ORIGINAL ARTICLE

Isolation and Characterization of Multiple Antibiotic Resistance Streptococci Isolated From Blood Samples of Diabetics

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ABSTRACT

Background: Diabetic patients are considered to be immunocompromised and at high risk of developing group B streptococcal infection.

Aim: To isolate and characterized gram positive bacteria Streptococci from diabetic's blood samples.

Methods: This study was included a total 30 diabetic patients (both male and female) with age range between 19-60 years. Case history of patients was recorded. Blood samples were collected from each patient and were stored at 4° C until used. Bacterial strains were identified using different by biochemical test based on their morphological and biochemical characteristics. Antibiotic susceptibility pattern of multi-drug resistant Streptococci was analyzed using ciprofloxacin, gentamycin and amoxicillin.

Results: Duration of diabetes was 8-9 years. Positive family history was observed among (12) male and (11) female. Biochemical test including catalase test is negative, whereas 71.4% blood sample of patients showed beta hemolytic streptococci. It is observed that streptococcal isolates from 60% male and 37.5% female were sensitive towards ciprofloxacin. In case of gentamycin streptococci was sensitive among 28.62% male and 36.8% females, while Streptococci showed 100% resistant against amoxicillin-clavulanic acids.

Conclusion: It is therefore concluded that diabetic patients are at high risk of developing streptococcal infection. Antibiotic susceptibility pattern showed that ciprofloxacin is highly sensitive against the streptococcal infection as compared to gentamycin. Whereas streptococci are 100% resistant to amoxicillin-clavulanic acids. However further research is needed on large number of diabetic subjects to use more antibiotic to reach a better conclusion.

Keywords: Antibiotic resistance, streptococci, diabetese

INTRODUCTION

Diabetes Mellitus is a metabolic disease result from imbalance between insulin sensitivity and insulin secretion, secondary to insulin resistance[1]. According to WHO report, diabetes will be the 7th major cause of death in 2030[2]. In 2012, an approximately 1.5 million deaths were caused by diabetes. Greater than 80% of diabetes deaths occur in developing countries[3].

Diabetic patients are considered to be immunocompromised and at high risk of developing a variety of infectious complications, such as genitourinary infections, lower extrememity infections, respiratory infections caused by Escherichia coli, Salmonella, Streptococcus,S. aureus, S. pyogenes, and Mycobacterium tuberculosis[4,5].

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Infectious diseases are more frequent in patients with diabetes mellitus, which potentially increases their morbimortality[6]. Frequency of infections in diabetic patients is caused by the hyperglycemia that favors immune dysfunction, neuropathy decrease in the antibacterial activity of urine and GIT[7]. The infections affect all organs and systems especially in diabetic people, such as foot infections, malignant external otitis, and gangrenous cholecystitis. Additionally infection processes may be the precipitating factors for complications, such as diabetic ketoacidosis[8].

Group B ß-haemolytic streptococcus (GBS) (Streptococcus agalactiae) has been the leading pathogen causing serious infection in both developed and developing countries. Control of this infection has therefore become a major priority[9].

Diabetes is one of the most important underlying diseases for the development of group B streptococcal (GBS) infection. Strep B can cause urinary tract infections, skin infections and blood infections especially in susceptible people including old age people and diabetics[10].

Group B streptococci usually colonize skin and mucosal surfaces and may be isolated from infected sites along with other virulent organisms, their role in pathogenesis has often been questioned[11].

Although group B streptococci are susceptible to penicillin, minimum inhibitory concentrations are 4-fold to 8-fold higher than for group A streptococci. Its resistance to erythromycin and clindamycin is increasing. Streptococci may identify and classify on the basis of their hemolytic action on blood cells. They showed three distinct hemolytic pattern alpha, beta, and gamma hemolysis catalyzed by enzyme hemolysin[12].

Many bacteriostatic and bactericidal antibiotics such as Aminoglycosides, beta lactams, fluoroquinolones etc are known that are used for the treatment of Streptococcal infections.

The objective of present study was to isolate and characterized Streptococci from blood and to find out the role of ciprofloxacin, amoxicillin- clavulanic acid and gentamycin against streptococci in patients with chronic diabetes.

MATERIALS AND METHOD

This study was included a total 30 diabetic patients (both male and female as diagnosed by clinical physicians with age ranging between 19-60 years who attended outdoor department of Sir, Ganga Ram Hospital, Lahore, Pakistan during the period from April 2014 to June 2014. Case history of patients involving patient age, BMI, family history, life style and duration of diabetes were recorded. Blood samples were collected from each patient and were stored at 4° C until used.

To investigate Streptococci, blood samples were cultured on nutrient agar and incubated at 37°C for 24 hours. The isolation and identification of bacterial isolates were performed through gram staining. The bacterial strains were appeared as Cocci in chains. The cultures were purified by repeated streaking and the purified cultures were used for further studies.

Bacterial isolates was further confirmed by biochemical test. Catalase test was used to distinguish the gram positive Streptococci from other gram negative Cocci. Hemolytic test was used to identify each class of streptococci alpha, beta, or gamma.

Antibiotic susceptibility pattern of Streptococci was determined by using this diffusing method. Commercially prepared antibiotics, from Roseto (TE) Italy was used. Antibiotics of known potencies include gentamycin (10µg), amoxicillin- clavulanic acid (30µg) and ciprofloxacin(5µg) were used. Results were recorded by measuring zone of inhibition in (mm).

RESULTS

Table 1 demonstrated the demographic characteristics of male and female diabetic patients. Mean age of female and male was 39.78 and 44.5 respectively. Value of BMI of male (26.35kg/m^2) was less thanthe BMI of female (30.25kg/m^2). Duration of diabetes was long in male (9 year) as compared to female (8 years). The frequency of diabetes was higher among female of class (B) as compared to male of same socio-economic status. (5) male and (3) female patients were belongs to class (C). Positive family history was observed among (12) male and (11) female. Negative family history was also observed among (2) male and (5) female patients with diabetes.

According to table 2, gram staining of blood samples revealed that (14) male and (16) female patients showed gram positive bacteria (cocci in chains). Catalase test of blood samples showed that (10) male and (10) female were highly catalase negative. Results of hemolytic test of blood samples showed that (20%) male (28.57%) female were alpha hemolytic. Beta hemolysis was observed among (71.42%) male and (66.66%) female blood samples. Gamma hemolysis was observed among female (13.33%) while no gamma hemolysis were observed among male patients

Results in table 3, showed the antibiotic resistance/susceptibility of Streptococcus isolates from diabetic's blood. Results revealed that streptococcal isolates from 60% male and 37.5% female highly sensitive towards ciprofloxacin. In case of gentamycin streptococci were highly sensitive among 28.62% male patients while 71.4% male samples were highly resistant to it. On the other hand streptococci showed 100% resistant against amoxicillin-clavulanic acid.

Table 1: Comparison of demographic characteristics of male and female diabetes mellitus. No of cases in parenthesis.

Characteristics	Male	Female
Age (year)	39.8±11.4 (14)	44.5±69.4 (16)
BMI (Kg/m2)	26.35±4.5	30.25±5.79
Duration of diabetes	9 year	8 year
Socio-economic status	(09) Class 'B' (05) Class 'C'	(13) Class 'B' (03) Class 'C'
Family history	12+ve 02-ve	11+ve 05-ve

Table 2. Comparison of biochemical test of streptococci among diabetic patients.

Gender	Gram staining	Catalase test	α –hemolysis	β -hemolysis	γ -hemolysis
Male	14 gram positive	Negative (10) Slightly negative (4)	4 (28.57%)	10 (71.42%)	0
Female	16 gram positive	Negative (2) Slightly negative (14)	3 (20%)	10 (66.66%)	2 (13.33%)

Table 3. Percentage of antibiotic resistance/sensitivity of Streptococci in blood of diabetic patients.

Antibiotic	Male		Female	
	R	S	R	S
Ciprofloxacin	40%	60%	62.5%	37.5%
Amoxicillin (Clavulanic acid)	100	0	100%	0
Gentamycin	71.4%	28.6%	64.2%	36.8%

DISCUSSION

Streptococci is a significant pathogen especially in advanced age, diabetes, and liver failure, may increase severity of disease as much as 30 fold in individual[13].

Results of blood cultures revealed that positive bacterial blood cultures were observed in all diabetic patients. Present study revealed that Gram positive bacteria (100%) were the major pathogen, 2(6%) samples showed the Gram positive bacteria with 1-2 rods. However a group of workers observed that[14] Gram positive bacteria were the major pathogen (70%) in blood of diabetics. It is suggested that GBS is likely to induce bacteremia and also invade every parts of body especially in diabetics[10].

Present study found that out of 30 samples, 23% were alpha hemolytic, 67% exhibited beta hemolytic and only 7% exhibited the gamma hemolysis. Compared with present study, it is reported[15] that most of the blood samples showed the presence of beta hemolytic streptococci[15]. However a group of workers identified both alpha and beta streptococci in diabetic patients[16].

According to our study ciprofloxacin as compared to gentamycin was more effective for male patient, as compared to female but in case of amoxicillin clavulanic acid both group showed high resistance against GBS in blood of both sexes.

Present study observed that streptococci was 60% sensitive to ciprofloxacin. These findings are in agreement with those obtained by other researcher[15] (Al-Saadi) who mentioned that Streptococci was 64% sensitive to ciprofloxacin. Another researcher[15] mentioned that resistance to ciprofloxacin was (17.5%). Our results revealed that gentamycin was found to be little effective (28.62%) against streptococci. However a study observed that[16] streptococci were highly resistant to gentamycin.

Results expressed that streptococci were highly resistant (100%) to amoxicillin-Clavulanic acid. Results are in common with a study[14] who observed streptococci highly resistant to amoxicillin. However a study reported that Streptococci can be treated with amoxicillin[17].

Limitation of the study: Patients included in the study were very small. More drugs should be tried against streptococci.

CONCLUSION

It is therefore concluded that diabetic patients are at high risk of developing streptococcal infection. Antibiotic susceptibility pattern showed that ciprofloxacin is highly sensitive against the streptococcal infection as compared to gentamycin. Whereas streptococci are 100% resistant to amoxicillin-clavulanic acids. However further research is needed on large number of diabetic subjects to use more antibiotic to reach a better conclusion.

REFERENCES

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2009 Jan; 32(Suppl 1): S62–S67.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med, 2006, 3(11):e442
3. World Health Organization. Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012. Geneva, WHO, 2014.
4. Stephen c, Braithwaite s, et al. "Management of Diabetes and Hyperglycemia in Hospitals." Diabetes Care. 2004;27(2) 553-91.
5. Dennis S, Bisno A, et al. "Oxford Journals: Clinical Infectious Diseases–Guidelines for Skin and Soft-Tissue Infections." Oxford Journals: Clinical Infectious Diseases–Guidelines for Skin and Soft-Tissue Infections. 2005: 1373-1406
6. Rantala, S.; Vuopio-Varkila, J.; Vuento, R.; Huhtala, H.; Syrjänen, J., Clinical presentations and epidemiology of β-haemolytic Streptococcal bacteremia: a population-based study, Clinical Microbiology and Infection. 2009; 15, 286-288.
7. Aguilar F, Rayo MD. Part 1, Diabetic Neuropathy: Classification, physiopathology and clinical manifestations. Rev Med 2000:257-266

8. Casqueiro J, Casqueiro J and Alves C.Infections in patients with diabetes mellitus: A review of pathogenesis. Indian J Endocrinol Metab. 2012 Mar; 16(Suppl1): S27–S36

9. Schrag SJ, Verani JR. Intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease: experience in the United States and implications for a potential group B streptococcal vaccine. Vaccine 2013;31 Suppl 4:D20-6.

10. Jackson LA, Hilsdon R, Farley MM et al. Risk factors for group B streptococcal disease in adults. Ann Intern Med 1995;123:415-20.

11. Farley MM. Group B Streptococcal Disease in Nonpregnant Adults. Clin Infect Dis. 2001; 33 (4): 556-568

12. Kilian, M. Streptococcus and Enterococcus. Medical Microbiology,2000; 17, 174-93.

13. Muñoz P, Llancaqueo A, Rodríguez-Créixems M, Peláez T, Martin L, Bouza E. Group B streptococcus bacteremia in nonpregnant adults. Arch. Int. Med 1997; 157: 213-216

14. Morales, MV, Navarro CJS, Lleti MS, Alonso MM, Belles CP, Aldeguer JL et al. Group A Streptococcal bacteremia: outcome and prognostic factors, Revista Espanola de Quimioterap 2006; 19, 367-375.

15. Al-Saadi M.A, Al-Charrakh AH, Al-Greti SH. Prevalence of bacteremia in patients with diabetes mellitus in Karbala, Iraq, Journal of Bacteriology Research 2011; 3, 108-116

16. Murdoch DR & Reller LB. Antimicrobial susceptibilities of group B Streptococci isolated from patients with invasive disease: 10-year perspective, Antimicrobial agents and Chemotherapy 2001; 45, 3623-3624

17. Edmond M. The treatment of diabetic foot infections: focus on ertapenem. Vasc Health Risk Manag. 2009; 5: 949–963.