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Research Article

Antibody and serum bactericidal response to *Burkholderia pseudomallei* in acute localized and septicemic melioidosis cases with diabetes mellitus

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Abstract

Background and objectives: Melioidosis, caused by the gram-negative bacillus *Burkholderia pseudomallei*, is a major cause of fatal community acquired infection in diabetic patients. Protective immune response in human melioidosis is not clearly understood yet. In this study, serum IgM/IgG and bactericidal antibody response to *B. pseudomallei* were determined in diabetic patients with acute localized abscess and septicemia.

Material and methods: Culture positive melioidosis cases with diabetes mellitus were included in the study. Blood samples were collected from the respective cases in active phase of the disease within 1 or 2 days of being culture positive. Anti- *B. pseudomallei* IgM and IgG and serum bactericidal antibody were measured by ELISA and microplate based bactericidal assay respectively.

Results: A total of 10 culture positive acute melioidosis cases with diabetes mellitus were included in the study. Out of 10 cases, 5 had abscess in different organs and 5 had septicemia. The mean age of the patients was 48.5 ± 3.91 years and 7 (70%) were male and 3 (30%) were female. The mean anti- *B. pseudomallei* IgM titer of septicemic and abscess cases were not significantly different (14,080 ± 4,489.13 vs. 19,200 ± 3,620.39; p = 0.4) while the mean IgG titers of two groups were > 204,800. Out of 10 cases, 9 (90%) were positive for serum bactericidal antibody. Mean serum bactericidal antibody titer of septicemia cases (66 ± 26) was not significantly (p = 0.72) different than those of localized infection (80 ± 28.28).

Conclusion: The results indicate that high anti- *B. pseudomallei* IgM/IgG and serum bactericidal antibodies are induced in diabetic patients with septicemia and suppurative infections. This immune response in diabetics might be important to contain the infection and help in recovery.

Introduction

Melioidosis, caused by gram-negative bacillus *Burkholderia pseudomallei*, is endemic in at least 45 countries across the tropical areas, and globally an estimated 89,000 deaths occur per year [1].

Studies in human have reported better survival of melioidosis patients in the presence of elevated anti-lipopolysaccharide II and anti-hemolysin co-regulated protein 1 IgG antibodies [2,3]. Also, humoral immune response to *B. pseudomallei*

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provided protection against infection in animal model [4]. Therefore, determining the role of antibody mediated protection in melioidosis would help in developing an effective vaccine and therapeutic monoclonal antibodies.

Antibody dependent complement mediated bacterial killing is an important immune defense against intravascular invasion of bacterial pathogens and is mediated by formation of membrane attack complex assembled from terminal complement components on the bacterial cell envelope [5,6]. By this mechanism of immune response, *Pseudomonas aeruginosa* infecting chronically infected cystic fibrosis (CF) patients are eliminated thus reducing the risk of pseudomonal bacteremia/septicemia in CF patients [7-9]. Moreover, antibody-dependent complement mediated killing of meningococci provide protection against invasive meningococcal disease was observed [10]. On the contrary, no association between presence of anti- salmonella bactericidal activity and protection against typhoid was seen following vaccination with Ty21a or M01ZH09 [11]. However, bactericidal activity after vaccination correlated significantly with delayed disease onset, lower bacterial burden and decreased disease severity.

Likewise in melioidosis cases, antibody dependent complement-mediated killing ability of host might be linked to the progression of localized infection to septicemia. The sensitivity or resistance of offending *B. pseudomallei* to antibody dependent complement mediated killing would help in understanding its association with disease progression. In view of the above, this study aimed to find out the serum bactericidal and antibody response against *B. pseudomallei* in diabetic melioidosis patients with different clinical manifestations. This would help to understand the role of serum bactericidal response on the progression and outcome of *B. pseudomallei* infection in diabetics.

Materials and methods

The study was approved by the Institutional Review Board of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) General Hospital, Dhaka, Bangladesh (Protocol code - BIRDEM/IRB/2017/69 and date of approval – 21/6/2017). Informed consent was obtained from all participants involved in the study.

Study population and collection of samples*:* Culture positive melioidosis cases with diabetes mellitus admitted in BIRDEM General Hospital were included in the study. Diagnosis of diabetes mellitus (DM) was based on HbA1c level ≥ 6.5% or fasting plasma glucose (FPG) ≥ 7.0 mmol/L or two-hour plasma glucose ≥ 11.1 mmol/L by oral glucose tolerance test (OGTT) or a random plasma glucose of ≥ 11.1 mmol/L [12]. Blood samples were collected from the respective case in active phase of the disease within 1 or 2 days of being culture positive. About 3-5 ml of blood was collected aseptically and serum was separated immediately and stored at -20^{o}C until tested. The serum samples of melioidosis patients were tested for anti- *B. pseudomallei* IgM and IgG antibodies and serum bactericidal antibody. Sera from healthy newborn babies (age less than 30 days) who were unlikely to get *B. pseudomallei* infection were used to determine the cut off for optical density (OD) value of ELISA test.

Determination of anti- B. pseudomallei IgM and IgG antibodies by ELISA: Serum anti- *B. pseudomallei* IgM and IgG antibodies were measured by an in-house indirect ELISA as described by Jilani et al [13]. The 96-well flat bottom ELISA plate (Greiner Bio-One GmbH, Germany) was coated with sonicated whole cell antigen in carbonate/bicarbonate coating buffer (pH 9.6; 100 µl/well) and incubated overnight at 4^{o}C. The plate was washed three times with phosphate buffered saline containing 0.05% Tween-20 (PBS-T, pH 7.4). To prevent non-specific binding, blocking buffer PBS-T containing 2% bovine serum albumin was added to each well (200 µl/well) and incubated at 37^{o}C for 2 hours. The plate was then washed three times with PBS-T. To detect anti- *B. pseudomallei* IgM and IgG, 100 µl of serially diluted serum sample in PBS-T (1:100 to 1:204,800) was added per well. The plate was incubated for 4 hours at 37^{o}C. After washing with PBS-T three times, 100 µl/well of horseradish peroxidase conjugated anti-human IgM and IgG antibodies at 1:4,000 dilutions (MP Biomedicals, USA) was added and incubated at 37^{o}C for 2 hours.

After washing three times with PBS-T, tetramethylbenzidine substrate (50 µl/well) was added and incubated at room temperature for 30 minutes in dark. The reaction was stopped by adding 50 µl of 2M H_2SO_4 per well and the optical density (OD) was read at 450 nm.

Interpretation: A cut off OD values for anti- *B. pseudomallei* IgM and IgG antibodies were determined with sera from 15 newborn babies of Dhaka city, who were unlikely to get *B. pseudomallei* infection. The mean OD + 2 × SD of newborn sera was taken as cut off OD. The calculated cut off OD values for anti- *B. pseudomallei* IgM and IgG were 0.14 and 1.24 respectively. Any sample showing OD above these cut off values was considered positive.

Bactericidal antibody assays: Serum bactericidal antibody assay was carried out with the strain of *B. pseudomallei* CS6887, MLST type ST56 [14]. The bacterial strain was isolated from a Bangladeshi melioidosis patient with septicemia. A microtiter plate based bactericidal assay was performed as described previously (15,16]. A single colony of *B. pseudomallei* grown overnight on MacConkey agar plate was inoculated in 5 ml of trypticase soya broth (TSB, Himedia Laboratories Pvt. Ltd., India) and incubated overnight at 37°C aerobically. The bacteria were harvested by centrifugation and suspended in cold phosphate-buffered saline (PBS; pH 7.4) having cell count of 3×10^8 CFU/ml. Guinea pig sera was used as external source of complement. A 1:10 dilution of guinea pig serum was prepared with cold PBS. A stock solution of bacteria plus complement containing 2.5×10^6 CFU/ml of bacteria was prepared by adding bacterial suspension to guinea pig complement and cold PBS. All serum samples were heated at 56°C for 30 minutes to inactivate complement in the test sera prior to use. Serial dilutions (two fold) of serum samples were prepared in cold PBS from 1:5 to 1:10,240 in sterile U bottom microtiter plate with lid (Greiner Bio-One GmbH, Germany). To each well containing 25 µl of serially diluted sera 25 µl of the mixture of bacteria, complement and PBS (2.5×10^6 CFU/ml) was added. Each plate had 4 control wells. Each control well contained 50 µl of (i) suspension of bacteria plus complement plus PBS, (ii) only serum, (iii), PBS and (iv) TSB. The control well (i) containing bacteria plus complement plus PBS without serum was included to determine bactericidal antibody titer of samples while control wells (ii), (iii) and (iv) were used to exclude the bacterial contamination in test procedure. The microtiter plate was incubated at 37°C for 1 hour. Then, TSB (150 µl/well) was added to each well and incubated overnight at 37°C. The OD values of the plates were measured at 595 nm.

Interpretation: The bactericidal antibody titer was measured as the reciprocal of the highest serum dilutions causing a greater than 50% reduction of the OD when compared with the OD of the control well containing bacteria, complement and PBS without serum. To further confirm bacterial killing, viable bacterial cell count was performed by sub-culturing the content of the wells on Trypticase Soya Agar plate.

Results

A total of 10 culture positive (*B. pseudomallei*) acute melioidosis cases with diabetes mellitus were included in the study. Out of 10 cases, 5 had localized infection in the form of abscess in different organs and 5 had septicemia (blood infection). *B. pseudomallei* was isolated from blood and from aspirated pus of septicemia and abscess cases respectively. The mean age of the patients was 48.5 ± 3.91 years and age ranged from 32 to 70 years. Mean age of the two groups was not significantly different (49.2 ± 5.7 and 47.8 ± 5.9 years; p = 0.86). Out of 10 cases, 7 (70%) were male and 3 (30%) were female (Table-1).

All 10 cases were positive for anti- *B. pseudomallei* IgM and IgG antibodies. The mean titer of anti- *B. pseudomallei* IgM and IgG of all cases were 16,640 ± 2995.04 and > 204,800 respectively. The mean anti-*B. pseudomallei* IgM titer of septicemic and abscess cases were not significantly different (14,080 ± 4,489.13 vs. 19,200 ± 3,620.39; p = 0.4) while the mean IgG titers of two groups were > 204,800. Out of 10 cases, 9 (90%) were positive for serum bactericidal antibody. Serum bactericidal antibody was negative in one 43 years old male patient with lung abscess. Mean serum bactericidal antibody titer of septicemia cases (66 ± 26) was not significantly (p = 0.72) different than those with localized infection (80 ± 28.28). Total mean bactericidal antibody titer of all cases was 72.22 ± 18.08.

Table-1: *Characteristics and* anti- *B. pseudomallei IgM, IgG and serum bactericidal antibody titers of study cases (N=10)*

Case	Gender	Age (Years)	Anti- *B. pseudomallei IgM*		Anti- *B. pseudomallei IgG*		SBA titer
			Titer Mean ± SE	Positive n (%)	Titer Mean ± SE	Positive n (%)	
Septicemia							
Septicemia	Male	50	3200		>204,800		1:80
Septicemia	Male	57	3200		> 204,800		1:10
Septicemia	Male	32	12,800	5 (100)	> 204,800	5 (100)	1:40
Septicemia	Male	42	25,600		> 204,800		1:40
Septicemia	Female	65	25,600		> 204,800		1:160
Mean ± SE		**49.2 ± 5.7**	**14,080 ± 4,489.13**		**-**		**66 ± 26**
Abscess in							
Lung	Male	43	25,600		> 204,800		0 (Neg)
Liver	Male	38	25,600		> 204,800		1:160
Spleen	Male	50	12,800	5 (100)	> 204,800	5 (100)	1:80
Kidney	Female	70	6,400		> 204,800		1:40
Left leg	Female	38	25,600		> 204,800		1:40
Mean ± SE		**47.8 ±5.9**	**19,200 ± 3,620.39**		**-**		**80 ± 28.28**

Note: SBA – serum bactericidal antibody; p = 0.87, compared age of septicemia and abscess groups; p = 0.4, compared anti- B. pseudomallei IgM of septicemic and abscess groups; p = 0.72, serum bactericidal antibody titer of septicemia and abscess cases; p value calculated by t test.

Discussion

B. pseudomallei is a highly pathogenic bacteria for human. The immune response in *B. pseudomallei* infection and its role in underlying pathology are not clear. Previous study has reported that there is no association between high antibody titers against whole cell *B. pseudomallei* antigens and protection against *B. pseudomallei* infection in human patients. In addition, the pathophysiology of being infected with *B. pseudomallei* in spite of having high background antibody titer in individuals residing in endemic zone is not clear [17]. On the other hand, an association of survival with high titer of antibodies to hemolysin co-regulated protein 1 was also reported [2]. Also, high antibody titer against *B. pseudomallei* is linked to survival of diabetic patients suffering from melioidosis [18].

Many studies used opsonic assays to investigate the role of antibodies in melioidosis [4,19]. In the present study, elevated levels of anti- *B. pseudomallei* IgM and IgG were found among all diabetic melioidosis patients and the antibodies were capable of complement mediated killing of *B. pseudomallei*. The lack of difference in antibody response between cases with septicemia and localized infection might be due to inoculating pathogen burden or undiagnosed underlying comorbidities in patients with localized abscess cases. Also, high antibody titer against *B. pseudomallei* has been reported among survivors of melioidosis patients with diabetes mellitus [18].

The mean bactericidal antibody titer of patients with septicemia and localized abscess was not significantly different from each other groups (66 ± 26 vs. 80 ± 28.28) except in one male patient with abscess where the bactericidal antibody was negative though IgM and IgG antibody titers were high. Similar magnitude of serum bactericidal antibody in both groups could be due to the fact that we could not ascertain the duration/

persistence of infecting bacteria in the hosts. Also, it indicates that in diabetic patients, serum sensitivity of bacteria due to complement mediated lysis might not be adequate to protect against bacteremia or septicemia. Also, the course of infection might depend on the type of infecting strains as serum sensitive and serum resistant strains were described previously for *Burkholderia cepacia* complex [20]. Negative bactericidal antibody in our patient could be due to the fact that the testing organism in our assay was serum resistant type. Further studies are necessary to exclude the above possibilities.

The limitations of the present study were small sample size and lack of melioidosis cases without diabetes. Besides, the cases of this study might be an acute exaggeration of chronic *B. pseudomallei* infection. So, actual scenario of antibody response and its role could be deciphered if kinetics of the antibody titer and serum bactericidal antibody titer could be performed from the entry of the bacteria and onset of the infection. In addition, we have used a single strain in our SBA assay for all serum samples. The magnitude of bactericidal antibody could be actually different in patients with either septicemia or localized infection if, we could use corresponding strains in the assay to exclude the possibility of serum resistance of the testing strain in our assay.

The study demonstrated that diabetic patients with melioidosis were capable of mounting good anti- *B. pseudomallei* IgM, IgG and bactericidal antibodies in both blood and localized infection types. Though there was equal bactericidal response in both groups, bactericidal antibody alone might not be enough to prevent the bacteremia in diabetic patients. Further studies with large number of cases are necessary to understand the complex immunopathology responsible for varied manifestation of melioidosis.

Authors' contributions

SM: laboratory investigation, analysis, writing original draft; MSAJ: methodology, resources, supervision and editing; LB: methodology, resources, supervision, KMSI: supervision, writing review & editing.

Competing interest

The authors declare no conflict of interest.

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References

1. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, et al. Predicted global distribution of and burden of melioidosis. *Nat Microb*. 2016; **1**: 15008. doi:10.1038/nmicrobiol.2015.8.
2. Pumpuang A, Phunpang R, Ekchariyawat P, Dulsuk A, Loupha S, Kwawong K, et al. Distinct classes and subclasses of antibodies to hemolysin co-regulated protein 1 and O-polysaccharide and correlation with clinical characteristics of melioidosis patients. *Sci Rep*. 2019; **9**: 13972. doi:10.1038/s41598-019-48828-4.
3. Charuchaimontri C, Suputtamongkol Y, Nilakul C, Chaowagul W, Chetchotisakd P, Lertpatanasuwun N, et al. Antilipopolysaccharide II: an antibody protective against fatal melioidosis. *Clin Infect Dis*. 1999; **29**: 813–818. doi:10.1086/520441.
4. Chaichana P, Kronsteiner B, Rongkard P, Teparrukkul P, Limmathurotsakul D, Chantratita N, et al. Serum from melioidosis survivors diminished intracellular *Burkholderia pseudomallei* growth in macrophages: a brief research report. *Front Cell Infect Microbiol*. 2020; **10**: 442. doi:10.3389/fcimb.2020.00442.
5. Walport MJ. Complement. First of two parts.*N Engl J Med*. 2001; **344**: 1058-1066. doi:10.1056/NEJM200104053441406.
6. Frank MM, Joiner K, Hammer C. The function of antibody and complement in the lysis of bacteria. *Rev Infect Dis*. 1987; **9Suppl 5**: S537-S545.doi:10.1093/clinids/9.supplement_5.s537.
7. Speert DP, Farmer SW, Campbell ME, Musser JM, Selander RK, Kuo S. Conversion of *Pseudomonas aeruginosa* to the phenotype characteristic of strains from patients with cystic

fibrosis. *J Clin Microbiol*. 1990; **28**: 188-194. doi:10.1128/jcm.28.2.188-194.1990.

8. Thomassen MJ, Demko CA. Serum bactericidal effect on *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Infect Immun*. 1981; **33**: 512-518. doi:10.1128/iai.33.2.512-518.1981.
9. Holby N, Olling S. *Pseudomonas aeruginosa* infection in cystic fibrosis. Bactericidal effect of serum from normal individuals and patients with cystic fibrosis on P. aeruginosa strains from patients with cystic fibrosis or other diseases. *Acta Pathol Microbiol Scand C*. 1977; **85**: 107-114. PMID: 404841.
10. Findlow J, Balmer P, Borrow R. A review of complement sources used in serum bactericidal assays for evaluating immune responses to meningococcal ACWY conjugate vaccines. *Hum VaccinImmunother*. 2019; **15**: 2491-2500. doi:10.1080/21645515.2019.1593082.
11. Juel HB, Thomaides-Brears HB, Darton TC, Jones C, Jones E, Shrestha S, et al. Salmonella Typhi bactericidal antibodies reduce disease severity but do not protect against typhoid fever in a controlled human infection model. *Front Immunol*. 2018; **8**: 1916. doi:10.3389/fimmu.2017.01916.
12. American Diabetes Association.Classification and diagnosis of diabetes mellitus. *Diabetes Care*. 2017; **34**: 2–7.
13. Jilani MSA, Robayet JA, Mohiuddin M, Hasan MR, Ahsan CR, Haq JA. *Burkholderia pseudomallei*: its detection in soil and seroprevalence in Bangladesh. *PLoSNegl Trop Dis*. 2016; **10**: e0004301. doi:10.1371/journal.pntd.0004301
14. Jilani MSA, Farook S, Bhattacharjee A, Barai L, Ahsan CR, Haq JA, et al,. Phylogeographic characterization of *Burkholderia pseudomallei* isolated from Bangladesh. *PLoSNegl Trop Dis*. 2023; **17**: e0011823. doi:10.1371/journal.pntd.0011823.
15. Boyd MA, Tennant SM, Saague VA, Simon R, Muhsen K, Ramachandran G, et al. Serum bactericidal assays to evaluate typhoidal and nontyphoidal Salmonella vaccines. *Clin Vaccine Immunol*. 2014; **21**: 712-721. doi:10.1128/CVI.00115-14.
16. Qadri F, Mohi G, Hossain J, Azim T, Khan AM, Salam MA, et al. Comparison of the vibriocidal antibody response in cholera due to *Vibrio cholerae* O139 Bengal with the response in cholera due to *Vibrio cholerae* O1. *Clin Diagn Lab Immunol*. 1995; **2**: 685-688. doi:10.1128/cdli.2.6.685-88.1995.
17. Chaichana P, Jenjaroen K, Amornchai P, Chumseng S, Langla S, Rongkard P, et al. Antibodies in melioidosis: the role of the indirect hemagglutination assay in evaluating patients and exposed populations. *Am J Trop Med Hyg*. 2018; **99**: 1378-1385. doi:10.4269/ajtmh.17-0998.
18. Kronsteiner B, Chaichana P, Sumonwiriya M, Jenjaroen K, Chowdhury FR, Chumseng S, et al. Diabetes alters immune response patterns to acute melioidosis in humans. *Eur J Immunol*. 2019; 49: 1092-1106. doi:10.1002/eji.201848037.
19. Zhang S, Feng SH, Li B, Kim HY, Rodriguez J, Tsai S, et al. In vitro and in vivo studies of monoclonal antibodies with prominent bactericidal activity against *Burkholderia pseudomallei* and Burkholderia mallei. *Clin Vaccine Immunol*. 2011; **18**: 825-834. doi:10.1128/CVI.00533-10.
20. Zlosnik JE, Gunaratnam LC, Speert DP. Serum susceptibility in clinical isolates of *Burkholderia cepacia* complex bacteria: development of a growth-based assay for high throughput determination. *Front Cell Infect Microbiol*. 2012; **2**: 67. doi:10.3389/fcimb.2012.00067.

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