THE ASSOCIATION BETWEEN ASYMPTOMATIC BACTERIURIA AND GLYCEMIC CONTROL IN TYPE 2

Reni Marlina[1], Ricke Loesnihari[1], Santi Syafril[2]

[1] Department of Clinical Pathology, School of Medicine, University of North Sumatera/Adam Malik Central Hospital Medan, Indonesia. E-mail: renimarlina81@gmail.com; [2] Department of Internal Medicine, School of Medicine, University of North Sumatera /Adam Malik Central Hospital Medan, Indonesia

ABSTRACT

The incidence of infection often occurs in patients with Diabetes Mellitus (DM) due to hyperglycemia that causes dysfunction of chemotaxis, phagocytic activity, malfunction of neutrophils and glycosuria. It is followed by other complications that lead to the malfunction of the bladder. Highly urinary glucose level is an exellent medium for pathogenic microorganisms growth. Asymptomatic Bacteriuria (ASB) is a risk factor for symptomatic urinary tract infection spontaneously or due to urinary catheters. This study aimed to analyze the association between the incidence of ASB of type 2 DM and glycemic control. The study was conducted during June-August 2016 with a cross-sectional method. Subjects were type 2 DM patients with age ≤ 60 years, who were treated in the Endocrinology Out-Patient Clinic of the Adam Malik Hospital. HbA1c was measured by Indiko automatic analyzer. Its association with urine culture results was analyzed. A total of 50 samples were enrolled, consisting of 25 females, and 25 males, with 19 having an exellent glycemic control (HbA1c <7%) and 31 with poor glycemic control (HbA1c ≥ 7%). Thirteen positive ASB were found, 5 with good glycemic control and 8 with poor glycemic control. Statistical analysis revealed a nonsignificant association between glycemic control and culture results (p = 1.000). Somers' d did not show a significant association between glycemic control and the incidence of ASB (p=0.968, d=-0.005). However, significant differences in culture results between gender, in which the ASB were found in samples of four females and two males (p=0.004). Somers' d revealed a significant association between culture results and gender (p=0.001; d=-0.360). Most of the bacteria found were Gram-negative. There was no significant association between glycemic control with an incidence of ASB. However, gender had significant differences in the incidence of ASB, which occurred more frequently in DM females than males. Urinalysis should be performed in patients with type 2 DM with ASB. However, further study was needed to analyze the relationship between glycemic control with the incidence of ASB and other factors that might affect the incidence of ASB.

Key words: Asymptomatic bacteriuria, type 2 diabetes mellitus, HbA1c

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases with hyperglycemic characteristics that happened due to insulin secretion disorders, the way insulin works or both.[1] Diabetes mellitus epidemiology threatens the health of a lot of people, both in advanced and in developing countries.[2] The International Diabetes Federation (IDF) predicts an increase in DM patients in Indonesia from 9,1 million in 2014 to 14,1 million in 2035, which at that point Indonesia will have the 7th highest total of DM patients in the world.[3]

Diabetes Mellitus patients with high blood glucose concentrations, easily get infected by various systemic infections, 4.4 times more than patients without DM.[4] Infections in DM are the most severe complications that have become world health problems.[5] Infections happen more in patients with DM due to the hyperglycemic environment that increases the pathogenic virulence, decreases interleukin production, causing chemotaxis and phagocytic activity dysfunction, damaged neutrophil function, glycosuria and gastrointestinal, and urinary tract dysmotility. These conditions can also be accompanied by other complications that are related to neuropathy causing malfunctions of the bladder that can cause the higher risk of infection, especially Urinary Tract Infections (UTI).[3,6,7]

Glycemic control is based on glycated hemoglobin (HbA1c) examinations. HbA1c is the gold standard to control a patients blood glucose concentration. Diabetes mellitus is stated as under good control if the levels of HbA1c < 7% and DM is unregulated the HbA1c ≥ 7 %. HbA1c is now used as an indicator and marker that is significant for glucose control describing the mean plasma glucose from 6 – 8 weeks before.[1,8] Bad glycemic control may cause kidney injury, and is a predisposition of UTI.[9]

Asymptomatic bacteriuria is a risk factor for symptomatic UTI and can cause a decrease in renal function, which needs serious handling.[10] Asymptomatic Bacteriuria (ASB) is bacterial colonization persistent in the urinary tract without clinical symptoms, that shows pure microorganism growth more that 10^5 Colony Forming Units (CFU/mL) in urine culture in every milliliter of fresh urine taken from midstream.[11,12]

High glucose levels in urine are an exellent growth media for pathogenic microorganisms.[13,14] Infection in DM patients is influenced by blood glucose control and high blood glucose concentration can increase vulnerability or aggravate the infection.

The purpose of the experiment was to determine, whether there was a correlation in asymptomatic bacteriuria in DM type 2 patients with glycemic control and learn the difference of asymptomatic bacteriuria in DM type 2 with regulated and unregulated HbA1c concentrations.

METHODS

This experiment was held during June – August 2016 by observational, analytical cross-sectional method at the UP HAM. HbA1c was measured by an automatic Thermo Fisher Scientific Indiko Analyzer with a turbidimetric inhibition immunoassay and correlated it with urine culture result. Urine was taken by midstream clean catch, using CLED Agar (Cystine Lactose Electrolyte Deficient) media. Inclusion criteria were DM type 2 patients who underwent therapy at the Endocrinology Out-Patient Clinic of the Adam Malik Hospital Medan, with no symptoms of UTI (fever, urgency, frequency, dysuria), age $\leq$ 60 years old. The exclusion criteria were DM patients with the urine catheter, DM patients with UTI that already received antibiotic therapy, anemia, pregnant patients, hemoglobinopathy and chronic kidney disease.

Statistical Analysis was done with Statistical package for social science (SPSS) Version 20 software. The variables of this experiment were shown in tabulation and were described. To observe the difference between asymptomatic bacteriuria in controlled HbA1c and uncontrolled HbA1c, X^2 (chi-square) test was used, unless there is an expected count < 5, whereas the Exact Fisher test was be used. To observe was the relationship between asymptomatic bacteriuria with glycemic control the Somers' d association was used.

RESULTS AND DISCUSSION

This experiment was done by an analytically observational cross-sectional method. This experiment was done from June – August 2016, 50 samples of DM type 2 patients were obtained that fulfilled the inclusion, and exclusion criteria. Subject's charateristic, can be seen in Table 1, where there were 25 females and 25 males with a mean of 54.16 $\pm$ 3.919 years old, HbA1c median 7.2% with a range of 5.3 – 15.1%.

As shown in Table 2, ASB was found in 11 female patients (44% of the total of female patients) and two male patients (8% of the total of male patients). The results of the x^2 test showed a significance from the sex variable towards ASB (p=0.004). Somers' d association from sex and culture results were very significant but low and negative. (p=0.001; d = - 0.360).

Table 1. Characteristics of experiment subjects

Characteristics	Total
n (people)	50
Age (years old)	54.16
Mean	(3.919)
(SD)	
HbA1c	
Median	7.2
(min-max)	(5.3-15.1)
Sex	
Female	25
Male	25

Table 2. Cross-tabulation of sex and ASB incidence

	Total	ASB incidence		P-value
		Negative (-)	Positive (+)	
n (people)	50	37	13	
Sex				0.004*
Female	25	14	11	0.001^
Male	25	23	2	

^ = Somers' d association tes
* = X^2 test (chi-square)
The results were significant when p < 0.05.

In Table 3, there were 19 patients with controlled HbA1c (<7%) and 31 patients with uncontrolled HbA1c (62%). Urine culture results showed that ASB was found in 5 patients with controlled HbA1c and 8 patients with uncontrolled HbA1c. According to the Exact Fisher examination, there was no significant ASB between controlled and uncontrolled DM type 2 (p=1.000).

As shown in Table 4 the calculation of association of Somers' d, also did not show any significant relationship or association between glycemic control and ASB (p= 0.968; d= - 0.0005).

For female patients with DM type 2, 13 patients (52%) were found with controlled HbA1c (<7%) and 12 patients (48%) with uncontrolled HbA1c (> 7%). As shown in Table 5, urine culture showed ASB in 4 female patients with controlled HbA1c DM type 2 and seven patients with uncontrolled HbA1c DM type 2.

The $x2$ test results showed that there was no significant difference between ASB in female patients with controlled, and uncontrolled DM type 2 (p=0.165). Somers' d association calculation also did not show any significant difference between glycemic control and female patient DM type 2 with ASB (p=0.150; d = 0.276).

Urine culture results as shown in Table 6 showed that the bacteria most found were Gram-negative bacteria (10 from 13 samples with significant bacteria growth were found). E.coli was found in 4 samples, Enterococus cloacae, Klebsiella Pneumonia and Acinetobacter baumannii, .

Table 3. The difference of ASB between controlled and uncontrolled DM type 2

	ASB		p-value
	Negative (-)	Positive (+)	
Glycemic control (HbA1C)			
<7%	14	5	1.000*
≥ 7%	23	8	
Total	37	13	

*Fisher Exact Test

Table 4. Association between glycemic control with ASB

	ASB	
	P-value	**Somers' d association quotient (d)**
Glycemic control	0.968*	-0.005

*Somers' d association test

Enterococus faecalis were found in two samples each, last of all, Streptococus pyogenes was found in one sample

The experiment was done with an analytical observational cross-sectional method to see the relationship between ASB, and glycemic control in DM type 2 patients. Samples were 25 female and 25 male patients with a mean age of 54.16 (3.919) years old. The median of HbA1c 7.2% was with a range of 5.3 – 15.1%.

Urine culture results found ASB samples in 13 people (26% of the total samples) consisting of 11 female patients (44% of the total of female patients) and 2 male patients (8% of the total of male patients). These results were consistent with the previous experiments in various countries where the prevalence of ASB in DM patients varied from 5.8 – 53 %.[15,16]

The x^2 test showed the significant difference from sex variables towards BAS (p=0.004). The Somers' d association between sex and culture results were significant but weak and negative (p=0.001, d= - 0.360). These results were not different from previous experiments where the prevalence of ASB in female DM patients was 9–29%, and higher than male DM (0.7 – 11%).[17]

Table 5. Difference between ASB and female patients with controll0ed, and uncontrolled DM type 2

	ASB		
	Negative (-)	**Positive (+)**	**P-value**
Glycemic control (HbA1c)			
<7%			
≥ 7%	9	4	0.165*
	5	7	0.276^
Total	14	11	

*X2 test (chi-square)
^ Somers' d association
Tests were significant if p was <0.05

This happened because the anatomical distance between the colon from the female urethra is closer than the male and the female urethra is shorter. Other than that, the urethra's orifice with the vagina is a space that is easier to become bacterial colonization. Infection that comes from bacterial pathogen in the gut flora, spreads from the perineal vaginal and periurethral to the bottom of the urinary tract to form a colony. There were 19 patients (38%) with controlled HbA1c (<7%) and 31 patients (62%) with uncontrolled HbA1c. There were 13 female patients with HbA1c < 7%, and DM type 2 and 12 patients with HbA1c ≥ 7%, and 6 male with HbA1c < 7 % DM type 2 and 19 with ≥ 7%.

Urine culture results showed 5 patients with ASB and controlled HbA1c and eight patients with uncontrolled

Table 6. Bacteria of urine culture result

Types of bacteria	Total
Gram-negative bacteria	**10**
Escherichia coli	4
Enterobacter cloacae	2
Klebsiella pneumonia	2
Acinetobacter baumannii	2
Gram-positive bacteria	**3**
Enterococcus faecalis	2
Streptococcus pyogenes	1
Total	**13**

HbA1c. The Fisher test showed that there was no difference between ASB in controlled and uncontrolled DM type 2 (p=1.000). The Somers' d association calculation did not find any significant association between glycemic control with ASB happenings (p=0.968; d = -0.005).

These experiment results were the same as the previous experiments by Boroumand.[7] Boroumand et al. examinations on Iran females with DM type 2 showed no significant relationship or correlation between HbA1c with ASB (p=0.75).[7]

E.coli was the most often organism found in patients with ASB. There were many organisms infecting the urinary tract, including Enterobacteriaceae (including Proteus, Klebsiella, Enterobacter, and Citrobacter species), Pseudomonas aeruginosa, Enterococcus species, Gardnerella vaginalis, streptococci, staphylococci, Candida albicans, and other fungi.

In this experiment, E.coli was found in 4 of 13 samples that had significant bacteria growth. Enterococcus cloacae, Klebsiella pneumoniae and Acinetobacter baumannii, with Gram-negative bacteria and Enterococcus faecalis as a Gram-positive that were found in every 2 samples.

Results from this experiment were consistent with experimental results by Turan et al. Viswanath et al. and Boroumand et al.[l7-18] In these experiments the bacteria that caused ASB were usually from enteric colonies like E.coli and Enterobacter sp. In a couple other experiments, Klebsiella was reported as the most likely bacteria to cause ASB.16 In this experiment, the bacteria found to be the most likely cause of ASB were Gram-negative bacteria, with the highest percentage of E.coli the normal flora of the gut.

The capability of E.coli to infect the urinary tract is due to the pili or fimbriae that specifically becomes a mediator (adhesion) between urinary tract epithelial cells. Uropathogenic bacteria can reach the tissues of the urinary tract and adhere to the epithelial and start to colonize, by excreting toxins that cause an inflammatory reaction in that location and eventually the whole body.[18]

CONCLUSION AND SUGGESTION

There was no significant relationship between glycemic control with ASB happenings in DM type 2 patients. There were significant differences due to sex towards ASB, where ASB was found more in female patients with DM type 2 than male patients with DM type 2. The bacterial pattern from

this experiment was 76.9% Gram-negative bacteria and 23.1% Gram-positive bacteria.

Urinalysis screening must be done in DM type 2 patients with ASB. Further experiments are needed to analyze the relationship between glycemic control with ASB and other factors that can influence ASB, so ASB in DM type 2 patients can be prevented.

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