The humoral mucosal immunity of urinary tract kelbsiella infections in menopausal women

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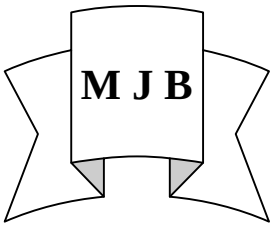

ABSTRACT

Seventy – one menopausal women patient (MWP) with urinary tract infections (UTI), thirty - two adulthood women patients (AWP) with UTI and twenty normal women subjects (NWS) were the study groups. Pyuria but not haematuria via examination of urine sediments. Kelbsiella like colony morphotypes were noted on blood agar and Mac (on key agar, in single and mixed infection patterns. K. pneumoniae and, K. oxytoca , were identified as uropathogens.

Klebsiella antigenic epitopes can be of B cell and of the Th_2 dependent types activating mucosal humnal immnal immune responsce in the urinary tract during Klebsiella infections. Lower mucosal antibody titers (4-16) were noted among MWP than that of AMP (16-64). This may attributed to local costimulatory signals, activation of suppresser T cells and disturbance of the Th1/Th2 activities leading to production of low activity and affinity antibodies.

الخلاصة

واحد وسبعون امرأة يائسة من الطمث تعاني من خمج السبيل البولي وأثنين وثلاثون امرأة مريضة بخمج السبيل البولي وعشرون امرأة سوية جعلت هي مجاميع الدراسة .

لوحظت البيلة القيحية دون عن البيلة الدموية في مجموعتين المرضى ولم تلاحظ في النساء السوية وتبين بأن طرز المستعمرات النامية على بوصفها *K. oxytoca* و*K.pneumoniae*الأوساط الزرعية تشبه مستعمرات الكلبسيلا وبهيئة أحادية او ثنائية المسبب كما وعزلت كل من مسبب مشارك لخمج السبيل البولي. وقد تكون الذرى وقد تكون الذرى المستضدية في الكليسيلا معتمدة على الخلايا اللمفية البائية او معتمدة الخلايا التائية المساعدة الثانية التي تنشط الاستجابة المناعية المخاطية في السبيل البولي .

وجرى إثبات ان عيار الضد المخاطي البولي أوطأ (4-16) في اليائسات من الشابات (16-64) وهذا ربما يعود إلى نقص إشارة التحفيز المشترك Thi/ Th، تنشيط الخلايا التائية الكابحة او الى اعتلال في النشاط

Introduction

The normal function of urinary tract have mucosa associated lymphoid aggregates (1,2) such aggregates forms the basis for mucosal immunity in both their humoral and cellular arms (2,3). Ascending , haematogenous and / or lymphogenous routes of infections are primarly combted by urine voding mechanisms mucosal trapping , mononuclear cell system , polymorphonuclear cells and urinary mucosal immunoglobulin (UMIG) (4,5). Imbalance, however, between The virulence factors of The uropthogen and the elements of mucosal immune system of the host lead to clinical infection (5) in the present work the possible effects of menopause on urinary mucosal immunity during women infection was being investigated

Materials and methods

1. urine samples
   Seven milliliters amounts of clean catch midstream urine samples were collected aseptically from 71 menopausal women and 32 adulthood women with urinary tract infections as a test group and 20 normal women subject as control group in Hilla maternity and childhood hospital.
2. urinary mucosal immunoglobulin separation: The urine samples were filtered through whatman no.1 filter paper ,then mixed with equal amount of Polyethylene Glycol (PEG) 6000 6% and precipitated as in (6,7) then partially characterized as in (8).
3. uropathogens
   The Klebsiella and the coexisting species were identified from primary plate cultures as in (9) using classical biochemical tests and API 20 E for conformation of isolate identification. Whole cell antigens were done as in (10).
4. serology :
   Slide and tube agglutination studies were done as in (10)
5. Biometry: statistical analysis was made as in (11)

Results

1-Infection

The MUP, AWP and NUS urine samples were deposited and wet mount prepared significant grades of pyuria rather than haematuria were noted among AWP and MWP but not NWS single monomicrobic infections were higher than dimicrobic infections. Intact uropathogens were found in higher rates than injured uropathogens *Klebsiella* – like clony morph types on blood and MacConkey ager plates K. pneumoniae and K. oxytoca were identified from MWP in higher rates than AWP and NWS . (Table 1) In dimicrobic infection The coexisting organisms were *E.coli, Eerobacter, S. epidermidisermidis and St . pyogenes.*

1- Urinary Mucosal Immune Responses ,

2-1 Urinary Mucosal Immunoglobulins,
The solution that were obtained using PEG 6000 6% precipitation and redisolution, were found to biurate positive , 2ME resistant and rected specifically with whole cell antigens of the corresponding uropathogens were considered as UMIG , one case from each of klebsiella species , the titer dropped to zero upon 2ME treatments indicating serum fraction (8 to zero , 4 to zero). Two UMIG negative cases noted among *K. pnumoniae* cases.

2-2.: specific mucosal antibody titres, most of the mucosal antibody titres were within 8 in MWP in contrast to the most noted titres 16 or more among AWP (Table 2)

2-3 : The Klebsiella specific urinary humoral mucosal immune responses in MWP.

2-3-1 : K. pneumoniae:-

Twenty- two and seven cases of K. pneumoniae of urinary tract infections were noted among MWP and AWP .The age ranges in MWP were 47-49 while for AWP were 18-29 years. The means, medians, and ranges were 1.69, 0.89, and 16.22 g/L for MWP, while they were 1.49, 0.90, and 4g/L for AWP. The mucosal specific antibody titre means, medians and ranges were 7.5, 8 and 28 respectively for MWP while they were 24, 16 and 56for AMP

2-3-2 : *K.oxytoca* :
Ten and three cases of *K.oxytoca* urinary tract infection among MWP and AWP respectively The age ranges were 47-49 for MWP and 22-30 for AWP The concentration means , medians and range of UMFO for MWP were 2.33 , 1.67 ,and 10.2 g/L while for AWP They were 1.11, 0.90 ,and 0.7 g/L respectively . The titre means ,medians and ranges of UMIG were 7.6 , 8 and 8 for MWP while They were for AWP as , 37.3 , 32 ,and 48 respectively .

2- Biometry :-
3-1 : Regression Analysis ;
The correlation between UMFG connection and titles were of simple linear type with significant r in both *K .pneumoniae* and *K. oxytoca*. It was positive in the two former cases and negative for the third cases (table 3).

3-2Paired observation t statistics;
The paired observation analysis t statistics for the differences between mucosal antibodies in AWP and AWP were significant at *K. pneumoniae* and *K. oxytoca* (table 4).

4-Mucosal Immunology of Mixed Infection;
Six *K. pneumoniae* and three *K. oxytoca* were noted in mixed infection states with other uropathogens. The Klebsiella specific UMIG titres were ranging among 4-16 and for the coexisting pathogen were 4-8 (Table-5).

Discussion

Klebsiella species can be the causals for descending from of an infections foci spreading through blood or lymph reaching bladder through glomerular filtration or ascending from the gut micro flora resident in perineum reaching urethral orofic, urethra then bladder (6,12,13).

Aging and/or menopause in women are associated with lack of estrogen that lead to atrophic changes in urethra, vagina causing atrophic urthritis and atrophic vaginitis. Together with ulteration of B lymphocyte and T lymphocyte function (15,16,17).

Such changes may predispose the bladder, urethra to colonization with Klebsiella. Through the action of virulence factors such as capsule, siderophores, pilli, serum resistance and lipo poly saccharine (17,18,19).

K. pneumoniae and K. oxytoca were with higher infection rates among MWP than AWP (table 1).

Such finding may be due to the lack of estrogen in urethra and vagina which could be terminated by colonization of these areas with Klebsiella (15).

Klebsiella may contained B cell or Th2 dependent antigenic epitopes associated with urinary tract or the antigen primed B cells migrates to an effector sites producing antibodies proximately to urinary tract (1,20).

The suppressive effect of menopause on mucosal immune response may be due to one or more of the followings; 1- an alteration in antigen uptake ,antigen reception by effector immune cells (21,22), 2- production of low affinity or low avidity antibodies, 3-lack of co stimulation signals 4- activation of suppressor T cells, 5- disturbance of Th1/ Th2 activities, 6- there

may be presence of peptide antigen T antagonizes (4,23,24).

There were 2ME sensitive fractions of mucosal antibodies among klebsiella UTI (table 1,2,3) can be serum transcudated fraction (7).

K. pneumoniae UTI in MWP, tow cases were with lack of UMIG may be due to ; 1-high catabolic rate of UMIG due to combate infection 2- Real lack of UMIG due to suppression of synthesis, 3-UMIG conc. too low to be precipitated by PEG 6000 6% (25,26,27).

Thus, on conclusion of this study one may state the followings:

1. Higher rates of klebsiella infections among MWP than AWP.
2. Low UMIG titres found among MWP than in AWP this may be due to ultred B and T cell functions.
3. 2 ME sensitive serum fraction was noted among some of cases.
4. Among, MWP, two cases with lack of UMIG were noted.

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Table -1:-urinary tract Infection among Menopause, adulthood and normal subject women.

I Parasite	K. Pneumoniae	K. oxytoca
Uropathogen		
Gram Rection	-	-
motility	-	-
Capsule	+	+
String test	+	+
Shape	OR	OR
Growth on Ma	LFC	LFC
Growth on BA		
Indole	-	+
Citrate	+	+
Vp	+	-
Urea	-	+
II Host-Parasite Interaction----------		
Pyuria	+	+
Haematuria	-	-
Injurd Parasite		
MWPs	2:71(2.816%)	1:71(1.40%)
m	1:71(1.40%)	0:71(0%)
AWP	0:32 (0%)	0:32 (0%)
Intact parasite		
	20:71 (28.169%)	9:71(12.676%)
	7:32 (21.675%)	3:32 (9.275%)
III Infection Rate ----------		
Single MWP	22:71(30.985%)	10:71(14.1%)
AWP	7:32 (21.875%)	3:32 (9.275%)
NW	1:20 (5%)	0:20 (0%)
Mixed MWP	8:71(11.267%)	3:71(4.225%)

OR : Oval Rods

LFC : Lactose Fermenting Colonies
MA : MacConkey Agar BA : Blood Agar

Table 2: Urinary Mucosal Immunoglobulin titres specific for Klebsiellae.

Titre	K. Pneumoniae		K. oxytoca	
	M	A	M	A
2	-	-	-	-
4	7	-	3	-
8	11	1	6	-
16	1	4	1	1
32	1	1	-	1
64	-	1	-	1
128	-	-	-	-

M = Menopause
A = Adulthood

Table 3: Humoral Mucosal Immune Responses of Urinary Tract Infected Women.

No.pt.	Age	MWP		No. pt.	Age	AWP	
		Conc. g/L	Titre			Conc. g/L	titre
	K. Pneumoniae						
		Mean 1.69	7.5		18		
22	47	Median 0.89	8	7	↓	1.49	24
	↓	Range 16.22	24		29 years	0.9	16
	49	r= 0.5				4.07	56
					r	=0.86	
						$g^{\wedge} = 146512 + 0.6X$	

$$g^{\wedge} = 0.5147 + 29.833X$$

	K. oxytoca						
10	47 ↓ 49	mean 2.33 median 1.68 range 10.80 r= 0.84 $g^{\wedge} = 0.5147 + 29.833X$	7.6 8 8	3	22 ↓ 30 Years $g^{\wedge} = 1.06055 + 1.5714X$	1.11 0.9 0.6756 0.7877	37.33 32 48

Table 4:'t' statistic for the Menopausal effects.

Organism	Menopausal Titre	Adulthood Titre	t statistic cal.	tab.	P.
K. pneumoniae	7.5	24	2.261	2.052	0.025
K. oxytoca	7.6	37.3	2.1010	1.296	0.05

Table 5: Mucosal Immune Responses of Mixed infections.

Mixed infection		UMIG g/L	Titre
K. pneumoniae	and E. coli	0.3396	4,4
	and S. epidermidis	0.5596	8,8
	and S. epidermidis	0.6112	4,4
	and S. epidermidis	0.6112	4,4
	and St. pyogenes	0.3391	4,8
	and Enterobacter	1.5923	8,4
K. oxytoca	and E. coli	1.069	8,8
	and S. epidermidis.	1.98	8,4
	and St. pyogenes	11.153	16,8