ADHERENCE OF TYPE 1 FIMBRIATED ESCHERICHIA COLI TO UROEPITHELIAL CELLS OF WOMEN WITH DIABETES MELLITUS

Rita N. Rammo                  Alice K. Melconian

Biotechnology Department, College of Science, Baghdad University

ABSTRACT

Adherence of type 1 fimbriated Echerichia coli to uroepithelial cells of diabetic and non-diabetic women as well as the inhibition of adherence by the presence of anti-type 1 fimbriae at different dilutions have been studied. One hundred thirty nine isolates were collected from different clinical sources. Ninety isolates were characterized as E. coli by morphological, microscopic and biochemical tests. Sixty one % of the E. coli isolates were identified as being type 1 fimbriated, mannose sensitive hemagglutination (MSHA) and the rest (39%) as being non-type 1 fimbriated, mannose resistant hemagglutination (MRHA). The number of type 1 fimbriated E. coli adhered to uroepithelial cells isolated from diabetic women (41.04 ± 2.43) was about twice that of control (16.48 ± 2.3). Partially purified type 1 fimbriated E. coli was mixed with Freund's adjuvant (CFA and IFA) and subsequently injected subcutaneously into rabbits via three injections in 2 weeks intervals. Anti-type 1 fimbriae (antibodies ) that had been raised in rabbit blood serum were pre-incubated with type 1 fimbrated E. coli. Inhibition in adherence ranged between 62 to 72 % depending on the dilution of antisera. Moreover, it has been found that low dilution of antisera causes higher inhibition of type 1 fimbriated E. coli to diabetic uroepithelial cells.

Key words: Adherence ; E. Coli, Diabetes mellitus, Fimbriae, Antisera
التصاص بكتريا القولون Escherichia coli المهدية بخلع النمط 1 بالخلايا الطلانية البولية نساء مصابات بمرض السكري

الخلاصة

تم دراسة التتصاص بكتريا نساء مصابات بالسكري و آخر غير مصابات بالسكري (سبيطة) إضافة إلى دراسة تأثير تثبيط الالتهاب بواسطة وجود ضد خلع النمط 1 بتركيز مختلفة. جمعت 139 عينة من مصادر سريرية مختلفة و شخص 90 عينة بواسطة الصفات المظهرية و المجهرية و الكيميائية، أظهرت (61 %) من عينات E. coli منها كونها تحوي على خلع النمط 1 مصابة على اختبار التلازن الدموي و حساسيتها للمانوز، و (39 %) على أنها لا تعود إلى خلع النمط 1 مصدراً على اختبار التلازن الدموي ومقاومتها للمانوز. وجد بأن عدد المنتصص بالخلايا الطلانية البولية المزوجة من نساء مصابات بالسكري (41.04 ± 2.43) هو أكثر من الضعف لحالة عينات السيطرة (16.4 ± 2.3). مرج خلع النمط 1 بكتريا الملحق جزئياً مع مساعد فروند (الكامل و غير الكامل) وحقن تحت جلد الارنب ثلاث فترات أبدلاً، أجرى التحقيق في نسب مريحة لخلع النمط 1 (أجسام مضادة) الناشئة في مصل الدم المستحصل من الأرنب مع بكتريا المهدية بخلع النمط 1. وجد أن التثبيط في الالتهاب يكون في المدى بين 62 - 72 % اعتناءً على درجة تركز مصل الضر، فضلاً عن ذلك فقد وجد بأن التركيز الواطيء لمرض السكري 1 يسبب تثبيط أعلى مع الخلايا الطلانية البولية المتخصصة بالسكري.
INTRODUCTION

Women with diabetes mellitus (DM) have an increased prevalence of asymptomatic bacteriuria (ASB) and an increased incidence of systematic urinary tract infections (UTIs) compared to women without DM (1). The cause of this increased prevalence, however, is not yet clear. One factor may be microbial adherence because the adherence of micro-organisms to host cells is an important step in the pathogenesis of many infections. The adherence (the first step in the pathogenesis of UTI) of *Escherichia coli*, expressing type 1 fimbriae, is higher to uroepithelial cells of women with DM compared to the adherence to uroepithelial cells of women without DM (2). Pili (fimbriae) are filamentous organelles that can be found at the surface of bacterial cell. The adhesive part of type 1 pili is the Fim H adhesin, located at the distal end of the pilus. Fim H adhesin is highly conserved in different strains and it has essential role in the pathogenesis of lower UTIs. Upon inoculation of *E. coli* into the bladder type 1 fimbriae binds to mannosylated glycoproteins lining the bladder mucosa (3). It has been resanoned that antibodies against Fim H could prevent bacterial colonization and, therefore, could prevent UTIs in women with DM. A complex compound based upon Fim C-Fim H has been developed against UTIs (4). Specific bacterial antibodies can bring on inhibition in binding of bacterial fimbriae to the receptors on the membranes of epithelial cells since women with DM are prone to UTIs, possibly due to a higher adherence of type 1 fimbrated *E. coli* to their uroepithelial cells, this compound might be of value for the prevention of UTIs (5,6). The aim of the present study is to evaluate the adherence of *E. coli* to uroepithelial cells isolated from women with diabetes and fimbriae and to investigate the potency of antiserum raised in rabbits against partially purified type 1 for inhibition of adherence of *E. coli* to uroepithelial cells isolated from women with DM.

MATERIALS AND METHODS

Samples

A total of (139) clinical samples (urine, stool, blood, sputum, pus, and sperm) were collected from patients attending Al-Kadmiya and Al-Yarmok Hospitals. These samples were cultured immediately onto MacConkey agar and EMB agar plates, and then incubated at 37°C for 24 hours.

Bacterial isolates and culture conditions

*E. coli* isolates were cultured in LB broth and incubated at 37°C for 24 hours. The bacterial suspension was adjusted to yield (1*10^8 CFU/ml), and washed in phosphate buffer saline (PBS) and resuspended in PBS.

Hemagglutination (HA)

The abilities of bacterial isolates to agglutinate erythrocytes were determined by using 3% suspension of human erythrocytes, with or without 2.5% mannose. Twenty microliters of the erythrocytes suspension with or without mannose was placed on a glass slide, and an equal volume of bacterial suspension (1*10^8 CFU/ml) was added. The slide was gently rotated for 2 min while monitoring for HA to occur. HA was considered to be (MRHA) when it occurred with or without of mannose, whereas (MSHA) when it was inhibited by the presence of mannose (7).

Adhesion testing
Uroepithelial cells were obtained from the sediment of fresh urine from diabetic women and (control group) women without a history of UTI. The cells were washed, resuspended in PBS (pH 7.4). To 0.5 ml of uroepithelial cells were added to 0.5 ml (1*10^8 CFU/ml) of bacterial suspension. After incubation of bacteria and uroepithelial cells for 60 minutes at 37 °C, the suspension was washed four times (2000rpm, 10min) with PBS to remove any unattached bacteria. Final cell suspension dried, fixed on microscope slide, and stained with methylene blue. Cell suspension with adherent bacteria were examined using oil-immersion light microscopy (X 100). The number of \textit{E. coli} adhering to 50 uroepithelial cells was counted (2).

**Isolation of type 1 fimbriae**

Bacterial cells were harvested using Tris buffer (10 mM Tris-HCL pH 7.4), and heated at 56 °C for 20 min in magnetic stirrer to remove the fimbriae followed by centrifugation (10000 rpm, 20 min). The supernatant was added to equal volume of Sodium Dodecyl Sulfate SDS (2%) and heated in water bath at 60 °C for 30 min. Precipitated fimbriae were spun down by centrifugation (18000 rpm, 30min), dissolved in PBS and dialyzed against PBS for 4 hours (8).

**Antisera**

Antisera were raised in wild rabbits against partially purified type 1 fimbriae by subcutaneously three injections each containing 0.1 ml of fimbrial suspension (150 μg/ml PBS). The first injection was done by the addition equal volume (0.1 ml) of Freund complete adjuvant whereas the second and third injections with 0.1 ml of incomplete adjuvant at three-week intervals (9). Blood from each injected rabbit was collected (10 ml) and centrifuged to yield blood serum. The detection of serum for the presence of specific antibodies for type 1 fimbriae has been done by immuno-diffusion test (10).

**Adherence inhibition**

After pre-incubation of 1 ml of bacterial suspension (5x 10^7 CFU/ml) for 30 min with 1 ml of different dilutions:-(1:50, 1:100 and 1:200) of antisera, the suspension was washed with PBS and centrifuged (4000rpm, 12min); 0.5 ml of bacterial suspension was mixed with 0.5 ml of epithelial cells suspension and adhesion testing was completed as described above.

**Statistical Analysis**

Differences in the adherence of \textit{E. coli} to the uroepithelial cells from diabetic women and control subjects were calculated by using student's t test to find the significance in the adherence. A value of p < 0.05 was considered statistically significant (11).

**RESULTS AND DISCUSSION**

**Isolation and characterization**

From 139 clinical samples, 90 isolates were \textit{E. coli} according to biochemical tests and API 20 E, from which it was observed that the prevalence of \textit{E. coli} isolates among the total number of samples from different sources is (65%), and the prevalence of \textit{E. coli} isolates among a specific source is demonstrated in figure (1). As to the pus and sperm samples, no \textit{E.coli} was detected and therefore not included in the prevalence figure.
Hemagglutination

Hemagglutination (HA) to all of the *E. coli* isolates, and the detection of the type 1 fimbriated as indicated by MSHA and MRHA have shown that 61% is type 1, and 39% is non-type 1. In addition, the percentage of type 1 fimbriated *E. coli* in urine and stool isolates were (55%) and (82%) respectively. The high percentage of type 1 fimbriae in stool samples, in comparison to urine, is a consequence of *E. coli* being normal flora in gastrointestinal tract.

Adherence of *E. coli* to uroepithelial cells

The *in vitro* adherence of *E. coli* isolates to human uroepithelial cells (represented by the mean number of bacteria per uroepithelial cell) for diabetic and non-diabetic women (control) are shown in figure (2).

The mean number of bacterial adhesion of type 1 fimbriated *E. coli* to uroepithelial cells of diabetic women was (41.04 ± 2.43) (P < 0.001) is more than twice of control (17.81 ± 2.18), which could be an indication of the different structural nature of uroepithelial cells in diabetic and control. This may be inferred to the changes in the receptors of type 1 fimbriae and diabetic uroepithelial cells.
In the non-type 1 fimbriated *E. coli*, the number of bacterial adhered to uroepithelial cells of diabetic women (34.33 ± 2.7) is also more than twice that of the control (15.14 ± 2.4) and are statistically highly significant (P < 0.001). The more adherence in the type 1 fimbriated *E. coli* is inferred to the initiation of lower UTIs whereas non-type 1 fimbriated one is inferred to the upper UTIs. For the control subjects, the mean number of bacterial adhesion per cell in the type 1 fimbriated was 17.81 and non-type 1 fimbriated was 15.14, these differences are considered as statistically insignificant. The results are illustrated as a bar chart shown in figure (3).

![Figure (3): Mean number of *E. coli* per uroepithelial cell of diabetic and control women for type 1 and non-type 1 fimbriae. Bars indicate SE.](image)

The adherence of type 1 fimbriated *E. coli* to diabetic and control uroepithelial cells reported by other were 12.9 and 6.01 respectively (2), whereas those of non-type 1 fimbriated *E. coli* were 8.8 and 8.1 respectively. It appeared from the results of Geerlings *et al.*, (4) that the difference between the number of bacteria adhered per cell for type 1 fimbriated in diabetic women was twice that of the control. Our results are in agreement in respect of type 1 fimbriated *E. coli*.

**Immunological reactivity with antifimbriae antisera**

The results Figure (4) revealed a formation of precipitation lines between the prepared serum (antibodies) and the different dilutions of the partially purified type 1 fimbriae; whereas no precipitation line appears with the control.

![Figure (4): Gel diffusion pattern of antisera versus partially purified type 1 fimbriae (central well), antisera dilutions and control.](image)
These observations indicate that specific antibodies for type 1 fimbriae have been raised and that the antibody production protocol used was efficient.

**Adherence of E. coli to uroepithelial cells after addition of antisera**

*In vitro* adherence test between type 1 fimbriated *E. coli* on diabetic uroepithelial cells in the presence of anti type 1 fimbriae (antisera) has shown significant inhibition in adherence of type 1 fimbriated *E. coli* in comparison with the same isolates already examined for adherence of *E. coli* to diabetic uroepithelial cells. In fact, the adherence by the presence of antibodies is yet better from that of control. This gives an indication to the role of antibodies which play in inhibiting the adherence of type 1 fimbriated *E. coli*. In order to look at the effect of antibodies play in the adherence process, different antisera dilutions have been utilized namely 1:50, 1:100 and 1:200. The findings presented in figure (5) as a bar chart reveal in general that when antibodies are diluted with PBS the adherence of type 1 fimbriated *E. coli* increases *i.e.* for the dilutions 1:50, 1:100, and 1:200 the adherence become 11.4 ± 3.2, 13.4 ± 3.4, and 15.7 ± 4.3 respectively. These adherence values correspond to inhibition percentages of 72 %, 67 %, and 62 % for the 1:50, 1:100, and 1:200 antisera dilutions respectively. These results confirm that antibodies possess the specific capacity to bind with the receptors of fimbriae and prevent them from attaching to the uroepithelial cells; and also that their effectiveness are reduced by dilution.

![Figure (5): Mean adherence of type 1 fimbriated *E. coli* per uroepithelial cell for diabetic and control women in comparison with pre-incubated *E. coli* with different dilutions (1:50, 1:100, 1:200) of type 1 fimbriae antisera. Bars indicate SE.](image)

The adherence of type 1 fimbriated *E. coli* to uroepithelial cells of women with DM reported by Meiland *et al.* (12) was inhibited by 65%, 56% and 41% after incubation with anti-Fim H antiserum 1:50, 1:100 and 1:200 respectively.
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