Relationship between human blood group antigens and haemagglutination, adhesion properties of some urinary tract infection (UTI) bacteria

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Abstract

For this study a total of 135 healthy person (71 female, 64 male) were eligible. Their urine samples were assayed for adhesion test, and from 94 persons of them blood samples were taken for agglutination assay. Data from the results indicated that agglutination properties was found in *E. coli* and *Proteus* with (A^+, B^+, AB^+, AB^-, O^+, O^-) human erythrocytes. While *Klebseilla* and *Pseudomonas* were able to agglutinate few types of human erythrocytes. The adhesion mean of bacteria were increased significantly with age increasing. No significant differences were appeared between the types of bacteria used in the assay. Significant variation was noted between the bacterial adhesion mean to cells with six, the adhesion mean was apparently more in females than of males. The bacteria were adhered significantly more to uroepithelial cells obtained from positive blood group antigen persons, the highest rate was for AB^+, A^+ blood groups and the lowest was for B^- in both sexes for all types of bacteria used.

Key words: Blood groups, Haemagglutination, Adhesion, UTIs bacteria.

Relationship between human blood group antigens and haemagglutination, adhesion properties of some urinary tract infection (UTI) bacteria

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العلاقة بين الفصائل الدموية للانسان وخاصية ألتلازن ألدموي وألالتصاق لبعض أنواع بكتريا التهاب ألمجاري ألبولية

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الخلاصة

شملت هذه الدراسة 153شخاص اصحاء(71انثى,64ذكر) اخذت عينات ادرار منهم لاختبار خاصية الالتصاق كما واخذت من 94 شخص منهم عينات دم لاختبار ألتلازن ألدموي البكتري. بينت نتائج الدراسة بان بكتريا *E. coli* و *Proteus mirabilis* له القابلية على تلازن كريات دم حمر الإنسان للفصائل الدموية (A^+,B^+,AB^+,AB^-,O^+,O^-) بينما بكتريا *Klebsiella spp*, *P. aeruginosa* كان لهما القدرة على تلازن عدد قليل من الفصائل الدموية.

تاثرت قابلية ألالتصاق معنويا بالمراحل العمرية حيث ازداد معدل الالتصاق بزيادة العمر, في حين لم يلاحظ وجود فرق معنوي بين انواع البكتريا المختلفة المستخدمة في الدراسة,اثر الجنس معنويا في خاصية الالتصاق البكتيري, حيث كان معدل التصاق البكتيريا للخلايا ألظهارية البولية في ألاناث اعلى منه في الذكور. التصاق البكتيريا كان اكبر معنويا للخلايا التي تعود الى اشخاص ذوات فصائل دم موجبة. وكان معدل الالتصاق الاعلى لفصيلة الدمA^+,AB^+ والمعدل الاقل كان لفصيلة B^- في كلا الجنسين لكل انواع البكتيريا.

الكلمات المفتاحية: الفصائل الدموية, التلازن الدموي, الالتصاق, بكتريا المجاري البولية.

Introduction

Bacterial urinary tract infection (UTI) is the most common infection affecting the urinary tract [1]. The majority of infections is attributed to *E.coli* which is responsible for 85% of infections, *Proteus mirabilis, Klebsiella, Pseudomonas* and *Enterococcus fecalis,* are the next most frequent [2,3].Pathogenesis of urinary tract infection involves complex interactions between an organism, the environment and potential host [4]. Bacterial adherence to mucosa is thought to be an initial and important stage to cause urinary tract infection [5]. Among some mechanisms of bacterial adherence, the role of fimbriae and their receptors is worthy of

notice, in particular, type 1 fimbria [6].The receptors for uropathogenic bacteria are carbohydrate residues located on the surface glycolipids and glycoproteins of urothelial cells. Several of bacterial adhesions mechanisms which adhere to these urothelial cell surface antigens have been identified [7]. Pili or fimbriae are nonflagellar filamentous bacterial surface appendages composed of hydrophobic proteins. Piliated bacteria stick to surfaces, both inorganic latex particles and organic animal or plant tissues. Besides bacterial binding due to this general stickiness, specific attachment to certain hosts and tissues occurs [8-10] and is thought to be a virulence factor for bacteria colonizing or causing infection of mucous surfaces [9]. Bacterial adhesins have been classified according to the agglutination patterns resulting when bacteria bind to erythrocytes from various species. Bacteria causing agglutination of guinea pig erythrocytes inhibited in the presence of D mannose are defined as carriers of type 1 pili [11, 12]. For *E. coli* causing UTI in children, the capacity to attach to human urinary tract epithelial cells in vitro was related to the severity of the UTI produced by the strain in vivo [13].

Blood group antigens are genetically determined carbohydrate structures and may affect the availability of host cell surface receptors, the host blood group antigen status may therefore influence bacterial adherence and host susceptibility to UTIS [14,15]. Adhesion can be detected by hemagglutination reaction (essentially binding of bacteria to blood cells) and a classification system has been designed using hemagglutination. Their for the aim of the present study was to register the hemagglutination and adhesion of some UTI bacteria and its relationship with blood group antigens.

Materials and methods

1-Samples collection:

From 135 healthy person in Kirkuk city (71female, 64 male), urine samples were collected in clean cups for adhesion assay, and from 94 persons of them blood samples were collected in EDTA containing test tubes for agglutination assay. The collected blood was examined for viral and bacterial tests to ensure the clearance of any organisms at General Kirkuk Hospital.

2- Bacterial isolates.

E. coli, Proteus mirabilis, Klebseilla spp., Pseudomonas aeruginosa were obtained from culturing urine of patient who were suffering from UTI in Azadi Teaching Hospital. The bacteria were isolated and identified by using routine colony characters , staining and biochemical methods [16].

3- Bacterial suspension.

Two to three colonies from each bacteria were suspended in normal saline and washed three times ,finally the bacteria was diluted in normal saline to a concentration of 1.5x10 cell\ ml by calibrating it with Macferland tube number 4 [9].

4-Blood suspension:

The anticoagulant containing blood groups (A^+, B^+, AB^+, O^+, A^-, B^-, AB^-, O^-) were centrifuged to separate the blood cells. The RBC washed three times with normal saline, then suspended in saline to a concentration of 1% v/v.

5-Uro epithelial cells collection:

Thirty ml of urine from the females under the study were collected in clean, dry container, then centrifuged at 2000 rpm for 5 minutes and washed thrice with saline and finally suspended in 8 ml saline. The epithelial cells in this suspension were transferred on to the surface of cover slips by adding the epithelial cells suspension in a petri dish containing a filter paper No.1 and allowing the cover slips to attached to the filter paper for 10 minutes then dried [9, 10].

6-Adherence assay of epithelial cells:

For each experiment the air dried cover slips containing the uroepithelial or buccal cells were incubated with either 1 ml of bacterial suspension or 1 ml PBS (negative control), for 1 hour at 37°C. After incubation, the suspension was washed four times (250*g*, 10 min) with PBS to remove any unattached bacteria. Then fixed for 15 min in methanol, washed with

saline , stained by 3% Geimsa stain for 20 minutes and washed with saline to remove excess stain. The number of bacteria adhering to each of the first 50 epithelial cells was counted with oil immersion lens light microscopy (1,000×). Epithelial cells that overlapped other cells were excluded from evaluation. The mean number of bacteria per cell was calculated. All experiments were performed in duplicate [9,10].

7-Hemagglutination assay:-

This test was performed by mixing the bacterial suspension with the blood suspensions on a glass slide (20µl for each suspension). Agglutination was read after 3 minutes and graded as positive or negative [11,12]. The strength of the agglutination was determined further by serial two fold dilutions in saline of the bacterial suspensions with a microtiter plate. After addition of erythrocytes to each well and incubation for 2 hours at 37c°, the agglutination titer was determined, a small pellet of erythrocytes at the bottom were considered negative, and those containing an even sheet of erythrocytes across the well considered positive [11,12].

8- **Statistical analysis:** Statistical analysis was performed using Chi-square test which was used to compare categorical variables. A p-value less than 0.05 were considered significant.

Results

Data from the results indicated that agglutination properties was found in *E. coli* and *Proteus* with (A^+, B^+, AB^+, AB^-, O^+, O^-) human erythrocytes, (table 1). While *Klebseilla* and *Pseudomonas* were able to agglutinate few types of human erythrocytes.

Table 1 Haemagglutination ability of the bacteria

Human blood group	Samples No.	Bacterial type			
		E. coli	*Proteus mirabilis.*	*Klebseilla spp.*	*Pseudomonas spp.*
A^+	18	+	+	+	+
A^-	3	-	-	-	-
B^+	20	+	+	-	+
B^-	3	-	+	-	-
AB^+	20	+	+	+	+
AB^-	3	+	+	_	-
O^+	20	+	+	-	-
O^-	7	+	-	-	-
total	94				

+ = Positive haemagglutination to human erythrocyte, - = Negative haemagglutination to human erythrocyte

Table 2 shows the number of female and male samples according to blood group. In table 3,4 its clear that the adhesion mean of bacteria were increased significantly with age increasing. No significant differences were appeared between the types of bacteria used in the assay. Significant variation was noted between the bacterial adhesion mean to cells with six, the adhesion mean was apparently more in females than of males.

Table 2 Number of urine samples used in adhesion assay

Blood group	A^+	A-	B^+	B-	AB^+	AB-	O^+	O-	Total number
Number of female urine samples	7	9	8	8	10	7	13	9	71
Number of male urine samples	10	6	12	6	5	11	9	5	64
Total number	17	15	20	14	15	18	22	14	135

Table 3 Bacterial adhesion mean to female uroepithelial cells in relation to age group

Age group in years	Bacterial adhesion mean			
	E. coli	*Proteus mirabilis.*	*Klebseilla spp.*	*Pseudomonas spp.*
1-9	17.3	10.9	12.1	12.5
10->19	20.1	25.5	15.6	19.7
19-29	49.5	39.8	25.8	39.6
30-39	45.8	41.2	23.3	25.2
40-49	40.3	40.9	29.2	37.4
50-59	43.2	42.2	30.0	40.4
60-80	48.8	44.4	29.9	42.6

Table 4 Bacterial adhesion mean to male uroepithelial cells in relation to age group

Age group in years	Bacterial adhesion mean			
	E. coli	*Proteus mirabilis.*	*Klebseilla spp.*	*Pseudomonas spp.*
1-9	10.6	8.5	5.9	9.3
10->19	12.8	10.1	6.6	9.7
19-29	20.4	10.8	5.5	10.8
30-39	22.3	14.2	10.3	12.6
40-49	29.1	22.6	16.8	19.3
50-59	35.5	30.8	17.7	23.6
60-80	39.6	32.4	21.9	28.5

The bacteria were adhered significantly more to uroepithelial cells obtained from positive blood group antigen persons, the highest rate was for AB^+, A^+ blood groups. The lowest was for B^- In both sexes for all types of bacteria used, with significant differences between males and females. Being the females more than males Table 5,6.

Table 5 Bacterial adhesion mean to female uroepithelial cells in relation to blood group

Type of bacteria	Bacterial adhesion mean in each blood group							
	A^+	A^-	B^+	B^-	AB^+	AB^-	O^+	O^-
E. coli	39.6	24.4	25.2	10.3	44.2	20.9	30.8	24.8
Proteus mirabilis	35.3	30.9	23.6	10.9	40.8	22.6	30.1	20.7
Klebseilla spp.	19.9	13.5	12.8	8.8	19.3	12.6	14.2	10.7
P. aeruginosa.	23.5	17.2	21.1	16.4	29.4	21.9	20.8	16.5

Table 6 Bacterial adhesion mean to male uroepithelial cells in relation to blood group

Type of bacteria	Bacterial adhesion mean in each blood group							
	A^+	A^-	B^+	B^-	AB^+	AB^-	O^+	O^-
E. coli	26.1	21.3	20.5	19.9	33.6	27.6	25.2	16.8
Proteus mirabilis	29.2	21.1	20.8	16.8	32.3	20.9	25.7	17.7
Klebseilla spp.	12.6	10.2	9.7	6.6	14.5	11.6	12.8	9.9
P. aeruginosa.	16.2	11.1	17.3	14.6	18.9	14.4	27.7	10.8

Discussion

The results indicated that agglutination properties were found in *E. coli* and *Proteus mirabilis* with (A^+, B^+, AB^+, AB^-, O^+, O^-) human erythrocytes. While *Klebseilla* and *Pseudomonas* were able to agglutinate few types of human erythrocytes. This results was consistent with those of [17, 18] were they concluded that 261 of 453 strains of *E.coli* can agglutinate human erythrocytes and 91 of 148 strains of *proteus* could agglutinate human and some animals erythrocytes. The bacterial surface antigen (s) mediating mannose resistant hemagglutination of human erythrocytes may be one factor selecting for *E.coli* agglutinating ability [17]. *P. mirabilis* may express a variety of adherence organells which have been characterized by their ability to agglutinate various erythrocyte types [19].

Bacterial adhesion means was greater in females than those of males in all bacteria used this may due to that the women has UTIS more than the men [1] and this may leads to alteration and increased bacterial adherence, this has been described in a studies on vaginal and buccal epithelial cells isolated from patients (women) with recurrent UTIS [20]. In addition of some special factors acting in females may influence bacterial adhesions like estrogen hormans

levels. An increased bacterial adherence was occurred during the menstrual cycle and early pregnancy [21].

Adhesion mean of the bacteria to the uroepithilial in the present study was differed according to the blood group antigens. Blood group antigens are carbohydrate structures bound to membranes lipids or proteins are integral structure in uroepithelial cell membranes. This affect the ability of host cell surface receptors, the host blood group antigen status may therefore influence bacterial adherence and host susceptibility to UTIS [15]. It has been suggested that bacterial adherence may also be affected by the blood group antigens, which are found on the surface of uroepithelial cells. Individuals with the Lewis blood group phenotype Le(a_b_) secrete Leb and A, B, or H substances in their saliva and plasma and are called "secretors," whereas nonsecretors with the Le(a_b_) phenotype do have Lea antigens in their secretions but not A, B, or H substances. Several studies have shown a correlation between the Lewis blood group phenotypes and recurrent UTIs in adult women [14, 15]. ABH non-secretors are at a greater risk for recurrent urinary tract infections (UTI) and are much more likely to develop renal scars. This susceptibility is even greater among the Lewis negative subset (Le (ab-)).The ABH secretor phenotype conveys a measure of protection; cutting the risk of recurrent UTI by greater than 50% and dramatically decreasing the likelihood that renal scars will develop. ABH non-secretors appear to be at extra risk for recurrent urinary tract infections. In one study of women with recurrent UTI, 29 % of the women were the Lewis (a+ b-) non-secretor phenotype, while another 26% of the women were Lewis (a- b-) recessive phenotype. When the women with ABH non-secretor and recessive phenotypes were combined and considered collectively, the odds ratio (an estimate of relative risk of urinary tract infection) for those without the secretor phenotype (Lewis (a-b+) was 3. A form of synergy also appears to exist between UTI risk, secretor status and the lack of ability to create anti-B isohemagglutinin. Essentially, blood group B and AB and the non-secretor phenotype seem to work together to increase the relative risk of UTI among these women [22].

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