Prediction of Wound Healing in Diabetic Foot Ulcers: an Observational Study in Tertiary Hospital in Indonesia

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ABSTRAK

Tujuan: *mempelajari peran faktor risiko klinis, penanda fungsi vasodilatasi, respons inflamasi, dan aterosklerosis dalam memprediksi penyembuhan luka kaki diabetik.* ***Metode:*** *studi kohort dilakukan sejak Februari – Oktober 2010 terhadap 40 subyek penelitian dengan ulkus diabetik akut di ruang rawat inap RSUPN Dr. Cipto Mangunkusumo, Jakarta, Indonesia. Pada setiap subyek, dilakukan minimal dua kali pengukuran variabel, yaitu pada fase inflamasi dan proliferasi. Variabel yang diteliti berupa karakteristik klinis, pemeriksaan darah perifer lengkap (DPL) dan hitung jenis, HbA1c, ureum, kreatinin, profil lipid, glukosa darah puasa (GDP), penanda disfungsi endotel (asymmetric dimethylarginine/ADMA, endothelin-1/ET-1, dan flow-mediated dilation/FMD arteri brakialis), dan penanda kalsifikasi vaskular (osteoprotegerin/OPG).* ***Hasil:*** *waktu rerata tercapainya jaringan granulasi 50% pada penelitian ini adalah 21 hari. Terdapat sembilan faktor yang berperan dalam pembentukan 50% jaringan granulasi, yaitu riwayat DM pada keluarga, riwayat luka, luas luka, lama luka, pemakaian kaptopril dan simvastatin, konsentrasi ADMA, ET-1, dan OPG. Terdapat tiga dari sembilan faktor yang secara signifikan berkorelasi terhadap penyembuhan luka, yaitu luas luka, konsentrasi OPG, dan pemakaian simvastatin.* ***Kesimpulan:*** *pada ulkus diabetik akut, luas luka dan konsentrasi OPG memiliki korelasi positif terhadap penyembuhan luka, sementara pemakaian simvastatin memiliki korelasi negatif terhadap penyembuhan luka.*

Kata kunci: *ulkus diabetik, penyembuhan luka, disfungsi endotel, kalsifikasi vaskular.*

ABSTRACT

Aim: *to evaluate the role of clinical characteristics, functional markers of vasodilation, inflammatory response, and atherosclerosis in predicting wound healing in diabetic foot ulcer.* ***Methods:*** *a cohort study (February – October 2010) was conducted from 40 subjects with acute diabetic foot ulcer at clinical ward of Dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia. Each subject underwent at least two variable measurements, i.e. during inflammatory phase and proliferation phase. The studied variables were clinical characteristics, complete peripheral blood count (CBC) and differential count, levels of HbA1c,*

ureum, creatinine, lipid profile, fasting blood glucose (FBG), marker of endothelial dysfunction (asymmetric dimethylarginine/ADMA, endothelin-1/ET-1, and flow-mediated dilation/FMD of brachial artery), and marker of vascular calcification (osteoprotegerin/OPG). ***Results:*** *median of time achieving 50% granulation tissue in our study was 21 days. There were nine factors that contribute in the development of 50% granulation tissue, i.e. family history of diabetes mellitus (DM), previous history of wound, wound area, duration of existing wound, captopril and simvastatin medications, levels of ADMA, ET-1, and OPG. There were three out of the nine factors that significantly correlated with wound healing, i.e. wound area, OPG levels, and simvastatin medications.* ***Conclusion:*** *in acute diabetic foot ulcers, wound area and OPG levels had positive correlation with wound healing, whereas simvastatin medications had negative correlation with wound healing.*

Keywords: *diabetic ulcer, wound healing, endothelial dysfunction, vascular calcification.*

INTRODUCTION

Diabetic foot is one of the most feared chronic complication of diabetes mellitus (DM). The mortality rate of diabetic foot complication was reported to remain high even after life-saving amputation procedure.[1] Several previous studies held in Indonesia had documented the rate to be in range of 17-32%, while the proportion of amputation was 15-30%. One-year survival following amputation can be as high as 14.8%, but the rate dramatically increases up to 37% in the next three years. Though amputation was thought to be an effective treatment of complicated diabetic foot ulcer, preexisting clinical background does affect the final outcome of the subjects.[2]

Time prediction model has already been developed for wound healing process in diabetic ulcer. Longer healing time was found in subjects characterized by old wound that existed for longer than 2 months, involving area of more than 2 cm^2, and penetrating deeper than subcutaneous layer in extent.[3] Zimny et al.[4] developed a formula to predict healing time for neuropathic ulcer in subjects with type 1 and type 2 DM who had neuropathic ulcer without peripheral arterial disease (PAD). Other prospective studies constructed similar prediction rules, but no additional indicators were included except wound characteristics and the dynamics of the healing process.[5]

The underlying mechanisms leading to the impairment in diabetic wound healing are still not fully understood and hence requires more intense investigation. Several known factors, such as endothelial dysfunction, inflammation and immunological responses, as well as atherogenesis with its subsequent vascular calcification, are responsible for this phenomenon observed in many diabetic wounds. It was reported earlier that increase in endothelin-1 (ET-1) and decrease in nitric oxide (NO) production were found in many diabetic patients, while osteoprotegerin (OPG) plays a significant role in atherogenesis of smaller and larger vessels.[6-8] Increased OPG expression in type 2 DM has been reported to be correlated with micro- and macroangiopathy as well as impaired endothelium-dependent arterial vasodilation.[9]

While previous wound studies were conducted mainly on animals, current knowledge still needs further information about the role of clinical characteristics, markers of vascular tone regulation, and vascular calcification as the predictor of final outcome. It is proposed that there are differences in vasodilatory function and the level and expression of angiogenic factors in inflammatory phase compared to the proliferative phase among acute diabetic foot ulcer patients. This study aims to study the role of clinical characteristics, functional markers of vasodilation, inflammatory response, and atherosclerosis in predicting wound healing in diabetic foot ulcer.

METHODS

An observational cohort study was conducted in February-October 2010 among inpatients admitted for acute foot ulcer and type 2 DM in Dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia. Out of 40 subjects,

twenty five subjects successfully completed the study which met the criteria of minimum sample size. Fifteen subjects were unable to complete the study and excluded from further analysis – 4 subjects underwent amputation, 5 patients died, and 4 patients dropped out. There were missing data from 2 patients in the proliferative phase.

Patients with ulcer or gangrene who developed sepsis or critical limb ischemia (CLI) were excluded from the study as it was not possible to perform complete observation and due to other confounding inflammatory factors. CLI was diagnosed based on physical findings and/or ankle-brachial index (ABI) result less than 0.8. Wound severity evaluation was assessed based on the Modified Texas Criteria (**Table 1**).

The manuscript had been fully approved by team of ethics at Faculty of Medicine, Universitas Indonesia with the ethical number of 374/PT02. FK/ETIK/2009 and it had also been approved by the Director of Dr. Cipto Mangunkusumo National Central General Hospital. All subjects were first asked to confirm their participation in this study by signing on the provided informed consent in correspondence with International Conference on Harmonization, which included prior explanation about study risks.[10]

There were at least two measurements conducted in association with the stages of healing process in which the respective subject diagnosed to, i.e. inflammatory phase and proliferative phase. Information includes complete peripheral peripheral blood count (CBC) and differential count, HbA1c, ureum, creatinine, lipid profile, fasting blood glucose (FBG), asymmetric dimethylarginine (ADMA), ET-1, OPG, and flow-mediated dilatation (FMD) of brachial artery, were measured in both phases. The same measurements were carried out at proliferative phase or at the fourth week of wound care. Proliferative phase was characterized by a tendency of reduced leukocyte count or the presence of granulation tissue in >50% of wound area or the wound demonstrated to be in the proliferative phase based on histopathological examination and no sign of infection or inflammation observed. To measure the granulation area, transparant film was manually placed over the wound and the outline was traced with a permanent marker. The film was then put onto metric grids and the area was determined by counting the number of square it covered. All subjects received standard treatment and care for acute diabetic ulcer in Cipto Mangunkusumo Hospital.[11]

Table 1. The degree of wound severity based on Modified Texas Criteria[10]

Stage		Grade			
		0	1	2	3
A		No ulcer or post ulcer, intact skin or bone	Superficial ulcer, but it does not penetrate to tendon or joint capsule	The wound penetrates to tendon or joint capsule	The wound penetrates to bone/ joint
B Infection	1	Infection of skin and subcutaneous tissue			
	2	Erythema of >2 cm or infection involving subcutaneous structure, signs of SIRS (-)			
	3	Infection with systemic manifestations: fever, leukocytosis, shift to the left, metabolic instability, hypotension, azotemia			
C Ischemia	1	There are signs and symptoms of PAD, but none of CLI			
	2	CLI			
D Infection and ischemia	B1	Infection of skin and subcutaneous tissue			
	B2	Erythema of >2 cm or infection involving subcutaneous structure, signs of SIRS (-)			
	B3	Infection with systemic manifestation: fever, leukocytosis, shift to the left, metabolic instability, hypotension, azotemia			
	C1	There are signs and symptoms of PAD, but none of CLI			
	C2	CLI			

SIRS: systemic inflammatory response syndrome; PAD: peripheral arterial disease; CLI: critical limb ischemia

FMD measurement was carried out by B-mode Doppler Ultrasonography (US) MyLab 30CV, Esaote. Brachial artery scanning was performed at rest and hyperemia conditions, the latter in which was induced by inflating and deflating the sphygmomanometer manset until the pressure raised up to 50 mmHg above the patient's systolic. Luminal diameter was defined as the largest diameter calculated between two adventitia layers of the artery. We used the following equation to assess the endothelial function: [Dmax-D]/D; Dmax referred to the hyperemic diameter, while D referred to diameter during rest. The result was then considered to be normal if there was more than 10% change in the luminal diameter before and after compression.

Evaluation of vasodilation and vascular calcification markers were carried out by enzyme-linked immunosorbent assay (ELISA) using the following kits: Endotelin-1 (IBL International), ADMA (DLD Diagnostic GmBH), and OPG (IDS Lucron Bioproduct) at Integrated Laboratory of Immunoendocrinology, Faculty of Medicine, Universitas Indonesia.

Statistical Analysis

Data cleaning was meant to replace any missing values with the one predicted by linear regression model. Any new variables gained from univariate analysis was then adjusted for further analysis. Based on the results of univariate analysis, some variables were then classified into new variables with adjusted categories for further analysis. Bivariate analysis was carried out to observe the correlation between each independent variable and the output, i.e. wound healing.[12]

Multivariate analysis used linear regression for dependent variables, which were numerical data. Variables of interaction were included in the multivariate model when the p value was less than 0.25. Analysis on survival was performed to evaluate the time needed for the development of 50% granulation tissue as a parameter of healing and factors of independent variables associated with the incidence of healing. Analysis on survival was performed using Kaplan-Meier survival curve, which was followed by Cox proportional hazard regression when we found there was more than one independent variable (covariate) showing significant correlation with the incidence of healing.

Table 2. Clinical characteristics of subjects

Variables	Values
Sex, n (%)	
- Female	22 (55.00)
- Male	18 (45.00)
Age (years)	56.0 (49.25 – 59.00)
History of CAD, n (%)	
- Yes	11 (28.00)
- No	29 (72.00)
History of stroke, n (%)	
- Yes	3 (8.00)
- No	37 (92.00)
Body mass index (kg/m^2)	22.35 (18.54 – 25.16)
Systolic blood pressure (mmHg)	120.00 (120.00 – 130.00)
Diastolic blood pressure (mmHg)	80.00 (70.00 – 80.00)
Baseline leukocyte count (/mm^3)	15,800.00 (11,050 – 19580)
Total cholesterol (mg/dL)	113.50 (92.25 – 141.25)
Triglycerides (mg/dL)	107.50 (89.75 – 149.25)
LDL cholesterol (mg/dL)	67.00 (50.25 – 83.75)
HDL cholesterol (mg/dL)	19.50 (14.00 – 29.00)
Fasting blood glucose (mg/dL)	216.50 (130.25 – 291.50)
HbA1c (%)	9.85 (8.40 – 11.38)
Period of time of having DM (years)	4.00 (1.25 – 9.75)
Family history of DM, n (%)	
- Yes	20 (50.00)
- No	20 (50.00)
History of using DM medication before having wound, n (%)	
- OAD	24 (60.00)
- Insulin	4 (10.00)
- OAD and insulin	5 (12.00)
- No medications	7 (18.00)
Treatment using captopril during wound care, n (%)	
- Yes	25 (63.00)
- No	15 (37.00)
Treatment using simvastatin during wound care, n (%)	
- Yes	12 (30.00)
- No	28 (70.00)
Ankle Brachial Index	1.02 (0.95 – 1.11)
Subjective symptoms about foot	
- Tingling	22 (55.00)
- Numbness	24 (60.00)
- Intermittent claudication	8 (20.00)

RESULTS

Clinical characteristics of the subjects in this study can be seen on **Table 2**. About 55% subjects were female with the median age of 56 years (49.25 – 59). The median of BMI was 22.35 which is in overweight range referring to Asian BMI. Majority of the subjects had no history of coronary artery disease (CAD) and stroke. Median of HbA1c levels was 9.85 (8.4 – 9.38). Although the median of lipid profile levels was in normal range, we found as many as 70% of subjects with statin medications.

Wound Characteristics

Wound characteristics of the subjects were varied and can be seen on **Table 3**. Most of the wound were as deep as muscle (35%) and bone (35%), with sign of local inflammation. Analysis using Kaplan Meier survival curve (**Figure 1**) revealed that there were as much as 81% subjects had not achieved 50% granulation tissue on the 14-day. The median of time achieving 50% granulation tissue in this study was 21 days (95% CI, 18.4 – 23.6). There were 3 out of 40 subjects dropped out before the earliest healing time (10 days). Longest treatment care until full recovery was 54 days.

Marker of Endothelial Dysfunction and Vascular Calcification

Marker of endothelial dysfunction (FMD, ADMA, and ET-1) and vascular calcification (OPG) in the inflammatory and proliferative phase were compared (**Table 4**). There was significant difference of OPG levels as the vascular calcification marker between inflammatory and proliferative phase. However, there were no significant differences of endothelial dysfunction markers between the two phases.

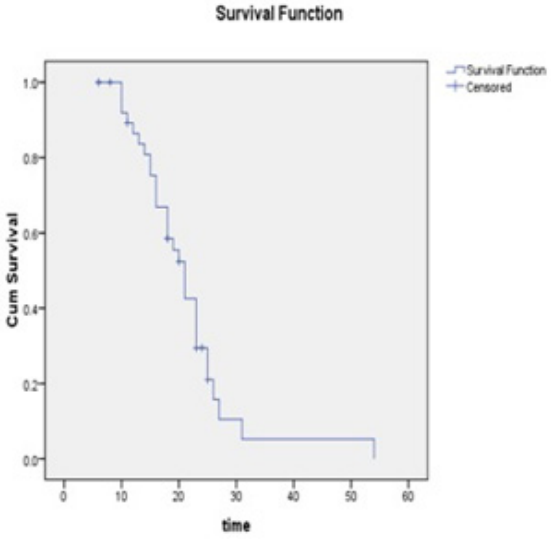

Figure 1. Survival curve of Kaplan Meier

Table 3. Wound characteristics

Variables	Values
Wound depth, n (%)	
- Subdermal	1 (2.00)
- Muscle	14 (35.00)
- Tendon	11 (28.00)
- Bone	14 (35.00)
Infection, n (%)	
- Yes	38 (95.00)
- No	2 (5.00)
History of amputation, n (%)	
- Yes	3 (8.00)
- No	37 (92.00)
History of previous wound, n (%)	
- Yes	17 (43.00)
- No	23 (57.00)
History of having wound (days)	14.00 (7.00 -21.00)
Median of wound area (cm^2)	47.30 (21.88 – 71.63)
Texas Wound Classification, n (%)	
- A0	0 (0.00)
- A1	12 (30.00)
- A2	14 (35.00)
- A3	14 (35.00)
- B1	0 (0.00)
- B2	38 (95.00)
- C1	12 (30.00)
- D	11 (28.00)

Bivariate and Multivariate Analysis

According to the bivariate analysis (**Table 5**), there were some factors which significantly correlated with wound healing ($p<0.25$), such as wound area, captopril and simvastatin medications, total cholesterol, fasting blood glucose, levels of ADMA, and OPG.

Multivariate analysis using Cox regression method was subsequently performed to the significant factors as well as clinical and laboratory characteristics which were considered important i.e. family history of DM, previous history of wound, and ET-1 (**Table 6**). There were

Table 4. Marker of endothelial dysfunction and vascular calcification in the inflammatory and proliferative phase

Variables	Inflammatory Phase	Proliferative Phase	p value
FMD (%)	2.97 (-2.63 – 5.58)	5.07 (0.80 – 8.62)	0.712
ADMA (μmol/L)	0.73 (0.61 – 1.07)	0.77 (0.64 – 1.07)	0.221
ET-1 (pg/mL)	4.38 (3.61 – 7.81)	4.40 (3.48 – 7.52)	0.757
OPG (pg/mL)	240.80 (185.12 – 314.61)	342.60 (246.60 – 387.10)	0.009

*Normal value: FMD ≥10%, ADMA 0.4-0.75 μmol/L, ET-1 0.3-0.9 pg/mL, OPG 37 pg/mL

Table 5. Bivariate analysis of factors related to wound healing

Variables	B	Hazard ratio	95% CI		p
			Lower	Upper	
Length of diabetes from diagnosis	-0.005	0.995	0.945	1.042	0.837
Family history of DM	0.191	1.210	0.576	2.540	0.614*
Previous history of wound	0.187	1.205	0.574	2.529	0.621*
Wound area	0.008	1.008	0.999	1.017	0.076
History of amputation	0.236	1.266	0.377	4.254	0.703
Infection	-0.441	0.663	0.153	2.863	0.582
History of CAD	0.186	1.204	0.534	2.717	0.655
Treatment using captopril during wound care	-0.708	0.492	0.217	1.116	0.090
Treatment using simvastatin during wound care	-0.508	0.502	0.265	1.366	0.225
Body mass index	0.035	1.036	0.940	1.142	0.476
Systolic blood pressure	0.010	1.010	0.983	1.037	0.487
Diastolic blood pressure	0.010	1.010	0.965	1.057	0.659
ABI	-1.052	0.349	0.018	6.810	0.488
FMD	-0.004	0.996	0.945	1.050	0.889
Total cholesterol	0.008	1.008	0.994	1.022	0.245
Total triglyceride	0.000	1.000	0.994	1.007	0.958
LDL cholesterol	0.008	1.008	0.992	1.025	0.302
HDL cholesterol	0.015	1.015	0.976	1.056	0.456
Fasting blood glucose	-0.002	0.998	0.996	1.001	0.143
HbA1c	-0.032	0.969	0.813	1.155	0.723
ADMA	1.338	3.813	1.116	13.023	0.033
ET-1	-0.002	0.998	0.868	1.146	0.975
Angiogenin	0.000	1.000	1.000	1.000	0.990
OPG	0.003	1.003	0.999	1.008	0.148

*variables were analyzed with Cox proportional hazard

three out of the nine factors that significantly correlated with wound healing, i.e. wound area, OPG levels, and simvastatin medications.

DISCUSSION

This study constitutes as the first observational cohort study aims to observe factors affecting the wound healing process which is commonly found in type 2 DM patients.

Median duration of DM among subjects was 4 years (1.25 – 9.75) with 60% subjects had already been using OAD for their medication before having wound, followed by 10% using insulin, and 12% using both OAD and insulin. Median of HbA1c levels was 9.85 (8.40–11.38)%, which showed that subjects had poor glycemic

Table 6. Multivariate analysis of various clinical and laboratory risk factors on wound healing

Variables	B	Hazard Ratio	Standard error	p value	95% CI
Family history of DM	0.908	2.480	0.615	0.139	0.744-8.273
Previous history of wound	-0.584	0.558	0.561	0.298	0.186-1.675
Wound area	0.014	1.014	0.006	0.027	1.002-1.027
Duration of existing wound (days)	-0.038	0.963	0.024	0.121	0.918-1.010
ADMA (µmol/L)	1.530	4.617	0.872	0.080	0.835-25.521
ET-1 (pg/mL)	0.119	1.126	0.095	0.214	0.934-1.358
OPG (pg/mL)	0.009	1.009	0.003	0.003	1.003-1.015
Using captopril	-0.685	0.504	0.478	0.152	0.197-1.288
Using simvastatin	-1.158	0.314	0.557	0.038	0.105-0.936

Overall chi square= 20.9, p=0.022; DM: diabetes mellitus; ADMA: asymmetric dimethylarginine; ET-1: endothelin-1; OPG: osteoprotegerin

control. Based on lipid profile, all subjects were categorized as hypocholesterolemia with low levels of low and high density lipoprotein (LDL and HDL). Some studies show that cholesterol levels (total cholesterol, LDL, and HDL are affected by inflammatory cytokines that may cause reduced synthesis or increased catabolism of cholesterol.[13,14]

The development of diabetic foot as a complication was quite too early considering the median duration of DM among subjects was 4 years. The median value of blood pressure was in pre-hypertension category which was consistent with most subjects receiving captopril. About 20% subjects were assumed to have PAD since they had signs of intermittent claudication regardless of the median value of ankle brachial index (ABI) falling within normal range. Normal ABI value indicating the undisrupted vascular integrity cannot exclude the presence of previous thromboemboli. The presence of vasodilation disorder in a relatively well macrovascular condition can explain the impaired healing process among subjects of our study.

About 60% of the subjects had numbness on their feet, 55% tingling, and 20% intermittent claudication, which showed neuropathy symptoms of the subjects.

Most of the wound were deeper than subdermal and coexisted with infection. Most of the subjects had no history of amputation. The median of wound duration was 14 days, which showed late treatment of the wound. Median of wound area was 47.30 cm^2.

There were 27 out of 40 subjects which followed the study until >50% granulation tissue (proliferation phase) achieved. Kaplan-Meier survival analysis showed the median of time achieving 50% granulation tissue was 21 days. Time achieving proliferation phase in normal people begins on day-4 and ends at day-21 after injury.[15] In this study, there was still 50% granulation tissue formation at day-21 which indicates that people with type 2 DM will have a longer duration of wound healing compared with normal people.

Marker of Endothelial Dysfunction in Inflammation and Proliferation Phase

In this study, marker of endothelial dysfunction was determined by FMD, ADMA, and ET-1 levels.

In proliferation phase, the metabolic disorder and cardiovascular risk factor are assumed to be improved thus produce better vasodilation. In this study, FMD median value was higher in proliferation phase than in inflammation phase, eventhough the increase was not statistically significant (p=0.712). However, the median FMD in proliferation phase was still below normal (<10%), showing despite acute metabolic factor improvement, endothelial dysfunction had not improved yet because of chronic inflammation affecting vascular elasticity. Type 2 DM subjects with inflammation phase of diabetic ulcer had FMD median of 2.97% (-2.63 – 5.58) which was very different with the study by Pribadi.[16] finding

7.46% as FMD mean in non-fasting healthy men or by Elliana.[17] finding 11.09% as FMD mean in prediabetes women. FMD measurement on those diabetic patients could not only show the change in local vascular function, but also systemic vascular function. In this study, FMD was measured on brachialis artery and associated with peripheral vascular disturbance.

Vasodilation function is important because vasodilation and vasoconstriction are dynamic mechanism of which are needed for optimal wound healing. Some studies have reported failure of vasodilation and vasoconstriction on diabetic patients can be detected through FMD measurement.[18,19]

Vasodilation and vasoconstriction balance on systemic vasculature are shown by two main components, i.e. NO and ET-1. However, NO is difficult to be measured due to its very short half life. Therefore, ADMA as another metabolite in vasodilation can be used after NO.

In our study, ADMA levels in proliferation phase was higher than inflammation phase. Increased ADMA was thought to be required for inhibition of NO as a physiological factor that is used against further inflammatory process in order to protect the endotelium.

Increased ADMA is equal to decreased NO as reported by Mahfouz et al.[20] This study found the median of ADMA levels is 0.73 (0.61 – 1.07) μmol/L in inflammation phase, which is higher compared with the mean of ADMA levels by Elliana.[17] in prediabetic women 0.65 (0.13) μmol/L or by Zoccali et al.[21] in acute infection 0.62 (0.23) μmol/L. An increase in ADMA levels is often found in diabetic patients,[20] and can precede vascular abnormalities in abscence of hypertension and dyslipidemia.[22] Hyperglycemia can increase ADMA levels through some mechanisms, i.e. polyol pathway activation that can decrease biosynthesis of NO, damage of DDAH as ADMA degradation enzyme, and increase arginine methyltransferase expression as ADMA synthesizer.[20,23,24]

In our study, we found a higher degree of wound healing, which is consistent with increased ADMA levels. Our findings were different from Gurdol et al.[25], i.e. subjects with worse wound healing actually had higher ADMA levels. The difference may be caused by limited number of sample in our study and therefore, further study is necessary to confirm the role of ADMA to determine the prognosis of wound healing of DM ulcer. Furthermore, no study has been reported about the direct effect of ADMA on wound healing.

Other factor which constitutes in FMD decrement is ET-1. The high level of ET-1 at initial phase can induce an increased NO production, which contributes in vasodilation process; the mechanism is also considered that it may affect the degree of wound healing. ET-1 is generally known as a vasoconstrictor substance. But then, some studies demonstrate that it has a role in wound healing and tissue repair. ET-1 has mitogenic activity on some types of cells, such as smooth muscle cells and fibroblasts. It affects the composition of extracellular matrix by inducing the synthesis of collagen I and III through ETA and ETB receptors. ET-1 has also been reported to have an effect of increasing contractility of normal fibroblasts, which is required for wound closure and dermal reconstitution.[26] Increase in ET-1 level has made the vasodilation and vasoconstriction balance prone to vasoconstriction. ET-1 levels in inflammation and proliferation phase was above normal. This study used subjects who at most had infection and impaired perfusion stimulation in inflammation phase. Once the inflammatory phase completed, it was assumed that both infection and impaired perfusion improved in proliferation phase. This study found that the average ratio of ET-1 in both phases ran in accordance with the processes described above. The value was higher than normal subjects in Lilyasari et al.[27] which showed ET-1 levels 0.88 (0.77 – 1.07) pg/mL. However, increased FMD value, ADMA, dan ET-1 levels in proliferation phase did not statistically significant. Correction of acute inflammatory conditions would not correct all metabolic and inflammatory factors because of ongoing chronic inflammatory process in DM.

Comparison of vasodilation function in this study was to show the importance of correction of acute inflammation as the basic distinction between inflammation and proliferation phase,

in association with vascular change. However, chronic inflammation in diabetic patients as a risk factor prior to the development of wound needs to be considered.

Marker of Vascular Calcification in Inflammation and Proliferation Phase

OPG levels increased seven-fold above normal value in inflammation phase. Besides basically diabetic patient has already increased levels of OPG, inflammatory process in subjects who participated in this study was believed to be the cause of increased OPG. High OPG levels in DM is associated with the presence of inflammation related to various inflammatory cytokines, such as IL-6 and TNF-α.[28,29] Chronic inflammation in subjects was not only observed in the vasodilation function, but also the character of atherosclerosis and vascular calcification, assessed by OPG levels. Several studies indicate that in DM population, OPG levels were lower by insulin and simvastatin therapy of which the mechanism was thought through anti-inflammatory effects. In this study, OPG levels remained significantly increased in proliferation phase, suggesting the process of angiogenesis.

Factors Related to Wound Healing

Earlier studies proposed their own prediction model of time for wound healing, but only put concerns of the wound closure at the beginning. Sheehan et al.[5] found that percent change in the area of ulcer after 4 weeks of observation can predict healing at 12 weeks, which can be used for early identification of standard care response and the need for additional treatment. Besides, Lavery et al.[30] found that percentage of wound area reduction at 1 week of observation can predict healing at 16 weeks. On the other hand, Zimny et al.[4] confirmed an equation that can reliably predict healing time in neuropathic foot ulcers.

In addition to wound area, this study tried to look up other factors affecting wound healing process, such as family history of DM, previous history of wound, duration of existing wound, medication history (including angiotensin-converting enzyme inhibitors and statins), serum levels of ADMA, ET-1, and OPG.

Based on multivariate analysis, there were 3 out of 9 factors that had significant role in wound healing. Greater wound area, higher levels of OPG, and absence of simvastatin medications increased wound healing. On the contrary, subjects with previous history of wound, longer duration of wound, as well as history of captopril and simvastatin medications showed decreased healing process.

Subjects with history of DM in the family, larger baseline wound area, high baseline ADMA, ET-1, and OPG levels will have impact to the high degree of healing. The effect of history of DM in the family on higher degree of healing is assumed caused by earlier and more intensive care when the wound develops in subjects who had family history of DM compared to those without.

It was assumed that subjects with previous history of wound had worse neuropathy and vascular disorder as well as metabolic control compared to subjects with no previous history of wound. Furthermore, previous history of wound increased the risk of recurrent ulcer and amputation.[32] Previous wound history also serves as a predictor for recurrent ulcer and higher the amputation risk of these subjects. Glycemic control was not significantly related to the healing process due to its long term effect in the otherwise acute process of ulcer in this study.

Subjects with longer duration of wound prior to treatment were estimated to have more severe infection and metabolic disorder since they relatively had a delay in receiving proper wound care. This finding is consistent with the study by Ince et al.[33] which showed lower degree of healing in subjects having long-standing wound. The longer the wound exists, the larger the area it occupies, and the higher the risk of developing secondary infection as well.

Regarding the correlation between high levels of cytokines such as ADMA, ET-1, and OPG in inflammatory phase and the degree of healing, it has been shown that the higher the level of those three cytokines, the higher the degree of wound healing.

OPG has been so far considered as a marker of vascular and bone calcification and it may be involved in the development of various

microvascular complications of DM. There has been no study explaining the correlation between wound healing of DM patients and OPG levels. Malyankar et al.[31] found that OPG protected endothelial cells from apoptosis and it had a role in angiogenesis in rats. Hence, it may explain the role of OPG in wound healing.

This study demonstrates that the medication history of captopril and simvastatin has a negative correlation with wound healing. Simvastatin is an LDL-lowering agent, whereas LDL stimulate fibroblast in human skin through activation of p38 mitogen-activated protein kinases (MAPKs), followed by interleukin-8 (IL-8) production that will finally increase the wound healing capacity of fibroblasts.[34] The presence of LDL has actually provided assistance in wound healing, and therefore lowering its level may impair wound healing.

Various results about the impact of captopril medication on wound healing have been reported. A study by Torgal et al.[35] in experimental animal showed that captopril and enalapril significantly increased the development of granulation tissue and hydroxyproline. On the other hand, Ilhan et al.[36] reported contrary results, i.e. high-dose lisinopril reduced hydroxyproline level, collagen deposition, and intestinal epithelization in experimental animals. However, our study was not aimed to show the direct effect of captopril or simvastatin on wound healing. Therefore, the results of our study regarding both issues should be interpreted wisely. Subjects who had captopril or simvastatin in our study were those with history of hypertension or dyslipidemia, in which both treatments were given to maintain controlled blood pressure and lipid profile. Hypertension and dyslipidemia are comorbidity besides DM that may affect wound healing through the process of endothelial dysfunction. Moreover, the presence of comorbidities other than DM also increases the risk of macroangiopathy that may also affect wound healing.[37-39]

The limitation of this study is the total number of subjects is less than the minimum sample size, thus causing low power of the study (P=50%) and higher type 2 error as well as lower possibility to find association between the studied variables. Besides, many subjects could not complete the whole study. Due to limited funds, marker of endothelial dysfunction and vascular calcification were only done in subjects which completed the study. Other limitation is there was no comparisons between type 2 DM and normal population.

CONCLUSION

In acute diabetic foot ulcers, wound area and OPG levels had positive correlation with wound healing, whereas simvastatin medications had negative correlation with wound healing.

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