STUDY OF SYSTEMIC IMMUNITY AND BACTERIOLOGICAL PROFILE OF SUBJECTS HAVING URINARY TRACT INFECTION IN INDIAN SUBJECTS

Dr. Shalini Gupta,[1] Dr. Basavaraj Savadi,[2] Dr Amit Rangari,[3] Dr Amresh Kumar [4*]

[1]MBBS, MD, Assistant Professor, Department of Microbiology, Venkateshwara Institute of Medical Sciences, Gajraula,Uttar Pradesh

[2]MBBS, MD, Professor and Head, Department of Biochemistry, Basaveshwara Medical College and Hospital, Chitradurga, Karnataka

[3]MBBS, MD, Professor and Head, Department of Microbiology, Nandkumar Singh Chouhan Government Medical College, Khandwa, Madhya Pradesh

[4*]MBBS, MD, Assistant Professor, Department Biochemistry, Darbhanga Medical College and Hospital, Darbhanga, Bihar

Corresponding author
Dr Amresh Kumar
Email id: dramreshmch@gmail.com

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ABSTRACT

Background: UTIs (urinary tract infections) are the most frequent bacterial disorder requiring medical management with a reported incidence of 8 million where nearly 20% need an emergency visit.

Aim: The present study was done to study the systemic immunity and bacteriological profile of subjects having urinary tract infections in Indian subjects. The study focused on isolating the uropathogenic bacteria, assessing their antibiotic sensitivity, and evaluating the serum TLR2 levels in subjects with UTIs and healthy controls.

Methods: In 200 blood and mid-stream urine study samples and 100 healthy samples of urine and blood as controls were subjected to TLR-2 detection. The samples from subjects with urinary tract infections were assessed for the presence of Uropathogenic bacteria (both gram-positive and gram-negative).

Results: The most common isolated bacteria were E. coli seen in 80% (n=160) study subjects followed by enterococcus faecalis in 50% (n=100) study subjects, Klebsiella pneumonia, enterococcus facium, and pseudomonas aeruginosa in 40% (n=80) subjects each, staphylococcus aureus in 36% (n=72) subjects. The mean TLR 2 level was significantly higher for cases subjects with urinary tract infections with a mean value of 3.875±1.495 compared to the control healthy subjects without urinary tract infections where the mean serum TLR2 value was 3.316±4.731. This difference was statistically significant with p=0.04
Conclusion: The present study concludes that subjects with active urinary tract infection commonly show more gram-negative bacteria than gram-positive bacteria with a

predominance of E. coli, Klebsiella pneumonia, and staphylococcus spp. Also, toll-like receptor 2 is significantly higher in subjects with urinary tract infections compared to their control subjects.

Keywords: Antibiotics, bacteria, TLR-2, toll-like receptor 2, urinary tract infection

INTRODUCTION

UTIs (urinary tract infections) are the most frequent bacterial disorder requiring medical management with a reported incidence of 8 million where nearly 20% need an emergency visit. As per 2006-2009 data in the United States, nearly 10 million subjects took treatment for UTIs as an emergency measure.[1] Most common pathogens causing UTI include Gram-negative bacteria staphylococcus, Enterobacter, Serratia, coagulase-negative enteric bacteria, S. epidermis, staphylococcus saprophyticus, streptococci, pseudomonas, proteus, klebsiella, E. coli, S. epidermidis, S. saprophyticus, and other coagulase-negative staphylococci.[2]

UTO prevalence differs with antibiotic use, hospitalization, catheterization, gender, and/or age. Bacteria are the most common etiologic factors for UTIs, however, parasites, fungi, and viruses also have a role in causing UTIs. Nearly 90% of the UTIs are caused by gram-negative bacteria and the remaining 10% by gram-positive bacteria. Previous literature data suggest that E. coli alone accounts for 65%-90% of all UTIs.[3] Other commonly associated uropathogens are coagulase-negative staphylococcus, pseudomonas aeruginosa, Citrobacter species, Klebsiella pneumoniae, and Enterococcus species. Clinical symptoms and laboratory test helps in diagnosing UTIs. Owing to the routine use of antibiotic medicine, most UTI pathogens are becoming MDR (multi-drug resistant). Also, increasing drug resistance is one of the most common causes of treatment failure. In treating common infections, antimicrobial drug-resistant has significantly increased making antibiotics such as quinolones ineffective against E. coli which is the most common etiology for UTIs globally including in the united states and India. It is seen that many subjects managed with carbapenem antibiotics are ineffective despite it being the last-line antibiotic for treating high resistant gram-negative microorganisms.[4]

Toll-like receptors (TLRs) are part of the natural innate immune system and PRRs (pattern recognition receptors) are the receptors identifying the patterns. TLRs seen can be TLR1, TLR2, TLR4, T, and B cells (TLR7, TLR9, and TLR10), natural killer cells, immature dendritic cells, and macrophages. These TLRs are involved in TLR activation of mesangial cells, epithelial kidney cells, and enterocytes. TLRs use heterophilic and homophilic interactions to assess molecular patterns and allow the protection of mucosal barriers by proinflammatory cytokines and immune cells.[5] 12 TLRs have been identified in humans. TLR binds to several ligands (both synthetic and natural). TLR4 binds to LPS (lipopolysaccharides), oligodeoxynucleotides, synthetic lipopeptides, heat shock proteins, extracellular matrix components, TLR3 to DNA motifs or viral RNA, and TLR2 to peptidoglycan. TLRs after binding activates nuclear factor beta (NF-B) making cells secrete TNF-like cytokines. In UTIs, both host defense and pathogen identification are done by TLRs.[6]

TLRs are necessary for bacterial virulence factor defense in subjects with UTIs. For recognition of specific ligands, human TLR 4 is the first PRR. LBP (lipid-binding protein) carries lipopolysaccharides to mCD14 (membrane-bound CD14) that distributes it to a

receptor complex named TLR4-MD-2. The function of TLR is based on MD2. However, TLR function is seen even in the absence of MD-2 and CD14 in some situations.[7] The present study was done to study the systemic immunity and bacteriological profile of subjects having urinary tract infections in Indian subjects. The study focused on isolating the uropathogenic bacteria, assessing their antibiotic sensitivity, and evaluating the serum TLR2 levels in subjects with UTIs and healthy controls.

MATERIALS AND METHODS

The present clinical study was done to study the systemic immunity and bacteriological profile of subjects having urinary tract infections in Indian subjects. The study focused on isolating the uropathogenic bacteria, assessing their antibiotic sensitivity, and evaluating the serum TLR2 levels in subjects with UTIs and healthy controls. The study population was comprised of subjects from the Department of Medicine of the Institute.

The study included two hundred blood and mid-stream urine samples along with 100 age and gender-matched controls. After the final inclusion of the study participants, detailed history was taken for all the subjects followed by a clinical examination. The included study participants were in the age range of 18 years to 50 years and the mean age of 29.6±6.42 years.

From the collected urine and blood samples, to identify the bacteria and make a diagnosis causing the urinary tract infections, the subjects with positive urine culture were further tested. The collected urine samples were inoculated into Muller-Hinton agar plate, blood agar base, and MacConkey agar. To identify the unknown microorganisms, Simmons citrate agar based on citrate utilization was used which is especially used for identifying the Enterobacteriaceae spp.

After collection, all the agar placed were at a temperature of 37^{o}C for nearly 18-24 hours in the aerobic environment. This was followed by cultivation with enough bacterial population. Also, bacteria were isolated and sub-cultured on the selective plates having a selective medium. The cultures were then assessed biochemically and under the microscope. Different approaches were used for bacteria identification including the study of growth features of bacteria as growth pattern, texture, and color. Gram staining was also used for bacterial identification.

In serum, TLR-2 levels were also assessed using the sandwich ELISA test which was calculated using the standard curve. This test was performed using the manufacturer's instructions.

The collected data were statistically analyzed using the SPSS (Statistical Package for Social Science) software version 22.0; Chicago, IL, USA, and ANOVA, chi-square, and t-test. The significance level was considered at the p-value of >0.05.

RESULTS

The study assessed two hundred blood and mid-stream urine samples along with 100 age and gender-matched controls. In the urine culture of the study subjects, there were 52% (n=104) gram-negative microorganisms and 48% (n=96) gram-positive. The most common isolated bacteria was E.coli seen in 80% (n=160) study subjects followed by enterococcus faecalis in 50% (n=100) study subjects, Klebsiella pneumonia, enterococcus facium, and pseudomonas

aeruginosa in 40% (n=80) subjects each, staphylococcus aureus in 36% (n=72) subjects, proteus mirabilis in 20% (n=40) study subjects, staphylococcus epidermidis in 14% (n=28) study subjects, morganella morganii in 10% (n=20) study subjects, and Enterobacter cloacae and streptococcus agalactiae in 2% (n=4) subjects as shown in Table 1.

For the antibiotic susceptibility testing in the present study, the diffusion disc method was used for the isolated bacteria to the commonly used antibiotics for the treatment of urinary tract infections. The bacteria isolated from the study subjects showed high sensitivity to tobramycin and amikacin, whereas, high resistance was seen for other antibiotics like trimethoprim. A large number of bacteria isolated from the study subjects were found to be resistant to antibiotics such as gentamycin, trimethoprim, and erythromycin. For assessing the antibiotic sensitivity to E. coli in the study participants, they were seen resistant to gentamycin, amikacin, and meropenem. The sensitivity to Proteus mirabilis was seen to ciprofloxacin, gentamycin, amikacin, and gentamycin.

On assessing the mean serum TLR2 values in cases and control subjects of the present study, it was seen that the mean TLR 2 level was significantly higher for cases subjects with urinary tract infections with a mean value of 3.875±1.495 compared to the control healthy subjects without urinary tract infection where the mean serum TLR2 value was 3.316±4.731. This difference was statistically significant with p=0.04 as depicted in Table 2.

The study included subjects within the age range of 18 to 50 years. On assessing the effect of age on the TLR2 levels in the study subjects, it was seen that for the age group of 18-28 years, mean TLR2 levels were higher for cases with 3.951±3.752 compared to control subjects, where TLR2 level was 1.558±0.875. This difference was statistically non-significant with p=0.24. Similar results were seen for the age range of 29-39 years where mean serum TLR2 levels were higher for cases with 6.029±6.641 compared to the control subjects where levels of TLR2 were 3.138±2.068. This difference was also statistically non-significant with p=0.29 as shown in Table 3.

DISCUSSION

The present clinical study was done to study the systemic immunity and bacteriological profile of subjects having urinary tract infections in Indian subjects. The study focused on isolating the uropathogenic bacteria, assessing their antibiotic sensitivity, and evaluating the serum TLR2 levels in subjects with UTIs and healthy controls. The study assessed two hundred blood and mid-stream urine samples along with 100 age and gender-matched controls. In the urine culture of the study subjects, there were 52% (n=104) gram-negative microorganisms and 48% (n=96) gram-positive. The study found more prevalence of gram-negative bacilli in subjects with urinary tract infections compared to gram-positive microorganisms. These results were consistent with the studies of Ibrahim I et al[8] in 2014 and Kothari A[9] in 2008 where authors reported that gram-negative bacilli predominate in the urine culture of the subjects with UTIs.

The most common isolated bacteria was E.coli seen in 80% (n=160) study subjects followed by enterococcus faecalis in 50% (n=100) study subjects, Klebsiella pneumonia, enterococcus facium, and pseudomonas aeruginosa in 40% (n=80) subjects each, staphylococcus aureus in 36% (n=72) subjects, proteus mirabilis in 20% (n=40) study subjects, staphylococcus epidermidis in 14% (n=28) study subjects, morganella morganii in 10% (n=20) study

subjects, and Enterobacter cloacae and streptococcus agalactiae in 2% (n=4) subjects. These findings were similar to the studies of Karananou P et al[10] in 2016 and Nurullaev RB[11] in 2004 where authors reported that the most common bacteria isolated in UTI include E. coli, Klebsiella pneumonia, and staphylococcus as also reported by the results of the present study.

Concerning the antibiotic susceptibility testing in the present study, the diffusion disc method was used for the isolated bacteria to the commonly used antibiotics for the treatment of urinary tract infections. The bacteria isolated from the study subjects showed high sensitivity to tobramycin and amikacin, whereas, high resistance was seen for other antibiotics like trimethoprim. A large number of bacteria isolated from the study subjects were found to be resistant to antibiotics such as gentamycin, trimethoprim, and erythromycin. For assessing the antibiotic sensitivity to E. coli in the study participants, they were seen resistant to gentamycin, amikacin, and meropenem. The sensitivity to Proteus mirabilis was seen to ciprofloxacin, gentamycin, amikacin, and gentamycin. The resistance to various antibiotics was also reported by the studies of Ghajiri HA et al[12] in 2020 and Karananou P et al[11] in 2016 to meropenem, gentamycin, and amikacin.

For assessing the mean serum TLR2 values in cases and control subjects of the present study, it was seen that the mean TLR 2 level was significantly higher for cases subjects with urinary tract infections with a mean value of 3.875±1.495 compared to the control healthy subjects without urinary tract infection where the mean serum TLR2 value was 3.316±4.731. These results were in agreement with the previous studies of Fischer H et al[13] in 2006 and Zanoni I[14] in 2013 where authors reported similar mean serum TLR 2 values in their study population. This difference was statistically significant with p=0.04. The TLR 2 identify and interact with various structures of gram-positive bacteria including mycobacteria possessed lipoproteins, mycoplasmas, lipoteichoic acid, peptidoglycan, and lipopeptides.

On assessing the effect of age on the TLR2 levels in the study subjects, it was seen that for the age group of 18-28 years, mean TLR2 levels were higher for cases with 3.951±3.752 compared to control subjects, where TLR2 level was 1.558±0.875. This difference was statistically non-significant with p=0.24. Similar results were seen for the age range of 29-39 years where mean serum TLR2 levels were higher for cases with 6.029±6.641 compared to the control subjects where levels of TLR2 were 3.138±2.068. This difference was also statistically non-significant with p=0.29. The previous study by Shimzu T et al[15] in 2004 and MacFaddin[16] in 2000 where authors reported no effect of age on the TLR2 levels in subjects with UTI.

CONCLUSION

Considering its limitations, the present study concludes that subjects with active urinary tract infection commonly show more gram-negative bacteria than gram-positive bacteria with a predominance of E. coli, Klebsiella pneumonia, and staphylococcus spp. Also, toll-like receptor 2 is significantly higher in subjects with urinary tract infections compared to their control subjects. The study had a few limitations of geographic area biases, a smaller study population, and short monitoring time.

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TABLES

Microorganism	Percentage (%)	Number (n)
Proteus Mirabilis	20	40
Pseudomonas aeruginosa	40	80
Morganella morganii	10	20
Enterobacter cloacae	2	4
Streptococcus agalactiae	2	4
Enterococcus facium	40	80
Enterococcus faecalis	50	100
Staphylococcus saprophyticus		
Staphylococcus epidermidis	14	28
Klebsiella pneumonia	40	80
Staphylococcus aureus	36	72
E. coli	80	160

Table 1: Bacteria isolated from the study subjects

TLR-2	Mean± S. D	p-value
Cases	3.875±1.495	**0.04**
Controls	3.316±4.731	

Table 2: Mean serum TLR-2 levels in controls and UTI study subjects

Age range (years)	Mean TLR-2 levels		p-value
	Cases	Controls	
18-28	3.951±3.752	1.558±0.875	0.24
29-39	6.029±6.641	3.138±2.068	0.29

Table 2: Age group effect on mean TLR-2 levels in cases and controls