abbr.	Full-term
AAP	: American Academy of Pediatric0073
ABU	: Asymptomatic bacteriuria
APN	: Acute pyelonephritis
AFA	: Afimbrial adhesion
Bp	: Base pair
CAUTIs	: Catheter-associated urinary tract infections
cDNA	: Complementary DNA
CFU	: Colony forming unit
CNF1	: cytotoxic necrotizing factor 1
CPS	: Canadian Paediatric Society
CRP	: C- reactive protein
CT	: Threshold Cycle
DMSA	: Technetium- 99m–dimercaptosuccinic acid
DNA	: Deoxyribonucleic acid
EAU	: European Association of Urology
ESPU	: European Society for Paediatric Urology
ESR	: Erythrocyte sedimentation rate
GAPDH	: Glyceraldehyde 3-phosphate dehydrogenase
HBSS	: Hanks Balanced Salt Solution
HIV	: Human immune-defficiency virus
HLYA	: Hemolysin A

HPMNLs	: Human polymorphonuclear leukocytes
IBCs	: Intracellular bacterial communities
IL-6	: Interleukin-6
IL-8	: Interleukin-8
LPS	: Lipopolysaccharide
MFI	: Mean fluorescence intensity
MR	: Methyl red
PAIs	: Pathogenicity-associated islands
PAMPs	: Pathogen-associated molecular patterns
PAP	: Pyelonephritis associated pili
PBMCs	: peripheral blood mononuclear cells
PBS	: Phosphate buffered saline
PCR	: Polymerase Chain Reaction
PMNs	: Polymorphonuclear neutrophils
PRRs	: Pattern recognition receptors
QPCR	: Quantitative Polymerase Chain Reaction
RAPN	: Recurrent acute pyelonphritis
RBCs	: Red blood cells
RCS	: Renal cortical scintigraphy
RNA	: Ribonucleic Acid
RPM (rpm)	: Revolutions per minute
RT- PCR	: Reverse transcription Polymerase Chain Reaction
RUTI	: Recurrent urinary tract infection
SD	: Standard deviation
SFA	: Sialic acid-fimbrial adhesin

SIM	: Sulphide-indole-mobility
SPA	: Suprapubic aspiration
TAE	: Tris acetate EDTA buffer
TLR4	: Toll-like receptor 4
UPEC	: Uropathogenic <i>E. coli</i>
UV	: Ultraviolet radiation
VF s	: Virulence factors
VUR	: Vesicoureteric reflux
WBC s	: White blood cell

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Abstract

Thanaa Saleh Mohammed Zaki

Interaction between *Escherichia coli* p fimbriae gene and host innate immune response in urinary tract infection

Context:

Uropathogenic *E. coli* is the pathogen mostly associated with urinary tract infections. Disease severity determined by the balance between the pathogen and the innate immunity of the host, producing either acute pyelonephritis or asymptomatic bacteriuria.

Aim:

To investigate the impact of *Escherichia coli* p-fimbriae gene and the host chemokine receptor CXCR1 on disease severity in APN and ABU patients and to assess the impact of CXCR1 gene expression on recurrence risk of APN.

Settings and design

50 children with APN (mean age 5.98 ± 2.54 years) and 50 children with ABU; as controls (mean age 6.98 ± 2.98 years).

Methods:

Detection of *E. coli* pap gene in *E. coli* isolates using conventional PCR, and quantification of CXCR1 gene expression using RT PCR.

Statistical analyses:

Independent-samples t-test, Chi-square (χ^2) test and Pearson's correlation coefficient (r) test.

Results:

pap gene was detected in 80% of the studied APN patients compared to only 16% of the ABU patients ($p < 0.05$). Comparing positive and negative pap APN patients regarding gender, number of recurrences, pus cells, RBCs, total leucocytes, CRP, bacteriuria, duration of fever, admission days and disease related renal scars, did not reveal any significant differences. CXCR1 gene expression was significantly ($p < 0.001$) lower in APN patients (0.464 ± 0.226) compared to their age matched ABU controls, (1.000 ± 0.428). There were no significant correlations between $2^{-\Delta\Delta CT}$ (CXCR1) and neither age, admission days or duration of fever.

Conclusion

Low CXCR1 gene expression may be a predisposing factor to APN as well as to increased risk of recurrence.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in humans (**Bischoff *et al.*, 2018**) and a common cause of morbidity and mortality which impairs the quality of life for large numbers of individual (**Ambite *et al.*, 2021**). Among the bacterial species involved in UTIs, uropathogenic *Escherichia coli* strains (UPEC) are the most common, accounting for about 80% of uncomplicated UTIs, 95% of community-acquired infections, and 50% of hospital-acquired infections (**Tabasi *et al.*, 2016**). UPEC also remains the most frequent pathogen in complicated UTIs (**Bartoletti *et al.*, 2016**).

Disease severity is determined by the balance between the pathogen and the host (**Ragnarsdottir *et al.*, 2008**). After invasion of the sterile urinary tract, *Escherichia coli* may either establish a state of commensalism or cause severe, symptomatic disease. The virulent strains break the inertia of the mucosal barrier and may cause acute pyelonephritis, characterized by a rapid innate host response with cytokine secretion, and rapid recruitment of immune cells to the infection site, which may lead to successful elimination of bacteria or to disease and tissue damage (**Svanborg *et al.*, 2001**). On the other hand, asymptomatic bacteriuria (ABU) occurs, in at least 1% of the population and *E. coli* ($> 10^5$ cfu mL⁻¹) may persist for months or even years without evoking a destructive mucosal host response.

Studies of innate immunity have identified genetic defects that increase the susceptibility to both symptomatic and asymptomatic UTI in patients (**Ragnarsdottir et al., 2008**). The innate immune response is initiated by activating Toll-like receptors (TLRs) (**Olson and Hunstad, 2016**). TLRs determine the efficiency of pathogen recognition while chemokine receptors, like CXCR1, are critical for bacterial clearance (**Ragnarsdottir et al., 2008**). CXCR1, the receptor for IL-8, is essential for the migration and activation of neutrophils (**Olson and Hunstad, 2016**).

The UTI severity also reflects the properties of virulence factors of the infecting UPEC strain. The ABU strains, in contrast, often fail to express those virulence factors, and hence, it has been suggested that many ABU strains are attenuated UPEC strains (**Ragnarsdottir et al., 2008**). UPEC strains possess a number of virulence factors that contribute to their ability to overcome different defense mechanisms and help to cause disease. These virulence factors that are located in virulence genes include fimbriae (which help bacterial adherence and invasion), iron-acquisition systems (that allow bacterial survival in the iron-limited environment of the urinary tract), flagella and toxins (which promote bacterial spread). The most important of them is the fimbriae, encoded by the *pap* gene, also known as pyelonephritis-associated pili (**Dadi et al., 2020**).