ASSOCIATION BETWEEN POLYMICROBIAL INFECTIONS AND SEVERITY OF DIABETIC FOOT INFECTION AMONG PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

by

DR SHARIFAH AISYAH BINTI SAYED HITAM

Dissertation Submitted In Partial Fulfillment Of The Requirements For The Degree Of Master of Pathology (Microbiology)

2016

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Background: Foot infections are a major complication of patients with diabetes mellitus (DM). The causative microorganisms are usually polymicrobial.The aim of the study is to determine the percentage of microorganism in diabetic foot infection, its susceptibility to antibiotic, association between polymicrobial infection and severity of diabetic foot infection and to determine the outcome of diabetic foot infection among patients infected with polymicrobial organisms in Hospital USM.

Materials and methods: This is a retrospective analysis conducted for one year duration starting from June 2014. A total of 104 patients selected from this study. Their folders were reviewed and assessed for severity and outcome of foot infection in association with polymicrobial infections. Parameters such as glycated haemoglobin (HbA1c), random blood sugar (RBS), total white cell (TWC) and haemoglobin (Hb) were analysed. The result were presented as descriptive and statistically analysed by Fisher exact test.

Results: There were 104 patients involved in this study, with a total 133 microorganisms were isolated with an average of 1.28 organisms per lesion. Gram negative (GN) and Gram positive (GP) microorganisms were 62% and 38% respectively.The most frequently isolated GN microorganism includes *Pseudomonas* spp (28%), *Proteus* spp (11%), *Klebsiella* spp (8%) and *E. coli* (4%). *Staphylococcus aureus* was the predominant (54%) among GP microorganisms followed by Group B *Streptococci* (26%) and *Enterococcus* spp (6%). GN isolates were sensitive to carbapenem and aminoglycoside groups while vancomycin showed good activity to GP microorganism. There was significant association between quantity of microorganisms and severity of diabetic foot infection using Fisher's Exact test (p=0.003). Thirty patients had polymicrobial infections. In severe diabetic foot infection, 77.8% with polymicrobial organisms undergone amputation, meanwhile monomicrobial infection was 33.3%. Majority of polymicrobial or monomicrobial infection was discharged well, 84.0% and 91.1% respectively. There was no significant association between polymicrobial or monomicrobial infection with patient's outcome of severe diabetic foot infection including amputation and discharge of patient (p=0.136 and p=0.465). The mean (SD) for HbA1c, RBS, TWC and Hb in severe polymicrobial infections were 11.8(2.1) %, 16.3 (5.2) mmol/L, 16.6 (2.4) x 10^9 and 9.0 (1.3) g/dL respectively.

Conclusion: GN microorganisms were predominantly isolated from diabetic foot infections and antibiogram showed that the common organisms remain sensitive to a number of widely used agents. Polymicrobial infections were associated with the severity of its infection meanwhile quantities of organisms was not associated with patient's outcome of diabetic foot infection. There were higher glucose level and TWC count with lower Hb in severe polymicrobial diabetic foot infection.

Professor Madya Dr Siti Asma’ binti Hassan : Supervisor

Dr Nurahan binti Maning : Co-Supervisor

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TABLE OF CONTENT

ACKNOWLEDGEMENT ii
TABLE OF CONTENT iii
LIST OF TABLES vi
LIST OF FIGURES vii
LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS viii
ABSTRAK ix
ABSTRACT xii

CHAPTER 1 INTRODUCTION 1
1.1 Overview 1
1.2 Complications 2
1.3 Burden 4
1.4 Significance of Study 5

CHAPTER 2 LITERATURE REVIEW 6
2.1 Epidemiology of Diabetes Mellitus 6
2.1.1 Global View 6
2.1.2 Asian View 7
2.2 Diabetic Foot Infection (DFI) 8
2.3 Risk for Infection 13
2.4 Microbiology of Diabetic Foot Infection 14
2.4.1 Quantity of Microorganism in Diabetic Foot Infection 14
2.5 Pathogenesis 17
2.6 Clinical Features and Diagnosis 19
2.7 Treatment 22
2.8 Conceptual Framework 29

CHAPTER 3 OBJECTIVE 30
3.1 Research Questions 30
3.2 Research Hypothesis 30
3.3 Objective(s) of the Research 31
3.3.1 General Objective 31
3.3.2 Specific Objectives 31

CHAPTER 4 METHODS 32
4.1 Study Design 32

4.2 Study Period 32
4.3 Study Location 32
4.4 Reference Population 32
4.5 Source Population 32
4.6 Study Participants 33
4.7 Inclusion and Exclusion Criteria 33
4.7.1 Inclusion Criteria 33
4.7.2 Exclusion Criteria 33
4.8 Sample Size Determination 33
4.9 Sampling Method 36
4.10 Flow Chart of Study 37
4.11 Measurement Tool 38
4.11.1 Quantity of Microorganism 38
4.11.2 Microorganism 38
4.11.3 Severity Diabetic Foot 39
4.11.4 Outcome 39
4.12 Data Collection 39
4.13 Flow Chart of Data Collection 41
4.14 Statistical Analysis 42
4.14.1 Descriptive Analysis 42
4.14.1.1 Data Entry, Cleaning and Exploration 42
4.14.2 Categorical Data Analysis 42
4.14.2.1 Fisher's exact test 43
4.14.2.2 Pearson Chi-square test 43
4.14.2.3 Assumption Checking 43
4.14.2.4 Data Presentation, Interpretation and Conclusion 44
4.15 Flow Chart of Statistical Analysis 45
4.16 Ethical Consideration 46

CHAPTER 5 RESULTS 47
5.1 Overview of the Data 47
5.2 Descriptive Analysis 47
5.2.1 Demographic Data 47
5.2.2 Microorganism by Isolates 48
5.2.3 Antibiotic Sensitivity in Diabetic Foot Infection 49

5.2.4 Association between Polymicrobial Infections and Severity of Diabetic Foot Infections. 52
5.2.5 Outcome of Polymicrobial Infection in Severe DFI..... 53

CHAPTER 6 DISCUSSION..... 54
6.1 Introduction and Overview 54
6.2 Distribution of Microorganism 54
6.3 Antibiotic Sensitivity 57
6.4 Association between polymicrobial infections and severity of DFI..... 58
6.5 Glucose Level, TWC and Haemoglobin Results in DFI..... 59
6.6 Outcome of DFI 61

CHAPTER 7 CONCLUSION AND RECOMMENDATION..... 63
7.1 Conclusion 63
7.2 Limitations 63
7.3 Recommendation 64

REFERENCES..... 65

APPENDICES..... 76

APPENDIX A: DATA COLLECTION SHEET..... 76

APPENDIX B: PERMISSION LETTER FROM DIRECTOR OF HOSPITAL UNIVERSITI SAINS MALAYSIA..... 79

APPENDIX C: APPROVAL FROM HUMAN RESEARCH ETHICS COMMITTEE USM..... 80

LIST OF TABLES

Table 2.6.1 Clinical manifestation with severity diabetic foot infection [Adopted from (Lipsky *et al.*, 2012)] 22

Table 4.11.3.1 Clinical manifestation with severity of diabetic foot infection [Adopted from (Lipsky *et al.*, 2012)] 39

Table 5.2.2.1 Descriptive statistics for Gram-positive and Gram-negative pathogen by isolates (n=133) 49

Table 5.2.3.1 Antibiotic sensitivity by Gram-positive pathogen (n=50) 50

Table 5.2.3.2 Antibiotic sensitivity pattern of Gram-negative microorganism (n=83) 51

Table 5.2.4.1 Associated factors between quantity of microorganisms among patients and severity of DFI (n=104) 52

Table 5.2.4.2 Descriptive statistics for polymicrobial DFI (n=30) 52

Table 5.2.5.1 Associated factors between quantity of microorganisms and amputation in DFI (n=15) 53

Table 5.2.5.2 Associated factors of quantity of microorganisms among discharge patients of diabetic foot infection (n=104) 53

LIST OF FIGURES

Figure 2.2.1 Diabetic foot infection [Adopted from (Frykberg *et al.*, 2006)] 12

Figure 2.8.1 Conceptual framework of association between polymicrobial infection and severity in diabetic foot infection patients in Hospital USM 29

Figure 4.10.1 Study flow chart of association between polymicrobial infection and severity in diabetic foot infection patients in Hospital USM 37

Figure 4.13.1 Data collection flow chart of association between polymicrobial infection and severity in diabetic foot infection patients in Hospital USM 41

Figure 4.15.1 Statistical flow chart of categorical data analysis 45

Figure 5.2.1.2 Diabetic foot infection patients based on gender 47

Figure 5.2.1.2 Diabetic foot infection based on age group 48

Figure 5.2.2.1 Distribution of microorganism among DFI patients 48

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

CDC	-	Center for Disease Control and Prevention
CRP	-	C-reactive protein
DFI	-	Diabetic foot infection
DGGE	-	Denaturing Gradient Gel Electrophoresis
DM	-	Diabetes mellitus
ESR	-	Erythrocyte sedimentation rate
Hb	-	Haemoglobin
HbA1c	-	Haemoglobin A1c
IDSA	-	Infectious Diseases Society of America
IGT	-	Impaired Glucose Tolerance
GN	-	Gram negative
GP	-	Gram positive
PAD	-	Peripheral arterial disease
RBS	-	Random blood sugar
TWC	-	Total white cell
WHO	-	World Health Organization

Faktor-faktor Berkaitan Di antara Infeksi Polimikrobial dan Keseriusan Jangkitn Kaki Diabetes dalam kalangan Pesakit di Hospital Universiti Sains Malaysia

ABSTRAK

Latarbelakang: Jangkitan pada kaki adalah antara komplikasi utama yang di alami oleh pesakit diabetes mellitus. Mikroorganisma penyebab biasanya adalah polimikrobial. Tujuan kajian ini dilaksanakan adalah untuk mengenalpasti peratus mikroorganisma dalam jangkitan kaki pada pesakit diabetes, berserta sensitiviti antibiotik terhadap mikroorganisma, hubungkait di antara polimikrobial dan keseriusan jangkitan kaki diabetes dan juga untuk mengenalpasti kesudahan jangkitan kaki diabetes dalam kalangan pesakit yang dijangkiti oleh polimikrobial mikroorganisma di Hospital USM.

Metodologi dan bahan kajian: Ini adalah kajian analisis retrospektif di mana ianya dijalankan dalam tempoh setahun bermula daripada Jun 2014 sehingga Jun 2015. Sejumlah 104 pesakit yang memenuhi kriteria-kriteria yang diperlukan telah direkrutkan. Fail pesakit yang mengalami jangkitan kaki diabetes dirujuk bagi kes-kes yang mempunyai jangkitan monomikrobial atau polimikrobial, penilaian tahap keseriusan dan kesudahan infeksi kaki mereka direkodkan. Parameter seperti kawalan gula dalam darah, bilangan sel darah putih dan hemoglobin dianalisa. Keputusan analisis ini disampaikan dalam bentuk diskriptif dan secara statistik menggunakan ujian "*Fisher exact"*.

Keputusan: Seramai 104 pesakit telah menyertai kajian ini. Sebanyak 133 mikroorganisma dikenalpasti dengan purata 1.28 mikroorganisma pada setiap jangkitan. Gram negatif (GN) menyumbang sebanyak 62% manakala Gram positif (GP) sebanyak 38%. Peratus mikroorganisma GN yang paling kerap didapati adalah *Pseudomonas* spp (28%), *Proteus* spp (11%), *Klebsiella* spp (8%) dan *E. coli* (4%). *Staphylococcus aureus*mendominasi (54%) di kalangan GP diikuti oleh Group B Streptococci (26%) and *Enterococcus spp* (6%). Bakteria GN adalah sensitif kepada antibiotik dari kumpulan carbapenem dan aminoglycoside manakala bakteria GP sensitif kepada vancomycin. Ujian "*Fisher's exact"* menunjukkan perkaitan yang signifikan antara kuantiti mikroorganisma dan keterukan jangkitan kaki diabetes (p=0.003). Tiga puluh pesakit dijangkiti oleh polimikrobial. Di kalangan jangkitan kaki diabetes yang serius, 77.8% dijangkiti polimikrobial mengalami amputasi, manakala yang dijangkiti monomikrobial adalah 33.3%. Majoriti pesakit daripada jangkitan polimikrobial atau monomikrobial didiscaj dengan baik, masing-masing pada kadar 84.0% and 91.1%. Kaitan antara jangkitan polimikrobial atau monomikrobial dengan kesudahan pesakit jangkitan kaki diabetes yang serius dikaji iaitu amputasi dan discaj pesakit, di mana keputusan adalah tidak signifikan (p=0.136 dan p=0.465). Purata (SD) untuk kawalan gula jangkamasa panjang (HbA1c), kawalan gula secara rawak (RBS), bilangan sel darah putih (TWC) dan hemoglobin (Hb) dalam jangkitan polimikrobial yang serius adalah masing-masing pada kadar 11.8(2.1) %, 16.3 (5.2) mmol/L, 16.6 (2.4) x 10^{9} dan 9.0 (1.3) g/dL .

Kesimpulan: Isolasi dominan daripada jangkitan kaki diabetes adalah GN dan antibiogram menunjukkan mikroorganisma yang biasa dijangkiti masih sensitif terhadap kebanyakan antibiotik yang digunakan. Jangkitan polimikrobial adalah

berkaitan dengan keseriusan jangkitan manakala tiada kaitan di antara kuantiti mikroorgnisma dengan kesudahan jangkitan kaki diabetes. Aras glukos dalam darah dan bilangan sel darah putih didapati tinggi dan aras hemoglobin didapati rendah dalam jangkitan polimikrobial yang serius.

Association between Polymicrobial Infections and Severity of Diabetic Foot Infection among Patients in Hospital Universiti Sains Malaysia

ABSTRACT

Background: Foot infections are a major complication of patients with diabetes mellitus (DM). The causative microorganisms are usually polymicrobial.The aim of the study is to determine the percentage of microorganism in diabetic foot infection, its susceptibility to antibiotic, association between polymicrobial infection and severity of diabetic foot infection and to determine the outcome of diabetic foot infection among patients infected with polymicrobial organisms in Hospital USM.

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CHAPTER 1

INTRODUCTION

1.1 Overview

As the incidence of diabetes mellitus (DM) is increasing globally, complications related to this endocrine disorder are also mounting. Foot infections are one of the common complications and are associated with high morbidity as well as risk of lower extremity amputation. Severe, chronic, or previously treated infections are often infected by polymicrobial infections. The diagnosis and severity of diabetic foot infection is based on the clinical signs and symptoms of local inflammation. Infected wounds should be cultured after debridement. Tissue specimens obtained by scraping the base of the ulcer with a scalpel or by wound or bone biopsy are strongly preferred to wound swabs. Imaging studies are indicated for suspected deep soft tissue purulent collections or osteomyelitis. Optimal management requires aggressive surgical debridement and wound management, effective antibiotic therapy, and correction of metabolic abnormalities (mainly hyperglycemia and arterial insufficiency). Treatment with antibiotics is not required for non infected ulcers. Mild soft tissue infection can be treated effectively with oral antibiotics. Severe soft tissue infection can be initially treated intravenously. Direct action of antibiotic towards targeted microorganism is better in treating diabetic foot infection.

1.2 Complications

Foot ulcers and their complications are an important cause of morbidity and mortality in patients with DM. Annual incidence of foot ulcers is 1%to 4% and prevalence is 5% to 10% in patients with diabetes (Control *et al.*, 2011). About 50% of patients undergoing non traumatic lower limb amputations have DM. These patients have a high mortality following amputation, ranging from 39% to 80% at 5 years (Hunt, 2009). Foot problems in DM continue to challenge the clinicianswho care for these patients. Not only are they associated with morbidity and disability, but they also lead to significant impairment of quality of life. Although a high mortality is well documented in patients with diabetic foot problems following amputation, there were long-term mortality in patients with new-onset diabetic foot ulcers (Moulik *et al.*, 2003).

Foot ulcers affect one in ten patients with DM during their lifetime(Lipsky *et al.*, 2012). They have increased risk of lower-extremity amputations and the main cause is diabetic peripheral arterial disease accelerated by the direct damage to the nerves and blood vessels by high blood glucose levels. The aim of primary amputation is to relieve pain and achieve rapid and successful mobility with an artificial limb. Peripheral arterial disease is an independent baseline predictor of the non-healing foot ulcer and along with progressing infection continues to be the main reason for lower extremity amputation. Although the intact foot may withstand markedly reduced skin perfusion, an ulcerated lesion requires a greatly enhanced blood flow to heal; therefore, many ulcers fail to heal where critical ischaemia exists. The progressive development of an abscess in the presence of ischaemia is an

ominous sign as it leads to irreparable tissue damage and amputation (Weledji and Fokam, 2014).

The 5-year mortality in patients with DM and critical limb ischaemia is 30% and about 50% of patients with diabetic foot infections who have foot amputations die within five years (Hunt, 2009). The mortality rate is similar to some of the most deadly cancers. Poor treatment can lead to lower extremity amputations. About half of these amputations can be prevented by proper care. It is vital that the diabetic condition in patients with infection is urgently controlled, otherwise the vicious cycle of infection leading to the instability of the diabetes and ketosis allows the spread of infection. Patients with a severe infection should be hospitalized immediately as these are often imminently limb-threatening and, in some cases life threatening (Weledji and Fokam, 2014).

Many DFI require surgical procedures, ranging from minor procedure which include drainage and excision of infected and necrotic tissues to major procedure, for instances, reconstruction of soft tissue or bony defects, revascularization of the lower extremity, and lower limb amputation (Lipsky *et al.*, 2012). Smokers, older patients with longer history of uncontrolled diabetes, and those with gangrenous infections and large ulcers have poorer outcome with amputations (Weledji and Fokam, 2014).

1.3 Burden

Medical expenses for people with DM are more than two times higher than for people without DM (Control *et al.*, 2011). Among United State residents aged 65 years and older, 10.9 million or 26.9%, had DM in 2010.About 1.9 million people aged 20 years or older were newly diagnosed with DM in 2010 in the United States. In 2005–2008, based on fasting glucose or HbA1c levels, 35% of United States adults aged 20 years or older had prediabetes while 50% of adults aged 65 years or older. Applying this percentage to the entire United States population in 2010 yields an estimated 79 million American adults aged 20 years or older with prediabetes. DM is the leading cause of kidney failure, non traumatic lower limb amputations, and new cases of blindness among adults in the United States (Control *et al.*, 2011).

The prevalence of DM in the Asia region is also clearly increasing, and so is the burden of this chronic disease and its complications. In Philippines, a true prevalence of about 7.2% among adults aged 20 years and older in 2008. On the other hand International Diabetes Federation Diabetes Atlas reports in 2010, Indonesia, Malaysia and Singapore prevalence rates of DM were 4.6%, 10.9% and 12.7%, respectively. These rates are consistent with global estimates, and considering the increasing populations in these countries, the absolute numbers are certainly staggering (Paz-Pacheco, 2014).

The Malaysian National Health and Morbidity Survey which monitors non communicable disease in Malaysian population was increasing in trend, they reported the prevalence of DM Type 2 increased from 11.6% in 2006, to 15.2% in 2011, which equates to approximately 2.6 million adults. This is parallel to three-fold

rise in the prevalence of obesity, from 4.4% in 1996 to 15.1% in 2011 for adult age 18 years and above in which equates to approximately 2.5 million Malaysians who in 2011 met the criteria for obesity (Mustapha *et al.*, 2014).

1.4 Significance of Study

In current practice, usually little attention is paid to the possible pathogen that causing the diabetic foot infection although some pathogens have different kind of virulence as well as response to different antibiotics. Polymicrobial infection of diabetic foot infection also contribute in severity of the disease therefore it can be one of prognostic factor and more vigilance management should be taken. In order to get true pathogen, a proper technique and correct site of sample is acquired.

Thus, by conducting this study, I hope that it will help clinician to improve patient's care of diabetic foot infection. This study can be considered to be included as one of the references in the national guidelines on management of diabetic foot infection. Since there is no local data available on common pathogens causing diabetic foot infection in Malaysia, I also hope that the result of this study can be used for proper selection of antibiotics in patients with DFI.

CHAPTER 2

LITERATURE REVIEW

2.1 Epidemiology of Diabetes Mellitus

The total number of people who have DM is projected to rise from 171 million in 2000 to 366 million in 2030. The most important contributor to the rise in DM prevalence globally appears to be the increase in the proportion of old people. However, given the increasing prevalence of obesity, these figures probably underestimate the future diabetes prevalence (Wild *et al.*, 2004). Asia has emerged as the 'diabetes epicenter' in the world, due to sudden economic development, urbanization and nutrition transition over a relatively short period of time. Among the 10 countries with the largest numbers of people predicted to have DM in 2030, five are in Asia including China, India, Pakistan, Indonesia and Bangladesh (Chen *et al.*, 2012) .

2.1.1 Global View

The number of people with DM is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Studies done for current and future estimation for DM prevalence and they are showed tremendously increasing in trend (King and Rewers, 1993; Wild *et al.*, 2004; Guariguata *et al.*, 2014). CDC reported, among U.S. residents aged 65 years and older 26.9%, had DM in 2010. About 215,000 people younger than 20 years had DM (type 1 or type 2) in the United States in 2010. About 1.9 million people aged 20

years or older were newly diagnosed with DM in 2010 in the United States (Control *et al.*, 2011).

2.1.2 Asian View

The Asia pacific region with an estimated DM-affected population of 135.4 million in 2010 is at the forefront of the current epidemic. The rise in prevalence is more in developing countries with an estimated projection, of 170% compared to 42% indeveloped countries to the year 2025. In Malaysia, the prevalence of DM has significantly increased from 0.6% in 1960 to 2.1% in 1982, 6.3% in 1986, 8.3 in 1996 and 14% in 1998 (Zaini, 2000). One of the factors including a rapidly developing economy, comprising a multi ethnic population possesses the entire favorable environment to support DM progression and its complications (Mafauzy *et al.*, 2011). It was reported, people with DM in Malaysia have almost doubled in a span of 20 years from 6.3% in 1986 to 11.6% in 2010 (Mafauzy *et al.*, 2011)

Zooming in to Kelantan state, the similar patterns were noted, a predominantly Malay village in the north-eastern state of the Peninsula. One study in 2000 reported which involved a total of 2508 subjects, aged 30 years and above, only 29.7% were in the urban areas. However, with the overall prevalence of DM at 10.5% and impaired glucose tolerance (IGT) at 16.5%, the urban–rural gap is getting narrower. There were several factors that responsible for the changes of the situation. Lifestyle, dietary and body mass changes are apparent and seemed to collaborate well with international experiences. Other than these factors the prevalence of concomitant hypertension and hyperlipidaemia were alarmingly high and should be a cause for concern. A total of 71.9% of the patient with DM subjects,63.0% of the

IGT and 57% in the normal group had hypercholesterolaemia. Meanwhile hypertension was noted in 12.9% of those with DM, 9% with IGT and 5.3% of the normal group (Zaini, 2000).

2.2 Diabetic Foot Infection (DFI)

DM is a chronic illness requiring continual health care and patient self-management, education to prevent the development of acute complications and reduce the risk of long term complications. As the prevalence of diabetes increases, the prevalence of long-term diabetes-related complications is also likely to increase. The rates of diabetic complications were cataract 27.2%, microalbuminuria 7%, neuropathy symptoms 45.9%, leg amputation 3.8% and history of angina pectoris was 18.4%. Quality of life evaluation showed that about one third of patients have poor quality of life. The 'diabetic foot' has been considered the "Cinderella" complication of diabetes care, and the International Diabetes Federation dedicated the year 2005 to foot care of people with diabetes in order to raise awareness of foot disease among people with diabetes (Boulton *et al.*, 2005). Diabetes-related foot ulcers represent challenges for the individual and for the health care system, as they increase the demand for specialized health care (Ortegon *et al.*, 2004).

Several predisposing factors for diabetic patients to develop a diabetic foot infection, including neuropathy, vasculopathy and immunopathy. The prominent risk factor for diabetic foot ulcer that occurs in early pathogenesis of DFI complication is peripheral neuropathy. Therefore DM patients with impaired protective sensation and altered pain response are vulnerable to trauma and extrinsic forces from ill-fitting shoewear. While the motor neuropathy causes muscle weakness and intrinsic muscle

imbalance leading to digital deformities such as hammered or clawed toes. This condition results in elevated plantar pressure due to metatarsophalangeal joint instability. On the other hand, autonomic dysfunction leads to altered in microvascular blood flow and arteriolar-venous shunting, diminishing the effectiveness of perfusion and elevating skin temperatures. Subsequently, the diabetic foot becomes dry and keratinized which cracks and fissures more easily, leading to a portal for infection due to loss of sweat and oil gland function (Hobizal and Wukich, 2012).

Apart from the obvious clinical predisposing risk factors, a few studies have revealed that very complex mechanisms are involved at the tissue-molecular level, which prevent normal healing processes (Lobmann *et al.*, 2002; Bennett *et al.*, 2003). There are several chemo-cytokines are involved, including matrix metalloproteinases, serine proteinases, integrins, chemokines, replicative cell senescence, growth factors and adult stem cells. Tissue injury in diabetic patients initially show impairment in the immune system response with reduced chemotactic effects to recruit inflammatory cells into the damaged tissues, thus, slowing down healing and increasing the risk of bacterial infection. After this initial step, when the inflammatory response is eventually established, the process switches to an exacerbation of proteolysis and inflammation. Glycation of proteins and disturbances of cell responses is also generated as the result of prolonged exposure to hyperglycemia, thus, further hindering the process of fibrosis and tissue repair. Recent molecular studies on chronic diabetic ulcers indicated that more specific processes may be involved. For instance, in the ulcer, it has been found that leucocytes are prevented from ready entry and accumulation, which, later, fail to

achieve normal healing (Leung, 2007). Other studies on the specific properties of fibroblasts from patients with chronic diabetic ulcers showed that these cells were different from those taken from patients without chronic ulcers in that the high molecular weight hyaluronic acid in the pericellular matrix was much more concentrated. The unique property of the fibroblasts might predispose these patients to chronic ulcer formation (Harding *et al.*, 2002; Yevdokimova, 2003; Yevdokimova and Podpryatov, 2005).

DM patients are commonly exposed to the infections and they are often more severe than infections found in non diabetic patients. Persons with DM have an increased risk for developing an infection of any kind and a several-fold risk for developing osteomyelitis (Shah and Hux, 2003). With an incidence of 36.5 per 1,000persons per year, foot infections are among the most common lower extremity complications in the diabetic population (excluding neuropathy), second only to foot ulcers infrequency (Lavery *et al.*, 2003). It is well documented that diabetic foot infections are frequently polymicrobial in nature (Armstrong and Lipsky, 2004; Peters *et al.*, 2012; Lipsky *et al.*, 2014). Hyperglycemia, impaired immunologic responses, neuropathy, and peripheral arterial disease are the major predisposing factors leading to limb-threatening diabetic foot infections (Lipsky *et al.*, 2004a). Uncontrolled DM results in impaired ability of host leukocytes to fight bacterial pathogens, and ischemia also affects the ability to fight infections because delivery of antibiotics to the site of infection is impaired. Consequently, infection can develop, spread rapidly, and produce significant and irreversible tissue damage. Even in the presence of adequate arterial perfusion, underlying peripheral sensory neuropathy

will often allow the progression of infection through continued walking or delay in recognition.

Foot infections in persons with DM are often initially treated empirically. The empirical antibiotics using are usually mend for broad spectrums organisms' coverage or according to local antibiogram study. Hence, therapy directed at known causative organisms may improve the outcome. Many studies have reported on the bacteriology of DFIs over the past 25 years, but the results have varied and have often been contradictory (Citron *et al.*, 2007). Therefore study on local causative organism and antibiogram of DFI is an essential tool for better management of diabetic foot patients. A number of studies have found that *Staphylococcus aureus* is the main causative pathogen (Lipsky *et al.*, 2004b; Raja, 2007; Eleftheriadou *et al.*, 2010), but more recent investigations reported a predominance of Gram-negative (Al Benwan *et al.*, 2012; Hefni *et al.*, 2013). The role of anaerobes is particularly unclear, because in many studies specimens were not collected or cultured properly to recover these organisms. Among those that did use appropriate methods, some report that anaerobes play a minimal role and *Bacteroides fragilis* is the predominant anaerobe isolated (Díaz *et al.*, 1992; Senneville *et al.*, 2006).

These discrepancies of aetiological agents could be partly due to differences in the causative organisms occurring over time, geographical variations, or the types and severity of infection included in the studies. In addition, some studies used a relatively small number of specimens, failed to report recent or concomitant antibiotic therapy, did not ensure that the specimen collection techniques would exclude superficial or colonizing organisms, or even make clear whether or not the

wound was clinically infected. Also, laboratory processing of the samples may have been inadequate to grow anaerobes or fastidious organisms, and protocols that classify potential pathogens for examples coagulase-negative staphylococci or *Corynebacterium* spp as colonizers may have been used (Lipsky *et al.*, 2012).

S. aureus and beta-hemolytic streptococci are widely recognized as pathogens in early DFIs, the role of other frequently isolated organisms is less clear to both the clinician and the microbiology laboratory. Previous studies have shown that when optimal specimen collection, transport, and culture techniques are used, multiple organisms are usually recovered from DFIs (Lipsky *et al.*, 2004a; Lipsky *et al.*, 2014). Furthermore, some studies suggest that the interactions of organisms within these polymicrobial mixtures lead to the production of virulence factors, such as hemolysins, proteases, and collagenases, as well as short-chain fatty acids, that cause inflammation, impede wound healing, and contribute to the chronicity of the infection. In such mixtures, biofilms that impede the penetration of antimicrobial agents into the infected site may also form (Peters *et al.*, 2012). Thus, the presence of multiple species can have important clinical implications that should not be overlooked.

Figure 2.2.1 Diabetic foot infection[Adopted from (Frykberg *et al.*, 2006)]

2.3 Risk for Infection

DM patients are commonly exposed to the infections and they are often more severe than infections found in non diabetic patients. Persons with DM have an increased risk for developing an infection of any kind and a several-fold risk for developing osteomyelitis (Shah and Hux, 2003). With an incidence of 36.5 per 1,000persons per year, foot infections are among the most common lower extremity complications in the diabetic population (excluding neuropathy), second only to foot ulcers infrequency (Lavery *et al.*, 2003). It is well documented that DFIs are frequently polymicrobial in nature (Armstrong and Lipsky, 2004; Peters *et al.*, 2012; Lipsky *et al.*, 2014).

Hyperglycemia, impaired immunologic responses, neuropathy, and peripheral arterial disease are the major predisposing factors leading to limb-threatening DFI (Lipsky *et al.*, 2004a). Uncontrolled DM results in impaired ability of host leukocytes to fight bacterial pathogens, and ischemia also affects the ability to fight infections because delivery of antibiotics to the site of infection is impaired. Consequently, infection can develop, spread rapidly, and produce significant and irreversible tissue damage. Even in the presence of adequate arterial perfusion, underlying peripheral sensory neuropathy will often allow the progression of infection through continued walking or delay in recognition.

2.4 Microbiology of Diabetic Foot Infection

2.4.1 Quantity of Microorganism in Diabetic Foot Infection

Microbes rarely exist as single-species planktonic forms. The majority are found thriving in complex polymicrobial biofilm communities attached to biotic and abiotic sites. Polymicrobial biofilm communities may be defined as a varied collection of organisms (fungi, bacteria, and viruses) that exist at a phase or density interface and are coated in a self- and/or host-derived hydrated matrix, often consisting of polysaccharide (Brogden *et al.*, 2005). Some microbes have evolved mutualistic or even synergistic relationships to facilitate cohabitation on epithelial surfaces and to efficiently utilize metabolic by-products, while others have developed competitive antagonistic approaches during colonization. These relationships are manifested by contact-dependent attachment, cell-cell communication via quorum-sensing cross talk, an enhancement of colonization, augmented virulence phenotypes *in trans*, immunomodulation, or a combination of these events (Peleg *et al.*, 2010).

Observations using the earliest microscopes revealed the colonization of multispecies communities on human tissues. However, people still do not have a solid understanding of how multi species interactions govern the scope, progression, and severity of human disease, and even less is known regarding how the host responds to polymicrobial infection compared to monomicrobial infection (He and Shi, 2009). It was previously believed that a single virulence factor sufficiently mediated disease caused by a single organism. While this is true for some human infections, immunization against single virulence factors of other organisms (i.e., *Staphylococcus aureus*) has proven much more difficult. Just as virulence can no longer be associated with a single virulence factor for some organisms, some

diseases can no longer be defined as an infection by a single species (Cripps and Otczyk, 2006)

Most diseases were previously characterized as being monomicrobial in nature, likely due to the extensive use of culture dependent isolation techniques. However, with the advent of culture-independent community analysis methodologies, several are becoming increasingly recognized as true polymicrobial infections, including diseases of the diabetic foot wound infections. In these cases, the composition of microbial populations predicts disease severity and outcome. Therefore, epidemiologic identification and comprehensive pyrosequencing surveys during human infection coupled with mechanistic studies of derived novel interspecies cross-kingdom microbial relationships should lead to the increased surveillance of potential disease risk factors (Peters *et al.*, 2012).

Another damaging effect of high glucose levels mediated by an insulin deficiency or resistance is the development of peripheral neuropathy and poor blood circulation, especially in extremities such as the hands and feet. Combined, these symptoms predispose diabetic individuals to an increased risk of infection, and if not identified or treated early, these infections may fulminate into chronic ulcerating polymicrobial biofilm-mediated wounds that often do not resolve with standard therapies and result in eventual limb amputation (Wu *et al.*, 2007). The development of such ulcers is often the synergy of two contributing factors, e.g., decreased neurosensory perception and stepping on a sharp object; it is this critical breach of the epithelial surface, coupled with neurological abnormalities, cardiovascular aberrations, and immune dysfunction, which facilitates polymicrobial colonization

and subsequent pathogenesis. Due to the inability of subjects with this disease to feel cuts and irritations on visually obscured areas of the feet, these infections often go unnoticed and progress to more serious illness (Boulton, 2010).

DFIs are often mediated by a mixture of several species of microbes coexisting as complex biofilm communities. A large multicenter analysis of 454 individual diabetic foot wound infection swabs and aspirates resulted in the identification of over 1,600 organisms by aerobic and anaerobic culturing techniques. Interestingly, of the specimens tested, 48.9% were infected with aerobic bacteria only, 1.3% was infected with anaerobic bacteria only, and 43.9% contained a mixture of aerobic and anaerobic bacteria. Bacterial growth was not identified in 5.9% of the samples. Of the positive cultures identified,16.2% harbored one bacterial isolate, 20.4% contained two bacterial isolates, 19.7% had three bacterial isolates, 13.3% demonstrated four bacterial isolates, and 30.4% supported the growth of five or more bacterial isolates. Of these, the most abundant aerobic isolates recovered were *Corynebacterium* spp., *Enterococcus* spp., *Escherichia coli*, *Staphylococcus epidermidis*, and *S. aureus*; among the most commonly isolated anaerobic bacteria were *Fusobacterium* spp., *Porphyromonas* spp., *Prevotella* spp., *Bacteroides*spp., and *Clostridium* spp (Citron *et al.*, 2007).

A smaller-scale study using denaturing gradient gel electrophoresis (DGGE), 16SrRNA gene sequencing techniques, and microscopy to examine debrided tissues from diabetic foot wounds resulted in the identification of highly polymicrobial communities and the detection of several species unidentifiable by standard culturing techniques (James *et al.*, 2008). Notably, DGGE analysis demonstrated the presence

of several unique bands (corresponding to unique species) for each sample tested, and banding patterns differed between individual samples. While no distinct relationships between coisolated organisms can be derived from these analyses, these studies demonstrate the tremendous diversity in microbial composition and the true polymicrobial nature of diabetic foot wound diseases. As of now, it is unclear whether diabetic foot wounds arise from specific combinations of pathogens or if a simple increase in the microbial loads of any opportunistic microbes can sustain infection.

2.5 Pathogenesis

Patients with DM were predisposed with many factors in developing DFI. Several factors including neuropathy, vasculopathy and immunopathy are important role in contributing this complication. In early pathogenesis, most prominent risk factor considered for diabetic foot ulcers is peripheral neuropathy (Reiber *et al.*, 1999). Patients with DM usually has an impaired protective sensation because presence of pain response alteration. Thus they are vulnerable to trauma and extrinsic forces from ill-fitting shoewear. On the other hand, motor neuropathy causes intrinsic muscle imbalance and muscle weakness leading to digital deformities such as hammered or clawed toes. Hence causing metatarsophalangeal joint instability that resulted in elevated plantar pressure. Autonomic dysfunction leads to changes in microvascular blood flow and arteriolar-venous shunting, diminishing the effectiveness of perfusion and elevating skin temperatures. Patients with DM, their foot becomes dry and keratinized which can cause cracks and fissures due to loss of sweat and oil gland function which can lead to a portal for infection (Snyder *et al.*, 2010).

The next element in pathogenesis of DFI is vasculopathy. Diabetic angiopathy is reported to be the most frequent cause of morbidity and mortality in patients with DM (Joseph and LeFrock, 1987). Infrapopliteal vessels typically involved as diffuse multisegmental in macroangiopathy manifestations and is also associated with compromised collateral circulation. This is considered an atherosclerotic obstructive disease of large vessels, which leads to peripheral arterial disease (PAD) of the lower extremities(Hobizal and Wukich, 2012). The PAD is considered an independent associated factor in increase risk in getting DFI (Peters *et al.*, 2005). Meanwhile, microangiopathy results in altered nutrient exchange, capillary basement membrane thickening, microcirculation ischemia and tissue hypoxia that leading to development of DFI (Association, 2014).

Immunopathy has been implicated in the patients with DM inherent susceptibility to infection as well as the potential to mount a normal inflammatory response. Impaired host defenses secondary to hyperglycemia include defects in leukocyte function and morphologic changes to macrophages (Bagdade *et al.*, 1974). In pathogenesis of DFI demonstrated that significantly reduction of leukocyte phagocytosis in patients with poorly controlled DM, and correction of hyperglycemia was directly correlated with improvement of microbiocidal rates. There were reduced chemotaxis of growth factors and cytokines, coupled with excess of metalloproteinases, delay normal wound healing by creating a prolonged inflammatory state leading to development of DFI (Hobizal and Wukich, 2012).

Catabolic state is associated with fasting hyperglycemia and the presence of an open wound. While gluconeogenesis from protein breakdown causing negative nitrogen balance as a result of secondary to insulin deprivation. This metabolic dysfunction impairs the synthesis of proteins, fibroblasts and collagen and further systemic deficiencies are propagated which lead to nutritional compromise. Research indicates impairment of the immune system with serum glucose levels >150 ml/dl (Inzucchi, 2006). Patients with DM tolerate infection poorly and infection adversely affects diabetic control. Thus, repetitive cycle leads to uncontrolled hyperglycemia, further affecting the host's response to infection (Hobizal and Wukich, 2012).

2.6 Clinical Features and Diagnosis

All skin wounds contain microorganism, making a diagnosis of infection is not an easy procedure. Therefore, infection must be diagnosed clinically, that is, by the presence of systemic signs including fever, chills and leukocytosis. Others are purulent secretions (pus), or local classical signs or symptoms of inflammation including warmth, redness, pain or tenderness, and induration. In chronic wounds, additional signs suggesting infection may include delayed healing, abnormal coloration, friability, or foul odor (Lipsky *et al.*, 2012).

In the presence at evidence of a systemic infection or of a metabolic disorder and at the first appearance of a foot problem, the infection should be suspected. However, sometimes the diagnosis of infection can be delayed by peripheral neuropathy or ischemia that causing either mask or mimic inflammation (Lipsky, 2004). Occasionally, inflammatory signs may be caused by other noninfectious disorders; on the other hand, some uninflamed ulcers may be associated with

underlying osteomyelitis (Newman *et al.*, 1991). In DFI signs of systemic toxicity are surprisingly uncommon, even those that are limb threatening.

Proper evaluation of a DFI requires a methodical approach. Whenever infection is considered, this diagnosis should be pursued aggressively; these infections can worsen quickly, sometimes in a few hours. Almost two-thirds of patients with a DFI have evidence of peripheral vascular disease, and about 80% have lost protective sensation (Lipsky, 2001). Forefoot is the most part of infections, especially the toes and metatarsal heads, particularly on the plantar surface. About half of the patients in reported series have received antibiotic therapy for the foot lesion by the time they present, and up to one third have had a foot lesion for more than one month. Many patients do not report pain, and more than half, including those with serious infections, do not have a fever, elevated WBC count, or elevated ESR (Lipsky, 2004).

Several classification systems have been proposed for diabetic foot lesions, none of which is universally accepted. Keys to classifying a foot wound are assessing the depth of the lesion by visually inspecting the tissues involved and by estimating the depth in millimeters as well as checking for ischemia by absent pulses or diminished blood pressure in the foot. Other key is by looking for infection. Whereas mild infections are relatively easily treated, moderately severe infections may be limb threatening, and severe infections may be life threatening. Assessing the severity of infection is essential to determine the need for hospitalization, selecting an antibiotic regimen and influences the route of drug administration. Severity of

infection also helps to assess the potential necessity and timing of surgery and the likelihood of amputation (Armstrong *et al.*, 1998).

The wound should be carefully explored to seek foreign or necrotic material, and it should be probed with a sterile metal instrument. Deep space infections often have deceptively few superficial signs. The clinician should suspect spread of infection when there is inflammation distant from the skin wound, or when suppurative lesions persist despite apparently appropriate therapy. A surgeon should evaluate any patient with systemic toxicity for an occult deep space infection. Clinical features that help define the severity of infection.

One of the first, and the most financially dominant, decisions when faced with a DFI is to determine whether a patient should be hospitalized. Several indications for hospitalization, patients with a serious infection and possible surgical interventions, fluid resuscitation, and control of metabolic derangements. Hospitalization should also be considered if the patient is unable or unwilling to perform proper wound care, cannot or will not be able to off-load the affected area, is unlikely to comply with antibiotic therapy, requires parenteral antibiotic therapy, or needs close monitoring of response to treatment. In the absence of these factors, most patients can be treated cautiously on an outpatient basis, with frequent (i.e., every few days, initially) reevaluation. Wound care (debridement, dressing changes, and pressure off-loading) and glycemic control should be optimized; antibiotics will not overcome inattention to these fundamentals (Lipsky, 2008).

Table 2.6.1 Clinical manifestation with severity diabetic foot infection [Adopted from (Lipsky *et al.*, 2012)]

Clinical Manifestation of Infection	PEDIS Grade	IDSA Infection Severity
No symptoms or signs of infection	1	Uninfected
Infection present, as defined by the presence of at least 2 of the following items: • Local swelling or induration • Erythema • Local tenderness or pain • Local warmth • Purulent discharge (thick, opaque to white or sanguineous secretion)		
Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema, must be >0.5 cm to ≤2 cm around the ulcer. Exclude other causes of an inflammatory response of the skin (eg, trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, venous stasis).	2	Mild
Local infection (as described above) with erythema > 2 cm, or involving structures deeper than skin and subcutaneous tissues (eg, abscess, osteomyelitis, septic arthritis, fasciitis), **and** No systemic inflammatory response signs (as described below)	3	Moderate
Local infection (as described above) with the signs of SIRS, as manifested by ≥2 of the following: • Temperature >38°C or <36°C • Heart rate >90 beats/min • Respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg • White blood cell count >12 000 or <4000 cells/μL or ≥10% immature (band) forms	4	Severe[a]

Abbreviations: IDSA, Infectious Diseases Society of America; $PaCO_2$, partial pressure of arterial carbon dioxide; PEDIS, perfusion, extent/size, depth/tissue loss, infection, and sensation; SIRS, systemic inflammatory response syndrome.

[a] Ischemia may increase the severity of any infection, and the presence of critical ischemia often makes the infection severe. Systemic infection may sometimes manifest with other clinical findings, such as hypotension, confusion, vomiting, or evidence of metabolic disturbances, such as acidosis, severe hyperglycemia, and new-onset azotemia [29, 43, 44].

2.7 Treatment

Several basic factors that should considered in determining an antibiotic regimen include clinical severity of infection, etiologic agents either known or presumed, bone infection and allergies to antibiotics (Lipsky *et al.*, 2004a). The initial antibiotic regimen must usually be selected empirically, and it may be modified later on the basis of availability of additional clinical and microbiological information. Choosing an empiric regimen involves making decisions about the spectrum of microorganisms to be covered, route of therapy and specific drugs to administer.

These decisions should be revisited when deciding on the definitive regimen and the appropriate duration of treatment. Initial empiric therapy should be based on the severity of the infection and on any available microbiological data, such as recent

culture results and the local prevalence of pathogens, especially antibiotic-resistant strains (Ramakant *et al.*, 2011). Available data suggest that 40%– 60% of diabetic patients who are treated for a foot ulcer receive antibiotic therapy (Jaegeblad *et al.*, 1999). One study reported a randomized trial in which 64 diabetic patients who received antibiotic therapy for clinically uninfected foot ulcers had a significantly increased likelihood of healing and had a reduced incidence of clinical infection, hospitalization, and amputation (O'meara *et al.*, 2001). Conversely antibiotic therapy is associated with frequent adverse effects, substantial financial costs, and the development of resistance and, thus, should currently be used only to treat established infection (Lipsky *et al.*, 2004a). The majority of mild, and many moderate, infections can be treated with agents that have a relatively narrow spectrum, usually covering only aerobic Gram positive cocci (Lipsky *et al.*, 1990). In countries with warm climates, gram-negative isolates (especially *P. aeruginosa*) are more prevalent. Obligate anaerobic organisms are isolated from many chronic, previously treated, or severe infections (Ng *et al.*, 2008).

For severe infections, and for more extensive, chronic moderate infections, it is safest to promptly commence therapy with a broad-spectrum regimen. The agent(s) should have activity against Gram positive cocci, as well as common Gram negative and obligate anaerobic organisms to ensure adequate tissue concentrations. For these more severe infections, it is usually safest to start with parenteral therapy, which can usually be switched to oral treatment within a few days when the patient is systemically well and culture results are available to guide the selection. Clinicians should consider the results of culture and sensitivity testing in light of the clinical response of the infection to the empiric regimen. Cultures may yield organisms that

are commonly considered to be contaminants (eg, coagulase negative staphylococci, corynebacteria), but these may be true pathogens in a DFI. Because these organisms are often resistant to the prescribed antibiotic, the clinician must decide if the preponderance of clinical and microbiologic evidence suggests they are pathogens that require targeted therapy. If the patient has had a good clinical response on the empiric therapy, the regimen may be continued, or even potentially narrowed ("deescalation" therapy) (Lipsky *et al.*, 2012).

The key to successful antibiotic therapy is achieving a therapeutic drug concentration at the site of infection. This typically requires first achieving adequate serum levels. Intravenous antibiotics are indicated for patients who are systemically ill, have a severe infection, are unable to tolerate oral agents, or are known or suspected to have pathogens that are not susceptible to available oral agents (Lipsky *et al.*, 2004a). The optimal duration of antibiotic therapy for diabetic foot infections has not been studied. For mild to moderate infections, a one to two week course has been found to be effective (Lipsky *et al.*, 1990), whereas for more serious infections, treatment has usually been given for about 2 weeks, sometimes longer. Adequate debridement, resection, or amputation of infected tissue can shorten the necessary duration of therapy. For those few patients with diabetic foot infection who develop bacteremia, therapy for at least 2 weeks seems prudent. Antibiotic therapy can generally be discontinued when all signs and symptoms of infection have resolved, even if the wound has not completely healed. Healing any skin ulcer is a separate, albeit important, issue in treating diabetic foot infections. In some instances of extensive infection, large areas of gangrene or necrotic tissue, or poor vascular supply, more prolonged therapy may be needed. Some patients who cannot, or will