Evaluation of urinary secretary immunoglobulin (sIg) level in diabetic patients at Karbala city

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Abstract

This study was designed to evaluate the prevalence of urinary tract infections (UTI) and measure the levels of urinary secretaryimmunoglobulin (sIg) in diabetic patients during the period from January until November 2009. A study was included (64) patients with diabetes mellitus (D.M.) and (30 person diagnosed by clinician as UTI without diabetes mellitus) as control. From both groupsurine samples were collected, general urine examination (GUE),countof pus cells, urine culture for bacterial isolates and measured the levelof urinary sIg. Results shows out of (64) diabetic patients, pyuria>10 pus cells/high power field was present in(39/64, 60.9%) cases. Out of these (39) cases, positive urine culture was seen only in (22, 56.4%) cases. Overall frequency of UTI was (34.44%) and the commonest microorganism isolated was *E.coli* (45.5%). Also result shows decreased significantly ($p<0.01$) of urinary sIg levels in D.M patients as compared with control subjects.

تقويم مستوى الامينوكلوبين المناعي الافرازي في ادرار مرضى داء السكري في مدينة كربلاء

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مفتاح البحث: الإمينوكلوبين المناعي الإفرازي

الخلاصة

صممت هذه الدراسة لتقويم مدى شيوع التهاب المجاري البولية وحساب مستوى الامينوكلوبين المناعي الافرازي في ادرار مرضى داء السكري للفترة من حزيران ولغاية تشرين الثاني 2009.تضمنت الدراسة (64) مريض بالسكري تراوحت اعمارهم بين (40-70 سنة) و(30) شخص مصابين بالتهاب المجاري البولية ليس لديهم داء السكري وبنفس المدى العمري كمجموعة سيطرة . من كلا المجموعتين تم اخذ عينات الادرار لغرض فحص الادرار العام وزرع الادرار وحساب تركيز الامينوكلوبين المناعي الافرازي.

اوضحت النتائج ان (64/39 ,60.9 %) كانو لديهم عدد الخلايا القيحية في الحقل المجهري الواحد اكثر من 10 خلاياومن هؤلاء ال (39) حالة مرضية فقط (22 , 56.4%) كانت النتيجة موجبة للنمو البكتيري على الاوساط الزرعية .بصورة عامة اوضحت النتائج ان نسبة التهاب المجاري البولية كانت(34.44 %) عند هؤلاء المرضى وكانت البكتيريا (*E.coli*) هي السائدة بنسبة (45.5%) كما ان النتائج اوضحت انخفاض معنوي في الكلوبين المناعي الافرازي في ادرارمرضى السكري مقارنة بمجموعة السيطرة .

INTRODUCTION

Mucosal surfaces such as saliva,tears,nasal,throat and urine represent a large part of entry pathogens and thus must be efficiently protected. This goal is achieved by combination of innate and acquired immune activity. Acquired immunity includes the production of antibodies(Abs) ,the primary Abspresent at mucosal level is secretaryimmune-globulin(sIg) which is important components of the first line of defense that operates at all mucosal sites [1,2].sIg is a major immune-globulin in the protection of mucosal surface against infections[3].Diabetes mellitus (DM) is a well recognized risk factor for occurrence of urinary tract infectionsUTI[4,5] the risk factors for UTI involve colonization with a different uropathogen in cases of recurrent UTI.Glucosureaand impaired granulocyte functions[6]. UTIs are the common bacterial infections of mankind [7]. A changed bacterial adhesion to the uroepithelium[8], granulocyte dyes-functions [9] and impaired antioxidant system involved in bacterial activity [10] are all involved in the pathogenesis of UTI in diabetic patients. In a large study of bacteremic patients, it was demonstrated that two thirds of the patients had D.M; the urinary tract was the most prevalent infection site[11].

This study was designed to determine the prevalent of UTI and evaluate concentration of urinary secretary(sIg) in diabetic patients atKarbala city.

Materials and Methods:

The study was conducted on 64 D.M.patients aged from (40-75 years) who were admitted to Al-Hussein hospital during a period from January 2009 up to November 2009 in addition to (30 patients UTI without D.M) as control to compareconcentrationof sIg.Fromboth patients and control about 10 ml of mid-stream urine samples were collected using sterile wide mouthed glass bottles with screw cap tops. On the urine sample bottles were indicated name, age, sex and time of collection.

Urine samples were divided into two divisions: First- (8 ml) for general urine examination (GUE) and measurement of sIg.Second- (2ml) for urine culture.The urine samples were mixed and centrifuged at 3000 r.p.m for 15 minutes .The deposits were examined using both 10X and 40X objective for qunntitating the number of leucocytes (pus cells) per high power filed(HPF),a sample with <10 pus cells/HPF was apyuria and a sample ≥10pus cell /HPF wasclassify aspyuria[12], and send forurine culture.

A loopful of well mixed urine sample was inoculated into duplicate plates of blood and MaCconkey agar and all incubated at 37˚c aerobically for 24 hrs. Then, the plates were examined for bacterial growth.The bacterialisolates were identified generally using a battery of tests [13].The supernant of urine sample taken for isolation of (sIg) by polyethylene-glycol (PEG,M.W 6400)as below:

1- Take 5 ml of supernant urine and filtered with Whatman-paper No.1
2- Take 3 ml of previous step and mixed with equal amount of PEG solution.
3- Centrifuged the mixture at 6000 r.p.m for 30 min.
4- Supernant was discarded and precipitate was dissolved in 0.5 of formal-saline [14].
5- sIg level was estimated by Biuret method.

Statistical analysis:

The mean, standard deviation (SD), and analysis of variance (ANOVA) test were calculated [15].

Results:

Among64patients with D.M. in this study (28 male and 36 female) as in table(1) which shows distribution the patients according to age groups and the highest rate of D.M. was among 50–59 years old which represent (34.38%).Microscopic examination of the urine sample revealedpatients with pus cells more than 10 /HPF were pyuria39 (60.9%) of the specimen, while 25 (39.1%) showed apyuria as in table (2).Patients with pyuria (39) were selected for urine culture, out of these (39)patients,(22, 56.4%) were positive urine culture; these considered UTI diabetic patients while (17(43.6%) were negative urine culture (sterile pyuria),also urine culture positive was highersignificantly ($p<0.05$) in female (23.44%) than male (11 %) as in table (3).The commonest organisms isolated from urine culture were*E.coli* (No. =10, 45.5%),*Pseudomonasaeroginosa* (No. =4, 18.2%),*Klebsilla pneumonia* (No. =2, 9%)and *Staphylococcusaureus*(No. =6, 27.3%) as in table (4). According to results of urine culture, positivecultures wereconsidered D.M.with UTI (22/64, 34.44%) and another (42/64, 65.6%) D.M. without UTI.

For all groupswere estimated theconcentration ofurinary sIgas in table (5), which show decreased significantly ($p<0.01$)sIg levels in diabetic patients (with or without UTI) as compared with control subjects, in addition there was no significant differences ($p>0.05$) between diabetic patients with or without UTI.

Discussion:

In our study that included (64) diabetic patients, the results shows the incidence of pyuria in these patients were (39/64, 60.9%). Out of them patients(22/39, 56.4%)were positive urine culture which indicate UTI in diabetic patients, and (17/39, 43.6%) were sterile pyuria that may be as result to fungal or mycoplasma effects, so may be to stress that increase adrenaline level and this lead to activation β2 which can cause incidence sterile pyuria, and this finding was agreement with[7,13,14]. UTIsare more common in diabetic patients as compared with non-diabetic patients [18].The mechanisms which potentially contribute to the greatestincidence of UTI in these patients are malfunctioning in the local urinary secretion and increase adherence of bacterium to the cells of uroepithelium cells [19]. On the other hand,the most common organisms isolate were *E.coli* ,*Staphylococcusaureus*,*Pseudomonas aeroginosa* and *Klebsillapneumonia*. This result agreement with [20,21] who recorded the most organism of UTI in diabetic patients was *E.coli*. The principal causative agents accounting for 85% of cases of UTI are Grams negative bacteria [22,23].Modification of chemical composition of urine in D.M. can alter the ability of urine and support the growth of microorganisms [24].*Pseudomonas aeroginosa* was prevalent in 18.2% of D.M. in this study, clearly indicates immune suppression by this opportunisticuropathogen which never cause any symptoms of UTI in non diabeticperson [5]. The obtained results shows decrease significantly ($p<0.01$) the mean levels of urinary sIg in diabetic patients with UTI as compared with control subjects. Also there was no significant differences between diabetic with UTI and diabetic without UTI ($p>0.05$). Diabetic patients were immune compromised and this lead to predispose to many infections such as UTI, sIg which is found on mucosal surface of epithelium cells that lining the lumen of urinary tract that protect against the colonization and invasion of pathogenic microorganisms[19,22]. Because we found decreased of urinary sIg levels in diabetic patients this would explain why relapses of UTI occur often in these patients.

Table (1) Distribution of patients according to age

Age group	No. of Male	No. of Female	Total	
			No.	%
40-49	0	12	12	18.75
50-59	10	12	22	34.38
60-69	6	6	12	18.75
≥70	12	6	18	28.13
Total	28	36	64	100

Table (2) Incidence of pyuria among diabetic patients

Group test	male	female	Total	%
Pyuria D.M. patients	17	22	39	60.9
Apyuria D.M. patients	11	14	25	39.1
Total No.	28	36	64	100

Table (3) urine culture positive according to sex in diabeticpyuria patients

Test groups	No. of patients	No. of positive culture	%
Male	28	7	11 %
Female	36	15	23.44%
Total	64	22	34.44%

Table (4) Types of microorganisms isolated from diabeticpyuria patients

Bacterial isolated	No.	%
E.coli	10	45.5
Staphylococcus aureus	6	27.3
pseudomonas aeroginosa	4	18.2
Klebsilla pneumonia	2	9
Total	22	100

Table (5) shows mean levels of urinary sIg

Test groups	No.	Conc.sIg mg/dl
D.M with UTI	22	231*
D.M without UTI	42	260
Control	30	580

*high significant (p=0.002)level

Reference

1. Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Ann. N. Y. Acad. Sci., 1089: 288-311.2007
2. Donald, B.K, Roger K. and Rawle M.M. B.M.J. 1970, 4:463-465.
3. Brandtzaeg P, Fjellanger I. and Gjeruldsen ST. Scand. J. Haematol. Suppl,12:1-3.1970
4. Manhal F.S. Al-kindy col Med J. 7(1):39-46.,2001.
5. Saleem;M, and Danial;B. Int.J.Emerg.Sci., 1(2),133-142 (2011).
6. Geerling, S.E. Int. J.antimicrobe agents, 31, s54-s57, 2008(medline).
7. Pargavi B, MekalaT,Thamaria A and Moorthy k.Inter.J.ofBiol.Technol. 2(2):42-45. 2011.
8. 8-Geerling SE, Erkelens DW, Hoepelman IM. Ned TijdschrGeneesked141:372-375.1999.
9. Valerius NH, EFF C, Hansen NE, Karle H, Nerup J, Soeberg B, et al. Acta Med scand 211:463-467.1982.
10. Muchova J, Liptakova A, Orszaghova Z, Garaiova I, Tison P, Carsky J. Diabet Med;16: 74-78. 1999
11. Carton JA, Maradona JA, Nuno FJ, et al. Eur J Med.(5); 21-7.1992
12. Smith Pj, Morris AJ, Reller LB. Pathol. 35(2); 161-165.2003.
13. Stark RP, Maki DG. New Engl. J Med. 311: 560-564.1984
14. Al-Saadi M.A.K. M.Sc. thesis Collage of science Babylon Uni. 2000.
15. Cochran, W.G. Sampling techniques textbook. 2nd ed. Whley eastern private limited. NewDelhi.1974
16. Akinyemi KO, Alabi SA, TaiwoMA,Omonigbehin,EA J. Hosp. Med. 1: 7-11. 1997.
17. Ebie M, Kandaki-Olukemi YT,Ayanbadejo J TanyignaKB J. Microbial. 15(1): 31-37. 2001
18. Muller LMA J,Gorter KJ, Hak E, Goudzwaard WL, et.al. Clin. Infect.Dis. 41(3): 281-288.2005
19. Geerlings,SE; Meiland R. and Hoepelman Al. Int.J. AntimicrobAgents.19(6):539-45.2002.
20. Brauner A, Foldin U,Hylander B, Ostenson CG. Diabetes Med. 10(6):550-554.1993
21. kolawole,A.S. ,Kolawole, O.M., Kandaki-Olukemi,Y.T.et al. Inter.J. ofMedi.andMedi.S. 1 (5):163-167.2009
22. Grunberg RN. J clin. Pathol. 33: 853-856.1980
23. BrandtzaegP. Int. J. Med. Microbiol, 293: 3-15.2003
24. LevisonM.E,Pitsakies,PG.J.infects.Dis.150
:554-560.1984