Journal of Infection and Public Health (2016) 9, 267—277

http://www.elsevier.com/locate/jiph

Association of bacteria in diabetic and non-diabetic foot infection — An investigation in patients from Bangladesh

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Received 27 April 2015; received in revised form 21 July 2015; accepted 7 October 2015

KEYWORDS
Diabetic foot infection;
Multidrug resistance;
C-reactive protein;
Bangladesh

Summary The microbial community on a host relies on its immune status and pathophysiological condition. *Diabetes mellitus* is a metabolic disorder associated with a 25% increased risk of developing foot infection. The pathophysiological differences between diabetic foot infection (DFI) and non-DFI patients may alter the microbial composition in infections. The present study aims to comparatively analyze the microbes colonized in DFI and non-DFI patients in Bangladesh. Pus specimens were collected from 67 DFI and 12 non-DFI patients to investigate the bacteria associated with foot infection. For this investigation, an array of microbiological, molecular biological and immunological approaches were performed. Common bacteria detected in both DFI/non-DFI samples were *Pseudomonas* spp. (22/29%), *Bacillus* spp. (12/3%), *Enterobacter* spp. (22/7%), *Staphylococcus* spp. (13/13%) and *Acinetobacter* spp. (10/10%). *Enterococcus* spp. (9%) and *Klebsiella* spp. (8%) occurred only in DFI patients, whereas *Citrobacter* spp. (29%) was only detected in non-DFI samples. The rate of occurrence of three organisms, namely, *Enterococcus* spp. $|Z| = 2.2125$, *Klebsiella* spp. $|Z| = 1.732$, *Bacillus* spp. $|Z| = 1.9034$, were also statistically significant. Most of the isolates from DFI patients were commonly resistant to the cephalosporin (Ceftazidime, Ceftriazone, Cefurozime) and monobactam (Aztreonam) groups of antibiotics. DFI patients had comparatively higher C-reactive protein (CRP) levels than non-DFI patients, and a positive correlation was observed between multi-antibiotic resistance and CRP levels (one of the markers of chronic

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http://dx.doi.org/10.1016/j.jiph.2015.10.011

subclinical inflammation). The present investigation implicated a complex association of the bacterial population in DFI compared with non-DFI with different antimicrobial resistance properties, which was linked with CRP levels.

Introduction

Diabetes mellitus (*DM*) is now a major health problem all over the world and is increasing globally at an alarming rate [1]. It has been declared an epidemic in developing countries, including Bangladesh [2]. Approximately 347 million people are suffering from *DM* worldwide, which is predicted to double by the year 2025 [3]. The prevalence of diabetes in Bangladesh is increasing rapidly, leading to complications of chronic diabetes. Diabetic foot infection (DFI) is one of the most serious complications of *DM*. Diabetic patients have a 25% increased risk of developing a foot ulcer [4]. The primary causes of DFI are microbial agents, and their early diagnosis is essential for appropriate antimicrobial therapy [5]. Once an infection has developed in DFI patients, it is difficult to treat because of impaired microvascular circulation to the lower limb, which limits the access of phagocytic cells and antibiotics to infected sites [6]. Common organisms reported in foot infection are mainly *Staphylococcus* spp. and *Enterococcus* spp. arising from the patient's own body [7]. Extensive tissue destruction and poor blood circulation are a result of infection with *Pseudomonas* spp., *Proteus* spp., and *Enterococcus* spp. bacterial groups [7]. The major predisposing factor associated with these infections is foot ulceration, which is usually related to peripheral neuropathy and peripheral vascular disease, and various immunological disturbances play a secondary role in the development of diabetic foot ulceration [8]. Chronic subclinical inflammation (CSI) reportedly has a significant association with the development of acute diabetic foot syndrome [9]. C-reactive protein (CRP) is an acute phase protein whose concentration increases in the blood in response to inflammation. CRP is a marker of CSI [10].

There is scant knowledge on the microbial composition inhabiting the DFI area and the correlation of these microbes to CSI. The present study was performed to determine the bacterial composition in foot lesions of DFI and non-DFI patients and their antibiotic resistance patterns. The investigation also explores the correlation of serum CRP levels with microbial composition and resistance properties. This study is expected to generate valuable information, which will be helpful in the management and prevention of foot infection in our population and will help clinicians to select and develop appropriate drugs.

Materials and methods

Specimen collection

Pus specimens were collected from infected foot wound sites of 67 DFI and 12 non-DFI patients from the Diabetic Foot Care Hospital (DFCH) and Dhaka Medical College Hospital (DMCH), respectively. All of the diabetic foot ulcers were included in this study irrespective of ulcer grading. According to the questionnaire prepared for this study, subjects suffering from diseases such as cancer, autoimmune diseases, cardiovascular diseases and renal diseases were excluded. Pus specimens were collected from the patients after ulcer base debridement. To avoid contamination, foot wounds and tissue debris were thoroughly cleaned with sterile normal saline followed by gentle rubbing of the wound site with 70% alcohol prior to swabbing the pus sample. Sterile cotton swab sticks were moistened with sterile normal saline before specimen collection. Then, the swab sticks were extended deeply into the depth of the lesion to avoid contamination from the wound surroundings. When copious volumes of pus existed, samples were collected aseptically by needle aspiration to avoid major exogenous contamination. After pus sample collection, the swabs were transported to the laboratory by immersion to maintain aseptic conditions (20-ml test tube containing 10 ml of peptone water). The samples were properly labeled and immediately transported to the laboratory for further investigation. Blood samples were also collected from these patients for investigation of subclinical inflammation. A detailed history was collected from each of the subjects and control patients, and the demographic data included age, sex, occupation, socioeconomic status, type of water used, and type of treatment used.