

CLINICAL RESEARCH ARTICLE

Risk factors for lower extremity amputation in patients with diabetic foot ulcers: a hospital-based case-control study

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Background: Diabetic foot ulcers (DFU) may cause significant morbidity and lower extremity amputation (LEA) due to diabetic foot problems can occur more often compared to the general population. The purpose of the present study was to use an epidemiological design to determine and to quantify the risk factors of subsequent amputation in hospitalized DFU patients.

Methods: We performed a hospital-based, case-control study of 47 DFU patients with LEA and 47 control DFU patients without LEA. The control subjects were matched to cases in respect to age (± 5 years), sex, and nutritional status, with ratio of 1:1. This study was conducted in Dr. Kariadi General Hospital Semarang between January 2012 and December 2014. Patients' demographical data and all risk factors-related information were collected from clinical records using a short structural chart. Using LEA as the outcome variable, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) by logistic regression. Univariate and stepwise logistic regression analyses were used to assess the independent effect of selected risk factors associated with LEA. The data were analyzed in SPSS version 21.

Results: There were 47 case-control pairs, all of which were diagnosed with type 2 diabetes mellitus. Seven potential independent variables show a promise of influence, the latter being defined as $p \leq 0.15$ upon univariate analysis. Multivariable logistic regression identified levels of HbA1c $\geq 8\%$ (OR 20.47, 95% CI 3.12–134.31; $p = 0.002$), presence of peripheral arterial disease (PAD) (OR 12.97, 95% CI 3.44–48.88; $p < 0.001$), hypertriglyceridemia (OR 5.58, 95% CI 1.74–17.91; $p = 0.004$), and hypertension (OR 3.67, 95% CI 1.14–11.79; $p = 0.028$) as the independent risk factors associated with subsequent LEA in DFU.

Conclusions: Several risk factors for LEA were identified. We found that HbA1c $\geq 8\%$, PAD, hypertriglyceridemia, and hypertension have been recognized as the predictors of LEA in this study. Good glycemic control, active investigation against PAD, and management of comorbidities such as hypertriglyceridemia and hypertension are considered important to reduce amputation risk.

Keywords: *diabetic foot ulcers; hospitalized patients; risk factors; amputation*

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Diabetes mellitus is the most common endocrine disorder known for its multifaceted complications, including diabetic foot ulcers (DFU) which often result in amputation as one of the worst outcomes (1). Among persons with diabetes, the prevalence of foot ulcers ranges from 4 to 10% and its lifetime incidence may be as high as 25% (2). Foot ulceration poses a distinct barrier to conservative therapies attrib-

uted to difficulty in properly offloading the wounds, inability to provide daily foot hygiene, and compromised distal vascular flow in diabetes. DFU are difficult to treat, frequently get infected, and become a leading cause of diabetes-related hospital admission (1, 3). Compared to healthy persons, diabetes mellitus holds a 15- to 20-fold increased risk of lower extremity amputations (LEA) and the majority of diabetes amputation are reported to be

preceded (up to 85%) by a poor healing ulcer (4). In the future, diabetes-related LEA will remain a source of significant morbidity and also mortality, considering the rapidly growing diabetes population worldwide and the high incidence of DFU (5).

According to the Global Lower Extremity Study Group, LEA can be defined as a complete loss of any part of the lower extremity irrespective of the causes (6). Approximately 82% of LEAs are performed on patients with diabetes, most of which follows foot ulceration (7). The pathway to ulceration and finally LEA may include essential contribution from underlying diabetes-related pathophysiology (neuropathy, peripheral arterial disease (PAD), foot deformity and limited joint mobility), initiating environments (trauma), subsequent infection, and healing complications (8). LEA is performed for various indications including severe soft-tissue infection, osteomyelitis, peripheral arterial occlusion, and gangrene. Following a LEA surgery, the impact of this procedure on an individual patient is very enormous so that amputation is always considered as the last resort of any unsalvageable limb (9). Apart from its causes, all attempts should be made to avoid amputation once DFU has developed or presents itself in the hospital (1, 4, 5).

The diabetic foot follows a common pathway that begins with a small ulcer or surgical wound. The majority of DFU (60–80%) will heal, whereas 10–15% of them will remain active, and up to 24% of them will finally lead to LEA (1, 4, 8). The question is why some patients with DFU be necessary for LEA while others were not. Previous studies have revealed that duration of diabetes mellitus (10, 11), previous amputation or foot ulceration (10, 12–14), poor glycemic control (10, 12, 13, 15–18), hypertension (15, 19), dyslipidemia (11, 15, 19), presence of PAD (11, 12, 14, 18, 20), peripheral neuropathy (13, 14, 20), osteomyelitis (19, 21), and wound severity (22, 23) are independent predictors for LEA. Additional factors include older age (18, 22), smoking history (22, 23), anemia (18), leukocytosis (18, 19, 22), hypoalbuminemia (20, 22), as well as presence of other microvascular (10, 11, 13–15, 17, 19, 21) and macrovascular comorbidities (13, 15, 22). However, different studies show different results and the published data that identify such risk factors for diabetes-related LEA in Indonesia are scanty. The risk factors have not been clarified in our center so that the scope for understanding the reasons for an LEA risk reduction is limited.

We performed a case–control study to assess the magnitude and common determinants of LEA in hospitalized patients with DFU from Dr. Kariadi General Hospital Semarang. The hypothesis underlying this analytical investigation was that there may be several differences in the risk factors pattern among DFU that warrant amputation surgery. The inclusion criteria were designed to allow the enrollment of a representative group of DFU

similar to ‘real world’ situations in developing countries where most patients were ambulatory, self-medicated at home, have a considerable delay before hospital admission, may have their diabetes poorly controlled, and have several sociocultural practices such as walking barefoot, use of herbal healer, and so on (5). Identification of variables and to suggest modifiable factors is the first step in the pathway for the creation of preventive and/or therapeutic programs to reduce LEA rates at institutional levels with local resources.

Material and methods

Study area and background

This study used an observational design and was conducted in Dr. Kariadi General Hospital, Semarang District, Central Java Province, Indonesia. Dr. Kariadi General Hospital is a tertiary care hospital, which is the central referral and main teaching hospital of the Medical Faculty of Diponegoro University. The incidence of LEA was determined by reviewing the medical records. For this study, the complete list of DFU and LEA population was identified from hospital databases (operating theater and medical record). Ulcer and gangrene due to reasons other than diabetes mellitus, and signs of acute peripheral arterial thrombosis were not included in this study. Traumatic amputations and those unrelated to diabetes mellitus were also excluded. The study was designed as a matched case–control study (24). Assuming the proportion of DFU with amputation to be 39.5% (25), a sample size of at least 23 in each group was needed to detect an odds ratio (OR) of 2.0 at 95% level of confidence interval (CI) with a power of 90% (two tails) (26). Ethical approval for this study was given by the Committee of the Medical Faculty of Diponegoro University and Dr. Kariadi General Hospital.

Subjects

In the present study, we have identified 232 hospitalizations involving 186 patients at our institutions who had foot ulcerations (International Classification of Disease, 10th Revision [ICD-10] codes E11.6 and E14.6) with diabetes. Diabetes was defined as at least one record of ICD-10 code E10 (type 1 diabetes) or E11 (type 2 diabetes). We designed the study to have 1:1 matching, with one subject control for each case (24, 27). The confounding factors such as age, sex, and nutritional status were considered in the case–control matching. The presence of the following factors was evaluated to determine if they predicted either amputation or not: demographic characteristics (duration of ulcer, duration of diabetes since diagnosed, sort of diabetes treatment), clinical features (presence and assessment of diabetic peripheral polyneuropathy, retinopathy, nephropathy, PAD, and type of diabetic foot), level of glycemic control, and several

laboratory data. These possible risk factors were chosen because they were common risk factors for LEA cited from the previous studies (10–23). The study period was January 2012 to December 2014 and medical records that contain missing data on any of the stratified information were excluded from analysis.

Treatment settings

We utilized a standard protocol for the management of patients hospitalized because of DFU which included off-loading, assessment of vascular status, assessment of neuropathy, treatment of PAD, and regular wound debridement. In general, DFU patients with signs of significant infection, such as extensive cellulitis, necrotizing fasciitis, deep abscess or osteomyelitis, septic foot, or presence of gangrenous tissue were hospitalized for intensive surgical management. All patients were placed on bed rest for pressure relief and appropriate antibiotic therapy was administered when infection was present. DFU subsequently were managed according to the severity of lesions; debridement, incision/drainage, and amputation were done as necessary. All of these patients were under the care of a multidisciplinary team of endocrinologist, infectious disease specialist, cardiologist, vascular surgeon, orthopedic surgeon, plastic surgeon, nutritionist, internal medicine residents, and nursing personnel.

Measurements of potential risk factors

We abstracted the medical records for each hospitalization and the operative reports were read to evaluate the exact surgical procedure performed. By using a pre-preformed customized chart, we collected the information regarding the patient's age, sex, body mass index (BMI), admission dates, duration of diabetes mellitus, therapeutic regimen, characterization of ulcer, ulcer duration, hemoglobin level, leukocytes count, creatinine serum, admission plasma glucose, fasting plasma glucose (FPG), HbA1c, and lipid profile (total cholesterol, fasting triglycerides, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol). Regarding lipid profile, the cut-off points for high total cholesterol (>200 mg/dL), high triglycerides (>150 mg/dL), high LDL-cholesterol (>100 mg/dL), and low HDL-cholesterol (<40 mg/dL) were based on The National Cholesterol Education Program (28). The cut-off points for high plasma glucose (≥ 200 mg/dL), high FPG (≥ 126 mg/dL), and high HbA1c ($\geq 8\%$) were based on the Indonesian Diabetes Association definition for poor glycemic control (29). BMI is defined as ratio of weight (in kg) to height (in meters squared). Diabetes micro- and macrovascular complications (retinopathy, nephropathy, neuropathy, cerebrovascular, cardiovascular, and PAD) were classified in accordance with the Diabetes Complications Severity Index created by Young et al. (30).

The diagnosis of diabetes mellitus was measured at the initial admission. We further classified participants with

diagnosed diabetes mellitus into the following treatment categories: 1) no pharmacological treatment, 2) oral hypoglycemic medication, and 3) use of insulin treatment (insulin alone or in combination with oral agents). Hypertension was considered to be present if patients were taking antihypertensive medicine or had elevated blood pressure measurement over systolic 140 and/or diastolic 90 mmHg. Retinopathy was defined as the presence of retinal hemorrhage exudate or microaneurysms on funduscopic examination by an ophthalmologist. The presence of diabetic nephropathy was defined by plasma creatinine >1.5 mg/dL or persistent proteinuria. Presence of coronary artery disease (CAD) was defined by its evidence on electrocardiography, echocardiography, or coronary angiography (30). The data regarding particular diseases such as myocardial infarction, congestive heart failure, cerebrovascular disease, and chronic renal disease also were collected from the patient's case sheets.

The presence of PAD (PAD; ICD-10 codes E11.5, E14.5 or unspecified PAD; ICD-10 code I73.9) was recorded by a history of intermittent claudication, non-palpable or weakly palpable pedal pulses, ankle-brachial index less than 0.9, or angiography showing significant stenosis in low extremity arteries. Vascular intervention at any time was also recorded as positive for PAD. Decrease or loss in sensation (vibration, light touch, pain, awareness of temperature differences) in a glove and stocking distribution, or loss of deep tendon reflex and absence of perception of the Semmes-Weinstein monofilament (10-g) at 2 of 10 standard plantar sites of either foot indicated peripheral neuropathy. The ICD-10 codes E11.4 and E14.4 (diabetes with neurological complications) were used for diabetic polyneuropathy. According to the presence of neuropathy and/or PAD, ulcers were divided into neuropathic, ischemic, and neuroischemic origin (1, 31).

Definition of wound grading and indications for LEA

The DFU were graded according to Wagner classification (grade 0: high-risk foot, grade 1: superficial ulcer, grade 2: deep ulcer penetrating to tendon, bone, or joint, grade 3: deep ulcer with abscess or osteomyelitis, grade 4: localized gangrene, and grade 5: extensive gangrene) (32). In this study, a foot ulcer was defined as a full-thickness skin break occurring distal to the malleolus at least to Wagner grade 1, applying definition from previous study (33). Depth of ulcer was categorized as: grade 1 (ulceration extending to subcutaneous tissue), grade 2 (ulceration involving the joint capsule or tendon), and grade 3 (ulceration extending into bone or within a joint) (34). The diagnosis of diabetic foot infection was made on clinical grounds and stratified using PEDIS system developed by the International Working Group on the Diabetic Foot (IWGDF). PEDIS itself stands for perfusion, extent (size), depth (tissue loss), infection, and sensation (neuropathy) (31, 34).

The primary outcome of interest in this study was an incident of LEA following DFU admission. Almost all LEAs were conducted in hospital settings so they could be properly registered in hospital discharge data. The indication for LEA included severe soft tissue infection, osteomyelitis, or gangrene (1, 9). This decision was made by internist-endocrinologist and surgeon conference; then the vascular or orthopedic staff executed the amputation surgery. Minor amputations were included if they were within one of the following categories: partial toe amputation, complete toe disarticulation at the metatarsophalangeal joint, ray (toe and metatarsal) amputation, or proximal foot amputation (transmetatarsal, Lisfranc's, Chopart's, and Syme's). Transtibial and transfemoral amputation were considered as major amputations (9). In our series, most major LEAs were performed in extensive gangrenous foot (Wagner grade 5) that associated with acute thrombosis occlusion. We excluded DFU in accordance to Wagner grade 5 in patient selection for statistical reasons.

Case-control classification

Cases

Case subjects included DFU patients admitted to Dr. Kariadi General Hospital with at least one subsequent lower extremity amputation (ICD-10 codes Z89.4, Z89.5, Z89.6, Z89.7, and Z89.9) during the study period. A manual review of operation theater database was conducted to identify LEAs performed between January 2012 and December 2014. In total 96 amputation surgeries were initially identified. Forty-nine patients were subsequently excluded for the following reasons: DFUs included in Wagner grade 5 lesion, 17; unable to retrieve a complete medical record, 21; unable to find a control suitable for matching, 11. This left 47 patients with LEA in confirmed diabetic patients available for the study. Of these, 37 patients (78.7%) had minor amputation and the remainder 10 patients (21.3%) had major amputation.

Controls

Control subjects were patients with DFU who had never undergone LEA during the time of hospitalization. Matching was done by pairing patients with sex and birth date within 5 years in the chronological order in which they were admitted to the study. The case and control subjects were also matched for nutritional status based on their BMI and classified as undernourished, normal weight, overweight, or obese. An attempt was made to individually match at least one control per case. In this process, 43 potential control subjects were excluded because the necessary data was incomplete or there was no corresponding match with the case subjects. The final 47 control subjects were verified after all studied patients had been evaluated and determined that a patient had not been paired with two matched controls; one control subject for each case with LEA.

Statistical analysis

Descriptive statistics were obtained to describe the characteristics of the studied population. The initial data analysis showed the distribution of key variables in all patients. Continuous variables were presented as the mean \pm standard deviation (SD) or geometrical mean and categorical variables were given as proportions. ORs and 95% CIs were calculated for various variables that have been previously reported to be independent LEA risk factors (10–23). The variables of interest were selected and these potential risk factors were compared on matched pairs of case and control subjects. ORs greater than 1 indicate an increased LEA risk for the corresponding variable using a conditional logistic regression. Accordingly, we created a dummy variable for each of the selected risk factors and examined their effects (adjusted to age, sex, and nutritional status) on LEA risk. Second, all potential predictors (variables selected through univariate analysis with $p \leq 0.15$) were entered simultaneously in a multivariable logistic regression model that was reduced using a backward selection method. In the multivariable logistic regression, the analysis was performed in a full model. The Hosmer–Lemeshow χ^2 goodness-of-fit test was used for model building (35). After the model creation, a multivariable score was computed using β coefficient values and the actual values for covariates for each variable. The ability of the score to discriminate between patients who did and did not develop an LEA was assessed using the Area under the Receiver Operating Characteristic Curve (ROC) with 95% CI. All tests were two sided with $p < 0.05$ considered statistically significant in both univariate and multivariate analysis. The Statistical Package for Social Science (IBM version 21.0; SPSS Inc., Chicago, USA) was used for all data analysis.

Results

Baseline characteristic and laboratory data

There were 47 cases with LEA at Dr. Kariadi General Hospital during the study period. In total 94 subjects were assessed as respondent to 1:1 matching according to sex, age, and nutritional state. Descriptive information that contains baseline characteristics and laboratory results are listed in Tables 1 and 2, respectively. All patients had type 2 diabetes mellitus and females were predominant (59.6%). Almost all of the patients included into the study were Javanese. The mean age of the patients and their matched control subjects was 52.6 ± 7.0 years and the median value of diabetes duration was 5 years. As for management of diabetes mellitus, the majority of patients (63.8%) were on oral hypoglycemic agents. Twenty patients (21.3%) were just diagnosed with diabetes mellitus at hospital admission.

Glycemic control was poor in the majority of subjects at the time of admission to the hospital as indicated by their admission plasma glucose (median value: 325.5 mg/dL),

Table 1. Baseline characteristics and laboratory data in the studied population^a

Variables	Overall (n = 94)
Sex	
Males	38 (40.4%)
Females	56 (59.6%)
Age (years)	52.6 ± 7.0
Hospital stay (days)	15.5 (5–69)
BMI (kg/m ²)	21.9 (17.5–32.0)
Systolic blood pressure (mmHg)	134.4 ± 23.9
Diastolic blood pressure (mmHg)	80.9 ± 12.2
Laboratory data	
Hemoglobin (gr %)	9.8 ± 1.7
Leukocyte × 10 ³ /μL	17.0 (4.9–39.5)
Albumin (g/dL)	2.4 ± 0.6
Creatinine (mg/dL)	1.1 (0.2–8.8)
Total cholesterol (mg/dL)	159.7 ± 40.6
Triglycerides (mg/dL)	153.0 (89–383)
LDL-cholesterol (mg/dL)	109.0 ± 26.6
HDL-cholesterol (mg/dL)	26.0 (10–58)
Discharge status	
Recovered (alive)	89 (94.7%)
Deceased (dead)	5 (5.3%)

Data are expressed as number (%), mean ± SD, or geometric mean (95% confidence interval). BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein. ^aCase and control were adjusted for patient's age, sex, and nutritional status.

mean FPG (220.6 ± 73.5 mg/dL), and mean HbA1c (11.3 ± 2.8%). Mixed dyslipidemia characterized by hypertriglyceridemia and low level of HDL-cholesterol could be observed in our patient population. The most common comorbidities were hypertension (53.2%) and chronic renal failure (43.6%, on dialysis in 4.2% patients). With respect to specific diabetes-related vascular complication, retinopathy can be observed in 92.6% of patients, 68.1% had peripheral neuropathy, 54.3% had nephropathy, and 40.4% had PAD. As for clinical outcomes, the median value of length of hospitalization was 15.5 days. The mortality rate was 5.3% involving total of five patients in both case and control subjects.

Type of diabetic foot

In our sampled population, DFU had developed within the median time of 2 weeks (ranged 1 to 72 weeks) before hospital admission. Thirty-two patients had a previous history of diabetic foot disease whereas most of them (66.0%) had never reported any previous ulcer. Fourteen patients (14.8%) had prior history of LEA due to diabetic foot. Of the total number of 94 patients with DFU, 40 (42.6%) were classified as neuropathic, 24 (25.5%) were neuroischemic, 14 (14.9%) were ischemic ulcers, and 16 (17.0%) had no identified underlying factors in respect to

Table 2. Characteristics of diabetic foot ulcer and diabetes complications in the studied population^a

Variables	Overall (n = 94)
Duration of ulcer (week)	2 (1–72)
Previous DFU	32 (34.0%)
Previous LEA	14 (14.8%)
Type of diabetic foot	
Neuropathic	40 (42.6%)
Ischemic	14 (14.9%)
Neuroischemic	24 (25.5%)
Wagner grade ≥ 3	71 (75.5%)
Diabetic foot infection	93 (98.9%)
Diabetes medication before admission	
Oral hypoglycemic agent	60 (63.8%)
Insulin	10 (10.6%)
Combination therapy	4 (4.2%)
Start at hospital	20 (21.3%)
Diabetes and its complications ^b	
Duration of diabetes (years)	5 (0–21)
Admission plasma glucose (mg/dL)	325.5 (113–740)
FPG (mg/dL)	220.6 ± 73.5
HbA1c (%)	11.3 ± 2.8
Hypertension status	50 (53.2%)
Retinopathy ^c	87 (92.6%)
Nephropathy ^c	51 (54.3%)
Peripheral neuropathy ^c	64 (68.1%)
Presence of PAD ^c	38 (40.4%)
Presence or history of CAD ^c	21 (22.3%)
Congestive heart failure ^c	3 (3.2%)
Cerebrovascular disease ^c	6 (6.4%)
Chronic renal failure ^c	41 (43.6%)
Dialysis ^c	4 (4.2%)

Data are expressed as number (%), mean ± SD, or geometric mean (95% confidence interval). DFU, diabetic foot ulcer; LEA, lower extremity amputation; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; PAD, peripheral arterial disease; CAD, coronary arterial disease. ^aCase and control were adjusted for patient's age, sex, and nutritional status; ^beither known or diagnosed during the course of hospitalization; ^cusing the Young et al. (30) proposed diabetic complications' classification.

either neuropathy or PAD. Most DFU (48.6%) had already penetrated into muscle or tendon, 43.6% of them penetrated into bone, and 7.4% of ulcers were categorized as superficial ulcers. When evaluated according to Wagner classification, the majority of patients (75.5%) were in grade 3 and grade 4 lesions, respectively 39.4 and 36.2% of patients. DFU corresponding to Wagner grade 5 were excluded from the population selection. Additionally, 98.8% of ulcers showed clinical evidence of infection at presentation. Deep abscess and osteomyelitis were found in 68 patients (72.3%) whereas in the most severe form, septicemia occurred in 12 (12.7%) of sampled patients.

Univariate analysis of LEA risk factors

To identify the significant risk factors for amputation, a conditional logistic regression was performed. Studied variables included older age, duration of diabetes, hypertension status, retinopathy, neuropathic foot, presence of

PAD, wound depth, gangrene, deep abscess, osteomyelitis, sepsis, admission plasma glucose, FPG, HbA1c, and lipid profile. Table 3 shows a comparison between the cases and control group to indicate the corresponding ORs for outcome. Significant risk factors were hypertension

Table 3. Univariate analysis of risk factors associated with lower extremity amputation^a

	Non-amputation <i>n</i> (%)	Amputation <i>n</i> (%)	OR	95% CI	<i>p</i>
Age ≥60 years	6 (6.4%)	12 (12.7%)	2.34	0.79–6.89	0.122
Duration of diabetes >5 years	20 (21.3%)	26 (27.6%)	1.67	0.73–3.77	0.217
Prior diabetes therapy, <i>n</i> (%)					
Not on previous treatment (reference)	11 (11.7%)	9 (9.6%)	1.00		
Oral hypoglycemic agents	31 (32.9%)	29 (30.8%)	1.14	0.41–3.15	0.796
Insulin use (insulin alone or in combination therapy)	5 (5.3%)	9 (9.6%)	2.20	0.54–8.95	0.271
Admission plasma glucose ≥200 mg/dL	39 (41.4%)	44 (46.8%)	3.00	0.74–12.14	0.122
FPG ≥126 mg/dL (mg/dL)	39 (41.4%)	46 (48.9%)	9.43	1.13–78.78	0.038*
HbA1c ≥8%	33 (35.1%)	45 (47.8%)	9.54	2.03–44.89	0.004*
Hemoglobin ≤10 gr%	29 (30.8%)	27 (28.7%)	1.19	0.52–2.72	0.674
Leukocyte count ≥15 × 10 ³ /μL	26 (27.6%)	27 (28.7%)	1.09	0.42–2.46	0.835
Albumin ≤2.5 g/dL	24 (25.5%)	28 (29.8%)	1.41	0.62–3.19	0.407
Serum creatinine ≥1.5 g/dL	15 (15.9%)	13 (13.8%)	1.22	0.50–2.97	0.692
Total cholesterol ≥200 mg/dL	6 (6.4%)	9 (9.5%)	1.07	0.69–1.67	0.736
Triglycerides ≥150 mg/dL	17 (18.1%)	33 (35.1%)	2.14	1.13–4.04	0.019*
LDL-cholesterol ≥100 mg/dL	23 (24.4%)	30 (31.9%)	1.42	0.76–2.62	0.277
HDL-cholesterol ≤40 mg/dL	39 (41.4%)	44 (46.8%)	2.67	0.70–10.05	0.147
Hypertension status	19 (20.2%)	31 (32.9%)	2.85	1.23–6.60	0.014*
Presence of CAD ^b	13 (13.8%)	8 (8.5%)	1.14	0.72–1.81	0.559
Diabetic retinopathy ^b	42 (44.7%)	45 (47.8%)	2.50	0.48–12.88	0.273
Diabetic nephropathy ^b	25 (26.6%)	26 (27.6%)	1.04	0.57–1.90	0.879
Diabetic neuropathy ^b	30 (31.9%)	34 (36.2%)	1.30	0.63–2.69	0.467
Diabetes with PAD ^b	9 (9.6%)	29 (30.8%)	2.11	1.20–3.69	0.009*
Type of DFU					
Pure neuropathic (reference)	25 (26.6%)	5 (5.3%)	1.00		
Ischemic/neuroischemic	9 (9.6%)	29 (30.8%)	3.22	1.52–6.80	0.002*
Wound depth					
Full thickness+deep to fascia or tendon (reference)	31 (32.9%)	22 (23.4%)	1.00		
Penetration to joint or bone	16 (17.0%)	25 (26.6%)	1.56	0.83–2.92	0.163
Osteomyelitis	18 (19.1%)	27 (28.7%)	2.17	0.95–4.96	0.065
PEDIS grade and IDSA infection severity score					
PEDIS grade 1 + 2 (reference)	10 (10.6%)	4 (4.3%)	1.00		
PEDIS grade 3	33 (35.1%)	35 (37.2%)	1.06	0.65–1.70	0.808
PEDIS grade 4	4 (4.3%)	8 (8.5%)	2.00	0.60–6.64	0.258
Wagner grade ^c					
Grade 1 + 2 (reference)	21 (22.3%)	2 (2.1%)	1.00		
Grade 3	23 (24.4%)	15 (15.9%)	1.53	0.80–2.93	0.198
Grade 4	3 (3.2%)	30 (31.9%)	10.00	3.05–32.76	<0.001*
Presence of foot necrosis or gangrene	3 (3.2%)	30 (31.9%)	25.88	6.97–96.13	<0.001*

Data are expressed as number (%). LEA, lower extremity amputation; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; LDL, low density lipoprotein; HDL, high density lipoprotein; CAD, coronary arterial disease; PAD, peripheral arterial disease; DFU, diabetic foot ulcer; PEDIS, acronym of perfusion, extent, depth, infection and sensation. ^aLogistic regression analysis was applied, data are adjusted for age, sex, and nutritional status; ^busing the Young et al. (30) proposed diabetic complications' classification; ^cDFU with Wagner classification grade 5 were excluded (see text); *denotes statistical significance ($p < 0.05$) compared to non-amputation group.

status (OR 2.85, 95% CI 1.23–6.60; $p=0.014$), presence of PAD (OR 6.80, 95% CI 2.67–17.32; $p<0.001$), foot necrosis or gangrene (OR 25.88, 95% CI 6.97–96.13; $p<0.001$), FPG ≥ 126 mg/dL (OR 9.43, 95% CI 1.13–78.78; $p=0.038$), HbA1c $\geq 8\%$ (OR 9.54, 95% CI 2.03–44.89; $p=0.004$) and triglycerides ≥ 150 mg/dL (OR 4.16, 95% CI 1.75–9.86; $p=0.001$). Other variables included in the logistic regression model were found not significant in determining the risk of amputation.

Multivariate logistic regression model

Univariate analysis of the amputation risk versus exploratory variables showed that, out of 27 variables, only seven showed a promise of influence, the latter being defined as $p \leq 0.15$ (see Table 3). The potential independent variables included hypertension status, diabetes with PAD, gangrene (Wagner grade 4), wound depth to bone and joint, osteomyelitis, FPG ≥ 126 mg/dL, HbA1c $\geq 8\%$ and triglycerides ≥ 150 mg/dL as independent variables. Gangrenous tissue implies extensive necrosis and poor circulation in the local tissue (36). In order to better elucidate the risk of LEA, we decided to exclude the variable of gangrene from further analysis.

Finally, in a stepwise manner, logistic regression analysis was performed of the amputation risk vs. the remaining seven variables simultaneously (not included gangrene), starting with a full model and removing non-significant variables one by one. The final result was a model with adjusted significant predictors of undergoing an LEA. Table 4 displays the adjusted multivariable logistic regression and, among others, the independent risk factors of LEA are hypertension status (OR 3.67, 95% CI 1.14–11.79; $p=0.028$), triglyceride ≥ 150 mg/dL (OR 5.58, 95% CI 1.74–17.91; $p=0.004$), diabetes with PAD (OR 12.97, 95% CI 3.44–48.88; $p<0.001$), and HbA1c $\geq 8\%$ (OR 20.47, 95% CI 3.12–134.31; $p=0.002$). The Hosmer–Lemeshow goodness-of-fit test statistic ($X^2=4.085$ with 8 degree of freedom, $p=0.849$) indicates that the model

created was appropriately fitted for the data (35). The multivariate analysis produced a score with an AUC value of 0.89 (95% CI 0.83–0.95; $p<0.001$) for the discrimination between those who did or did not experience an incident LEA.

Discussion

DFU is the most frequent cause of hospitalization among diabetic patients and LEA is the most feared consequence of foot ulceration (2, 3, 7). The present study examined whether or not certain baseline characteristics and laboratory measures can predict the risk of LEA. In Indonesia, studies of the incidence or determination of particular risk factors of LEA in the diabetic population are few. This study reports the results of an extensive subset analysis of the data collected during a period of hospitalization in the treatment of DFU. Our references at most will examine age, sex, and/or BMI as predictors of interest, however we considered such variables to be included in study matching criteria thus providing a difference from the previous research. The samples were limited to 94 patients treated by a diabetic foot team in a tertiary hospital in Semarang, Indonesia and the studied populations represented a diabetic population that constituted the highest risk of poor outcome.

To describe the severity of DFU, we used two of the diabetic foot classification systems: 1) Wagner grade (32), and 2) PEDIS system as classified following IDSA-IWGDF recommendation (34). In a Turkish cohort, Yesil et al. (22) reported that Wagner grade (Wagner grade 4 and 5) was a strong predictor for LEA with OR 23.95 (95% CI 14.04–40.87; $p<0.001$). A study from Pakistan also reported that the frequency of amputation increased with the higher grade (Wagner grade ≥ 3) of ulcers (37). According to Wagner classification, our study revealed that 95.7% of the cases were classified as high grade lesion (\geq grade 3) whereas in the control group, the number of

Table 4. Final logistic model for multivariate (adjusted) risk factors of lower extremity amputation^a

	Amputation ($n=47$)			
	β -coefficient	Adjusted OR	95% CI	p
Hypertension status	1.30	3.67	1.14–11.79	0.028*
Triglycerides ≥ 150 mg/dL	1.72	5.58	1.74–17.91	0.004*
FPG ≥ 126 mg/dL	2.16	8.67	0.74–101.11	0.085
Diabetes with PAD	2.56	12.97	3.44–48.88	<0.001 *
HbA1c $\geq 8\%$	3.01	20.47	3.12–134.31	0.002*

Area under the ROC curve = 0.89; Hosmer–Lemeshow goodness-of-fit test: $X^2=4.085$, $p=0.849$. In the multivariate model, the following variables were added as potential independent variables: patient's age ≥ 60 years, hypertension status, neuropathic foot, diabetes with PAD, admission plasma glucose ≥ 200 mg/dL, FPG ≥ 126 mg/dL, HbA1c $\geq 8\%$, triglycerides ≥ 150 mg/dL, wound depth and osteomyelitis. OR, odds ratio; CI, confidence interval; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; PAD, peripheral arterial disease. ^aBackward stepwise conditional logistic regression model was applied; *denotes statistical significance ($p<0.05$) compared to non-amputation group.

patients with high Wagner grade was 51.1%. By conditional logistic regression, we obtained a 10-fold increased risk of amputation when DFU severity at admission was at least Wagner grade 4 when compared to grade 1 and grade 2. We also found that DFU that penetrated to bone was not merely a risk factor but the presence of gangrene became a very strong reason for an LEA (OR 25.88, 95% CI 6.97–96.13; $p < 0.001$). The prevalence of overall patients with foot necrosis or gangrene was 35.1% (we excluded Wagner grade 5, see Methods section). Our hospital was considered as the main referral medical center in Central Java District, thus hospitalized patients contained complexities and more advanced DFU with an increased risk of extensive surgical management. This fact becomes a relatively common scenario in developing countries while there was a sequential timeline of patients before referred to the hospital and brings considerable delay for optimal management when an amputation surgery was inevitable (3–5).

After accounting for differences in the stage of presentation, we addressed the role of PAD on this matter regarding LEA risk in patients with DFU. PAD was identified by different studies as an independent risk factor for LEA; it is a point of almost universal agreement among studies (11, 12, 14, 18, 20). The Eurodiale study (38) has confirmed when stratifying patients according to the presence or absence of PAD, significantly fewer ulcers with PAD were healed than those without PAD (69 vs. 84%, respectively). In our study, the prevalence of PAD is about 40.4% of all studied population. There was also a significantly higher prevalence of PAD in the case subjects. As many as 61.7% of patients from the LEA group had various degrees of PAD compared to 9.6% on the control group ($p = 0.009$). PAD was associated with LEA because of impairment in wound healing due to inadequate circulation and its presence (PAD that did not present the possibility of revascularization) led to a significantly higher rate of LEA (OR 6.80; 95% CI 2.67–17.32; $p < 0.001$). The subsets of patients with most likelihood to present with LEA were those with neuroischemic ulcer (OR 3.22, 95% CI 1.52–6.80; $p = 0.002$) compared to only neuropathic ulcer, showing that combined risk factors put patients at a significantly higher risk. Because we did not differentiate the minor from major LEA, an interesting report by Calle-Pasqual et al. (39) shows that 100% of the major amputations, whereas a lower percentage (62%) of minor amputations in their population-based series were associated with PAD. Reiber et al. (20) also reported that the presence of PAD as indicated by Doppler vascular studies (OR 4.3 for mild to moderate PAD and OR 55.8 for severe PAD) was the most powerful predictor of amputation in diabetic subjects.

Another finding in our sampled population was the high prevalence (68.1%) of peripheral diabetic neuropathy. This finding was common in the countries of developing economics, where ischemic disease accounts for only

20–30% of cases (18, 40). In contrast, the nations of Western Europe and the USA have higher prevalence of PAD (usually around 50% or more) and reporting a lesser prevalence of peripheral neuropathy (38, 39). Ethnic differences in PAD and diabetes-related microangiopathy rates have been observed (41, 42). The vast majority of our patients were Javanese and that might be able to partly explain the relative difference between PAD and neuropathy prevalence in this study compared to other scientific literature. Our results signify an important pathway of foot ulceration through peripheral neuropathy. But contrary to the expectation, our study revealed that diabetic peripheral neuropathy was found to have no independent effect on the final outcome as determined by statistical analysis. As shown in Table 3, patients with peripheral neuropathy and PAD (i.e. neuroischemic ulcer-type) were more likely to undergo LEA but neuropathy alone was not independently associated with LEA. It has been suggested that neuropathy may precipitate an ulcer through decreased foot protective sensation, however it was the PAD that inhibited the ulcer from healing (1, 38, 43). In our study population, diagnosis of diabetic retinopathy and nephropathy did not prove to be significant and independent risk factors for LEA. The high prevalence of diabetes-related microangiopathy may indicate that the patients in both case and control subjects have already experienced an advanced diabetes stage altogether with their DFU occurrence in the hospital.

The most important finding in our study was that poor glycemic control had a major role in the development of LEA. In the results of our study, baseline glycemic control (median plasma glucose 325.5 mg/dL (range 113–740), mean FPG 220.6 ± 73.5 mg/dL, and mean HbA1c $11.3 \pm 2.8\%$, see Table 2) show that the diabetics in our studied population was poorly controlled. HbA1c above 8% was a significant risk factor for LEA (OR 20.47, 95% CI 3.44–134.31; $p = 0.002$) whereas admission blood glucose was not included in the final model, and FPG did not meet statistical significance in the final multivariate analysis. The role of chronic hyperglycemia as indicated by high HbA1c level as a marker of LEA incident is similar to several other studies, notably those reported by Moss et al. (10), Miyajima et al. from Japan (17), and Imran et al. (37). In contrast to admission plasma glucose, FPG, or post-prandial blood glucose; the level of HbA1c is directly related to the average glucose concentration over the life span of the hemoglobin (44). The strong association of HbA1c with LEA could reflect a greater pathogenic role of chronic hyperglycemia probably via neuropathy, autonomic dysfunction, PAD, and susceptibility to infection (44, 45). The United Kingdom Prospective Diabetes Study (46) reported that the hazard ratio of death from amputation declines 43% when HbA1c declines by 1%. The Steno-2 study (47) has shown that an intensified multifactorial intervention including tight glucose control

reduces the risk of vascular complication by half, and significantly lowers the amputation rate compared to standard treatment for patients with type 2 diabetes. The meta-analysis adds to the accumulating data on hyperglycemia as an independent risk factor for LEAs (45).

Because metabolic control in diabetic patients tends to deteriorate linearly with time after the diagnosis, the exposure to the harmful effects of hyperglycemia will increase with the longer duration of diabetes (11, 46). In a study from Finland by Lehto et al. (11), the duration of diabetes was related to the risk of LEA independently of the degree of hyperglycemia. However, from Table 3 of our study, we can conclude that as many as 27.6% of cases compared to 21.3% of control had diabetes for more than 5 years ($p = 0.217$) and the clinical duration of diabetes was not related to the risk of amputation. Our finding was similar to many other studies that claimed the duration of diabetes is not a baseline factor that predicts amputation (17, 19, 22). Reiber et al. (20) and Adler et al. (45) also reported the non-differences in the risk of LEA by the duration of diabetes but the risk can be explained better by the level of glycemia. However, the clinical duration of diabetes may contain an error because the initial diagnosis does not always coincide with the onset of the metabolic disease. Arguably, the diabetes duration calculated in this way is shorter than the real duration of diabetes (4).

Hypertension also contributes to the development and progression of chronic diabetes complications and it is considered as an established risk factor for atherosclerosis (30). The data concerning the importance of blood pressure as a predictor of LEA are somehow conflicting. In American Indians, systolic blood pressure was found to be an important predictor of LEA (16). Other previous cross-sectional and prospective studies also have shown an association between amputation with higher blood pressure parameter (15, 19). On the contrary, a population study conducted by Lehto et al. (11) reported that hypertension was not found to be a significant predictor for LEA incident. In our study, there were significantly more recorded diagnoses of hypertension in case subjects compared to control group (32.9 vs. 20.2%, $p = 0.013$) and we found that hypertension status was a major risk factor for LEA (OR 3.43; 95% CI 1.07–10.94, $p = 0.037$). Our finding was in accordance with Wisconsin Epidemiologic Study of Diabetic Retinopathy which shows that blood pressure and HbA1c were related to amputation risk but that nephropathy and retinopathy were at most only weakly correlated (10). Direct comparison of the role of hypertension as a risk factor for LEA between the studies is difficult because of diverse methods of defining hypertension, different demographics, and sample population.

Several lipoprotein abnormalities have been reported to be more prevalent among diabetic than non-diabetic persons (28–30). Only a few studies have been published regarding the effect of abnormalities in lipids and lipoproteins on the risk of amputation in DFU.

The recent study by Zubair et al. (19) reported that the levels of fasting triglyceride (> 150 mg/dL), cholesterol (> 150 mg/dL), LDL-cholesterol (> 100 mg/dL), and HDL-cholesterol (< 40 mg/dL) were associated with the risk of amputation. Our observation regarding plasma lipoproteins have demonstrated that of all the elements considered, only hypertriglyceridemia predicts LEA (OR 5.87, 95% CI 1.84–18.97; $p = 0.003$) whereas the other fractions do not seem to be associated with amputation. Another study by Lacle et al. (13) from Costa Rica and Chaturvedi et al. (41) from The WHO Multinational Study also failed to demonstrate that serum cholesterol, LDL-cholesterol, and HDL-cholesterol are significant risk factors for LEA. Hypertriglyceridemia has shown to be an independent stepwise risk factor in a cohort of 28,700 diabetic patients from Distance study (48). Increased plasma triglycerides were also reported by Lee et al. to be significant risk factors for LEA in American Indian women (15). However, there was no clear explanation about what extent and if there was a causative relationship or if triglycerides just merely serve as a risk marker (15, 48). Clearly, further studies are needed to ascertain the role of hypertriglyceridemia in these diabetic sequelae.

Benotmane et al. (40) reported that length of stay was increased in patients with high grade of Wagner classification. The length of hospitalization was 15.5 days in our study. In other studies, the length ranged from 20 to 40 days (40, 49). Currie et al. (50) studied the patients with PAD, infection, neuropathy, and ulceration and reported that diabetics had twice longer length of stay as compared with non-diabetic patients. History of previous ulceration and amputation in either foot can also predict amputation in previous reports (10, 12–14, 33). Such previous history (previous ulceration or amputation) was not an independent risk factor according to our analysis. The less obvious risk factors such as sex, older age, and lower BMI were not prominent because of the study matching criteria. The other risk factors that were not addressed in this study were smoking history and ulcer size. Although it was included in the study protocol, it was not feasible because such information was not found in most of our charts.

Diabetic foot problems develop on the basis of micro- or macroangiopathy and can present with infection (1, 31). Diabetic foot infections can threaten a limb when there is osteomyelitis and/or sepsis (34). In our study, infectious events occurred in nearly all lesions (98.8%). If compared to other studies, the prevalence of infection in our study was higher which may be related to uncontrolled hyperglycemia, presence of PAD, and cultural differences in foot care. More severe infection (PEDIS grade 3 and 4) is associated with higher rates of LEA than milder one (45.7 vs. 39.3%, $p = 0.138$). If compared to mild infection (PEDIS grade 1 and grade 2) as reference categories,

obviously the more severe infection only shows a step-up increase of OR which was not statistically significant. Previous studies agree that foot infection is a risk factor for diabetic foot amputation (10, 21, 51), however our data did not reveal a strong association. This substantiates that a septic foot does not inevitably lead to LEA and may explain the role of severe infection as dependent rather than independent of risk factors. In support of this observation was a previous study by Bamberger et al. in 1987 (52). Their group reported the success in eradicating osteomyelitis in 27 out of 52 patients (53%) by conservative approach and suggested a good outcome without the need for an ablative surgical procedure in the absence of extensive necrosis or gangrene. A more recent cohort study ($n=58$) by Yadlapalli et al. (53) also support in attempting a treatment based on local care and potent antibiotic regimens.

Overall, DFU and amputation could be considered as the marker of advanced stage of diabetes. Some authors hypothesized that DFU could be *per se* an independent predictive variable of LEA as well as mortality (14). Many factors influence the decision of whether or not an LEA should be performed on a patient with DFU, besides the ulcer severity as determined by high Wagner grade. The predictive estimate of our model was 0.89 (95% CI 0.83–0.95; $p < 0.001$); it was similar to that of a model suggested by Martins-Mendes et al., 0.81 (95% CI 0.74–0.87; $p = 0.001$) from Portugal (14) and a study by Lipsky et al., 0.72 (95% CI 0.67–0.77; $p < 0.001$) in diabetic foot infection (54). Martins-Mendes et al. (14) suggested the following risk factors for LEA: previous DFU, PAD complication history, neuropathy, and nephropathy. Lipsky et al. (54) reported that LEAs were higher for patients with surgical site infection, vasculopathy, amputation history, and high leukocyte count. We added a few more variables to this suggested model and identified a typology of risk for LEA in DFU patients with an average HbA1c $\geq 8\%$, along with the presence of PAD, hypertriglyceridemia, and hypertension. Accordingly, diabetic patients with foot ulcers with the above-mentioned profile should be considered to be at high risk of LEA and signal the need for close monitoring by health care professions. The variations in the extent and ranking of risk factors for the development of diabetic foot LEA between the present results and other research are probably due to differences in study settings and population selection.

Study limitations

This study has several limitations. First, missing data were inevitable because our analysis was a retrospective study. Hospital discharge database as a source of our information was administrative in nature and not primarily intended for research purposes, consequently, many variables that affected the outcomes were not recorded or considered. This included type of off-loading and description of foot

deformities. The degree of blood pressure control, lipid control, and previous foot care procedures prior to hospitalization was also difficult to estimate. Second, the specific type and duration of antibiotics for patients with infection were not well documented. Third, we did not address the severity of PAD in distinct gradation and this might have affected the final outcome. Fourth, the data used in this study was generated from one hospital, limiting its generalizability to other hospitals. Our studied population was mainly Javanese, therefore all our results may not apply directly to other racial or ethnic groups. This analysis, despite having limitations for a developing country with limited data on economics and a lack of continuous longitudinal data on LEA, could be justified by the fact that the studied risk factors can easily be assessed and are potentially modifiable during clinical practices. The present study, to our knowledge, is the first study sharing the experience of a DFU management in Semarang for the evaluation of risk factors for LEA.

Conclusions

In the results of our analysis, poor glycemic control, the presence of PAD, hypertriglyceridemia, and hypertension status were independent risk factors for LEA. Short of prevention of DFU itself, this study indirectly implies that early intervention before critical DFU has developed might help to prevent diabetes-related LEA. However, we believe that not all of these DFU can be prevented and still, clinicians will face patients in the hospital with DFU in advanced stages as ours. Diabetic patients with inadequately controlled blood glucose levels are at highest significant risk for serious complications affecting their lower limbs. Strict control of diabetes, which is the primary disease, is first of all required for the risk reduction. For the PAD, active investigation of each patient is necessary to assess the possibility of revascularization and the probability of wound healing. At the same time, this study indicates that triglyceride and hypertension control should not preclude the pursuit of limb conserving treatment options and both may be an important additional primary prevention effort. We suggest that prospective studies and multicenter designs involving more detailed vascular risk factors should be undertaken in the future for further conclusions.

Conflict of interest and funding

The authors have no conflict of interest to declare in relation to the content of this article.

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