BACTERIAL ADHERENCE AND HUMORAL IMMUNE RESPONSE IN WOMEN WITH SYMPTOMATIC AND ASYMPTOMATIC URINARY TRACT INFECTION

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Abstract

Purpose: To determine the role of humoral immune response and bacterial adherence in the pathogenesis of symptomatic and asymptomatic urinary tract infection in women. Methods: The study population consisted of 30 women with symptomatic UTI, 30 women with asymptomatic UTI and 30 healthy women as controls. Bacterial adherence to vaginal epithelial cells was studied and the concentration of serum and urine antibodies to mixed coliform antigen and clinical isolate was determined by ELISA. Surface hydrophobicity of the urine isolates was determined. Student’s unpaired t test and Pearson’s correlation coefficient test were used in the statistical analysis. Results: Compared to asymptomatic UTI, significantly more number of bacteria adhered to the epithelial cells of women with symptomatic UTI (P<0.001). All cases of UTI had significantly high concentration of urinary IgG antibody to mixed coliform antigens. Asymptomatic UTI cases had higher concentrations of urinary IgG, IgM and IgA antibodies to clinical isolate. Concentration of sIgA level was more in symptomatic UTI. Significant correlation was observed between urinary IgG and adherence of clinical isolate in case of asymptomatic UTI. Conclusions: The present study showed that greater receptivity of epithelial cells to bacteria may increase the susceptibility to UTI. Humoral immune response and local immunity may modify the pathogenesis of UTI.

Key words: Urinary tract infection, bacterial adherence, bacterial hydrophobicity, humoral immune response

Materials and Methods

Subjects

The study population consisted of 90 women belonging to Greater Mangalore. The subjects were further divided into different subgroups, each comprising of 30 individuals. Sample selection was done by random sampling method. The control and study groups were matched with respect to age, parity, history of prior UTI and gynaecological history. Informed consent was obtained from each human subject included in the study. The study was carried out in the Department of Microbiology, Kasturba Medical College, Mangalore, India.

Study Groups

Group 1 (control) consisted of 30 healthy women. Their age varied from 20-36 years (median age was 26 years). Group 2 consisted of 30 women with symptomatic UTI and yielded bacterial count of $\geq 10^5$ cfu/mL of urine. The age varied from 21-43 years (median age was 28 years). Group 3 consisted of 30 women with asymptomatic UTI. The individuals who did not show symptoms of UTI but whose urine culture yielded the growth of the same organism in count $>10^3$ cfu/mL in two consecutive samples were considered to have asymptomatic UTI. Their age varied from 18-40 years (median age was 24 years).

Bacterial isolation from urine

Clean catch, midstream urine was collected in a sterile
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Microscopic examination was performed using Gram smear and wet mount of the centrifuged deposit. Two milliliters of urine were used for the assay of urinary antibodies. Semi-quantitative calibrated loop technique was used to culture urine on blood agar and McConkey's agar. The inoculated plates were incubated at 37°C for 24 hours. Significant isolates (colony count >10^5 cfu/mL) were identified by standard procedures. Antibiotic sensitivity test was done by Kirby Bauer disk diffusion method using the following antibiotics: ampicillin (10 mg), cefotaxime (30 mg), chloramphenicol (30 mg), co-trimoxazole (30 mg), gentamicin (10 mg), nalidixic acid (30 mg), norfloxacin (10 mg), tetracycline (30 mg) and tobramycin (10 mg).

*Escherichia coli* strains were serotyped at the National Salmonella and Escherichia Centre, CRI, Kasauli, India.

**Bacterial adherence**

Vaginal epithelial cells collected from patients and controls were used to study their receptivity to bacteria by the bacterial adherence assay. Adherence of the clinical isolate to the vaginal epithelial cells of patients and the adherence of *E.coli* to the vaginal epithelial cells of patients and controls was studied.

**Assay of antibodies in serum and urine**

Blood (5 mL) was collected on the tenth day of infection for the estimation of antibody. Antibodies against the clinical isolate and mixed coliform antigen were determined in urine and serum. The mixed coliform antigen was prepared using *E.coli* serogroups O2, O25, O51, O75, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Proteus mirabilis*. The reagents for ELISA were procured from Sigma, USA and ELISA was performed according to the standard method. SIgA was detected by ELISA using antihuman secretory component (Sigma, USA) conjugated to alkaline phosphatase (Sigma USA).

**Determination of surface hydrophobicity of the isolates**

Surface hydrophobicity of bacterial isolates from symptomatic and asymptomatic UTI was determined by the hydrocarbon binding method.

**Statistical analysis**

Statistical analysis of the results was done using SPSS/PC, version 6.0. The data were analysed by Student’s unpaired t test and Pearson’s correlation coefficient test, wherever appropriate. The power of the test was 85% (0.85).

**Results**

The predominant organism isolated from the urine samples of women with symptomatic and asymptomatic UTI was *E.coli*. The urine from all the 30 (100%) patients with asymptomatic UTI showed the growth of *E.coli* whereas 25 (83.3%) urine samples from patients with symptomatic UTI had *E.coli* oh6, 2 (6.7%) had *K. pneumoniae*, 2 (6.7%) had *Enterobacter cloacae* and one (3.33%) showed the growth of *Proteus mirabilis*.

All cases of UTI had significantly high level of urinary IgG antibody to mixed coliform antigen (Table 1). The concentration of IgA was significantly high in symptomatic UTI, whereas s IgA level was significantly high in cases of asymptomatic UTI.

Compared to symptomatic UTI, asymptomatic UTI cases had higher concentrations of IgG, IgM and IgA antibodies to clinical isolate. Serum IgA level was more in symptomatic UTI. Level of serum antibodies to mixed coliform antigen and antigen of clinical isolate are shown in (Table 2). The concentration of IgG, IgM and IgA antibodies to mixed coliform antigen was significantly more in serum of patients.

The concentration of IgG and IgM antibodies was higher in the serum of cases of asymptomatic UTI whereas IgA was more in symptomatic UTI.

Significantly more number of bacteria adhered to the epithelial cells of women with symptomatic UTI compared to asymptomatic UTI (Table 3).

The surface hydrophobicity of all significant bacterial isolates was determined and the mean hydrophobicity index

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Urinary antibodies to mixed coliform antigen and clinical isolate in different study groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td>MCA</td>
</tr>
<tr>
<td>Control</td>
<td>0.36±0.27</td>
</tr>
<tr>
<td>Symptomatic UTI</td>
<td>1.57±1.01</td>
</tr>
<tr>
<td>Asymptomatic UTI</td>
<td>1.30±0.77</td>
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</tbody>
</table>

* P < 0.05 † P < 0.01 ‡ P < 0.001 ND: not done; CMA: mixed coliform antigen; CIA: clinical isolate antigen.

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was 9.58±9.48 in case of symptomatic UTI while it was 8.96±4.06 in women with asymptomatic UTI. There was no significant correlation between serum antibodies and adherence of clinical isolate to epithelial cells in symptomatic as well as asymptomatic UTI (P>0.05). Similarly, there was no significant correlation between urinary IgM, IgA and sIgA as well as adherence of clinical isolate in symptomatic and asymptomatic UTI (P>0.05). However, significant correlation occurred between urinary IgG and adherence of clinical isolate in case of asymptomatic UTI.

There was no correlation (P>0.05) between cell surface hydrophobicity and adherence of bacteria in both the study groups which is consistent with our previous report.21

Discussion

The predominant organism isolated from urine of women with different types of UTI was E.coli and this is comparable to the results of previous reports.21-23 The faecal flora serves as the source of E.coli which colonizes the vaginal introitus and urethra prior to infection of the bladder.24,25

We could demonstrate serum and urinary antibodies to the infecting organism and an independent pool of mixed coliforms. In the present study, there was significantly high level of urinary and serum IgG antibodies to mixed coliform antigen in asymptomatic UTI and symptomatic UTI when compared to controls, which is consistent with the results of our previous study.21 Urinary IgG and IgM antibody to the clinical isolate was significantly lower in cases of symptomatic UTI compared to asymptomatic UTI. The basis for the apparent inability of patients to produce IgG antibodies against E.coli is not clear. It is possible that UTI- susceptible women respond poorly to specific E.coli antigens because of a genetically determined restriction in antigen presentation or absence of gene coding for a specific antibody combining site.26

Antigens on mucosal surfaces can induce a state of tolerance in the host.27 Thus, alimentary or introital colonization with E.coli or repeated UTI may suppress host capacity to produce antibodies against bacterial antigens. Development of symptomatic UTI may partly be due to the poor production of IgG and IgM.

The present study showed an increase in urinary sIgA to the clinical isolate in symptomatic UTI. SlgA provides local immunity by preventing adherence of pathogens on to the epithelial cells.28 There was increased adherence of bacteria to the epithelial cells of women with symptomatic UTI. Similar observations were also made by previous workers.29-31 The absence of correlation between cell surface hydrophobicity and adherence of bacteria indicate that many microbial and host factors contribute to the pathogenesis of UTI. Greater receptivity of epithelial cells to bacteria may increase the susceptibility to UTI. The concept of bacterial adherence as the initial event of ascending UTI has allowed a new area of excitement in urologic research. Further studies are required to understand the effect of SlgA on bacterial adherence.

Table 2: Serum antibodies to mixed coliform antigen and clinical isolate in different study groups

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Serum antibodies (OD_{405} ± S.D)</th>
<th>Serum antibodies to MCA</th>
<th>Serum antibodies to CIA</th>
<th>Serum antibodies to MCA</th>
<th>Serum antibodies to CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.622±0.61</td>
<td>ND</td>
<td>0.253±0.23</td>
<td>ND</td>
<td>0.107±0.03</td>
</tr>
<tr>
<td>Symptomatic UTI</td>
<td>2.051±0.51*</td>
<td>1.48±1.0</td>
<td>0.809±0.46*</td>
<td>0.692±0.37</td>
<td>0.260±0.30*</td>
</tr>
<tr>
<td>Asymptomatic UTI</td>
<td>2.439±0.25*</td>
<td>2.115±0.64</td>
<td>0.824±0.35*</td>
<td>0.904±0.54</td>
<td>0.286±0.22*</td>
</tr>
</tbody>
</table>

*P<0.001. ND: not done; MCA: mixed coliform antigen; CIA: clinical isolate antigen.

Table 3: Adherence of clinical bacterial isolate and E. coli O6 to epithelial cells

<table>
<thead>
<tr>
<th>Source of epithelial cells</th>
<th>Number (Mean±SD) of clinical isolate adhering to epithelial cells</th>
<th>Number (Mean±SD) of E. coli O6 adhering to epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ND</td>
<td>3.595±2.56</td>
</tr>
<tr>
<td>Symptomatic UTI</td>
<td>26.22±7.80*</td>
<td>19.365±7.64*</td>
</tr>
<tr>
<td>Asymptomatic UTI</td>
<td>14.9±6.15</td>
<td>8.1±4.60*</td>
</tr>
</tbody>
</table>

*P<0.001. ND: not done.

References


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