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The association between hypoxia-inducible factor 1α tissue concentration and the risk of amputation in diabetic foot ulcer patients

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ABSTRACT

Background: Diabetic foot ulcer (DFU) is one of the complications of diabetes mellitus, with a high rate of disability and mortality. Amputation is the last option for DFU management with a poor healing prognosis; moreover, angiogenesis plays an important role in wound healing. HIF-1α is a key player in neovascularization and tissue formation. The present study aimed to investigate the role of HIF-1α in predicting the risk of amputation in DFU patients.

Methods: This study was a cross-sectional study conducted from June 2019-December 2020. Subjects were DFU patients indicated for amputation or debridement. The tissue sample was collected from the proximal side of the wound. The concentration of HIF-1α was calculated using the ELISA sandwich method. The multiple logistic regression was used to determine the independent risk of each patient's characteristic and HIF-1α in amputation.

Results: A total of 67 DFU patients were enrolled: 31 amputations and 36 debridements. HIF-1α concentration in the amputation was lower than in the debridement group (median: 5.77 versus 26.56, $p<0.001$). The cut-off of HIF-1α concentration is 8.807 pg/mg protein. Patients with HIF-1α concentration <8.807 pg/mg had a higher risk of amputation than those with HIF-1α concentration ≥8.807 pg/mg (OR 11.12, 95% CI 1.44–85.75).

Conclusion: HIF-1α has a significant role in predicting amputation in DFU patients.

Keywords: Amputation, DFU, Diabetic wound, HIF-1α.

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INTRODUCTION

Diabetic foot ulcer (DFU) is a microvascular complication of diabetes mellitus with a high rate of disability, morbidity, and mortality. The prevalence of DFU in Indonesia is approximately 7-24% of diabetic patients.[1] Approximately 24-37% of DFU patients undergo minor and major amputations at Cipto Mangunkusumo Hospital, Indonesia, during 2015-2017.

Amputation is the last option of DFU management. It is considered among DFU patients with a poor prognosis of healing in order to prevent further complications. However, the amputation procedure may result in disability and physiological, functional, and material burdens for the patient and their family, especially if the patient is at productive age and the family's main income source.[2]

The decision of amputation is based on the severity of infection, macrovascular, soft tissue, and bone conditions. In sepsis conditions, amputation is performed to control the source of infection.[2] Wound healing is another problem encountered by DFU patients. Infection and tissue oxygenation play an important role in the wound healing process. Chronic hypoxia will interfere with energy production and the signaling of neo-tissue formation in wound healing.[3]

Hypoxia-inducible factor 1α (HIF-1α) is a key transcription factor that responds to hypoxic stress and activates the expression of downstream genes related to neovascularization and tissue formation.[4] However, the association between HIF-1α and amputation has never been conducted.. We hypothesized that decreasing the concentration of HIF-1α expression would predict amputation in DFU patients because of its role in angiogenesis and wound healing processes. This study aimed to investigate the role of HIF-1α in predicting amputation in DFU patients by examining the expression of hypoxia-inducible factor 1α (HIF-1α) in DFU patients and its association with amputation.

METHODS

This cross-sectional study was conducted from June 2019 to December 2020. The Institutional Ethics Department of Medical Faculty, Universitas Indonesia, approved this research by protocol number 21010083; the date of approval was February 10, 2020. Each participating patient was provided written informed consent.

The DFU patients from the Emergency Unit and Surgical Polyclinic of Cipto Mangunkusumo Hospital. The inclusion criteria were DFU patients indicated to amputation or conservative therapy (debridement), not in sepsis condition, and willing to participate in this study. The amputation patients are those indicated to have an amputation or required amputation after debridement. The debridement patients are those indicated for debridement only. The exclusion criteria were DFU patients with a history of malignancy in the area around DFU, severe peripheral arterial disease, and other conditions which may affect the wound healing process, e.g., vasculitis, autoimmune disease, clinical sepsis shock. The amputation was determined by viability assessment using ABI, WIfI score, and duplex ultrasonography.

The tissue sample was collected from the operating room and emergency unit during the amputation or debridement procedures. A 2 cm x 1 cm x 1 cm tissue was collected during the biopsy from the proximal side of the wound. The HIF-1α protein was extracted from the tissue samples in the biomolecular laboratory. ELISA sandwich method was used to calculate the HIF-1α concentration quantitatively using the Human HIF-1 alpha ELISA Kit®.

Data analysis was performed on the Statistical Package for the Social Sciences (SPSS) 25.0. HIF-1α, a numeric variable, underwent the Shapiro-Wilk normality test to determine the distribution of the data. If the data had normal distribution, it presented in mean (standard deviation), while if not, it presented in median (range).

In bivariate analysis, categorical variables were examined using the chi-square test. As appropriate, numeric variables were examined using an independent t-test or Mann-Whitney U test. A test of normality of numeric variables was conducted using Shapiro-Wilk. HIF-1α was a numeric variable and dichotomized into a categorical variable using Receiver Operating Characteristic (ROC) curve, and the Youden index determined its cut-off point in Microsoft Excel Office.

The multivariate analysis was conducted using logistic regression with the Backward: LR method. Variables with $p \leq 0.25$ in bivariate analysis were included in the multivariate analysis. Odds ratio (OR) and 95% confidence interval were used to measure the association between the decision of amputation and predicting variables. All analyses were performed using SPSS version 25.0 with a p-value <0.05 considered statistically significant.

RESULTS

A total of 67 patients were enrolled: 31 underwent amputation and 36 debridements (Figure 1). One patient in the amputation group was excluded due to acute limb ischemia, and two in the debridement group due to acute limb ischemia and the tissue sample was not eligible. Overall, the proportion of female and male patients was comparable; most patients (75%) were <65 years, the nutritional status of the patients was only obese and normal based on Asia-Pacific body mass index classification[5], and the comorbidity was smoking, hypertension, and chronic kidney disease (CKD).

Table 1 compares patients' characteristics and HIF-1α concentration between amputation and debridement groups. All characteristics, except gender, were comparable between amputation and debridement groups. The proportion of female patients was higher in the amputation than in the debridement group.

HIF-1α tissue concentration data were not normally distributed; median (range) and Mann-Whitney U tests were used (Table 2). HIF-1α concentration in the amputation group was lower than in the debridement group (5.77 versus 26.56, p=<0,001). The ROC curve and Youden index analysis of HIF-1α concentration showed a cut-off value of 8.807 pg/mg protein (sensitivity: 82.4%, specificity: 66.7%) (Figure 2). The multiple logistic regression showed that patients with HIF-1α concentration <8.807 pg/mg had a higher risk of receiving amputation than those with HIF-1α concentration ≥8.807 pg/mg ([a]OR 11.12, 95% CI 1.44–85.75) (Table 3).

DISCUSSION

This study demonstrates the patient characteristic and HIF-1α tissue concentration among amputation and debridement patients. Other than gender features, no other patient characteristics are significantly associated with amputation and debridement. The proportion of the female gender is significantly greater in amputation than in the debridement group. This finding is similar to a case-control study by Kogani et al., which observed the

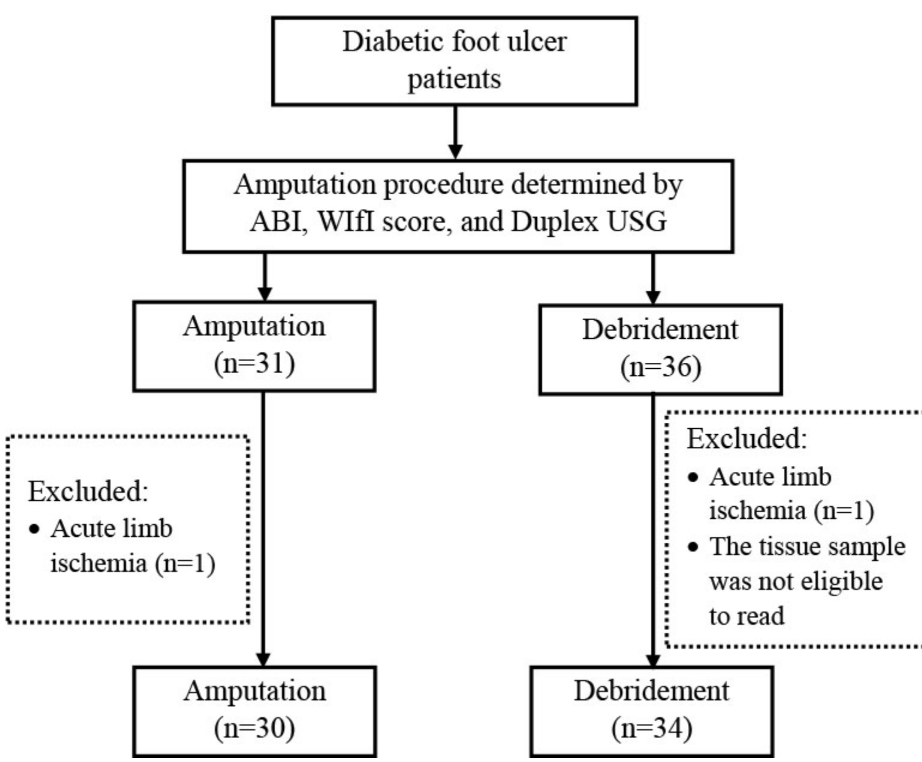

Figure 1. Subjects enrollment chart.

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factors that influence amputation, with 68% of the subjects in the amputation group were female.[6] However, multivariate analysis showed that gender did not have a significant risk of amputation.

HIF-1α is a transcription factor for response to hypoxia conditions; it also regulates genes involved in wound healing processes such as glycolysis, angiogenesis, apoptosis inhibition, cellular matrix, and cell proliferation.[7,8] The extreme loss of HIF-1a decreases energy metabolism, tissue proliferation, and vascularization; meanwhile, the overexpression of HIF-1α increases transcription activity and promotes tumor growth.[9]

This study demonstrated the role of HIF-1α in amputation. Its median tissue concentration in the amputation group significantly differs from the debridement group. We find the cut-off concentration was 8.807 pg/mL with a sensitivity of 82.4% and specificity of 66.7%. It shows that the concentration bellows the cut-off has a higher risk of amputation (^{a}OR 11.116 [95%CI 1.44–85.75]). These findings align with the theory that tissue oxygenation is achieved in an adequate concentration of HIF-1α.[9] It is also in line with the in vivo study, which found that inhibition of HIF-1α signaling contributed to wound healing impairment in diabetes.[10,11] When tissue has good perfusion, the tissue viability will be better, reducing the probability of amputation. The HIF-1α concentration, therefore, was lower in the group with chronic perfusion failure, leading to the need for amputation.

In this study, we succeeded in documenting the role of HIF-1α in maintaining tissue perfusion, as the concentration of HIF-1α was found to be higher in viable tissue than in tissue that needs amputation. This research is the first study that finds the role of HIF-1α tissue concentration in DFU patients and their amputation risk. However, this study has several limitations, such as the limited samples, the HIF-1α concentration having abnormal distribution, and a large odds ratio confidence interval.

Table 1. Characteristic of the subject in amputation and debridement group.

General characteristic		Amputation (n=30)	Debridement (n=34)	p-value
		n (%)	n (%)	
Gender	Male	10 (33.3)	20 (58.8)	0.041*
	Female	20 (66.7)	14 (41.2)	
Age (years)	≥65	6 (20.0)	10 (29.4)	0.386
	<65	24 (80.0)	24 (70.6)	
Nutritional status	Obese	18 (60.0)	13 (38.2)	0.082
	Normal	12 (40.0)	21 (61.8)	
Hypertension	Yes	10 (33.3)	8 (23.5)	0.384
	No	20 (66.7)	26 (76.5)	
Chronic kidney disease	Yes	6 (20.0)	2 (5.9)	0.133
	No	24 (80.0)	32 (94.1)	
Smoking	Yes	15 (50.0)	16 (47.1)	0.841
	No	15 (50.0)	18 (52.9)	

*p<0.05

Table 2. HIF-1a tissue concentration in amputation and debridement group.

	Amputation (n=30)	Debridement (n=34)	p
HIF-1a (pg/mL)	5.77 (0.55–53.47)	26.56 (2.23–211.12)	<0.001

Table 3. The odds ratio subject characteristic and HIF-1a concentration.

Variables		Amputation	Debridement	Bivariate analysis		Multivariate analysis	
		n (%)	n (%)	p†	OR (CI 95%)	p	^{a}OR (CI 95%)
Gender	Male	10 (33.3)	20 (58.8)	0.041	0.350 (0.12–0.97)	0.184	0.283 (0.04–1.81)
	Female	20 (66.7)	14 (41.2)				
Nutritional status	Obese	18 (60.0)	13 (38.2)	0.082	2.423 (0.88–6.62)	0.482	1.878 (0.32–10.85)
	Normal	12 (40.0)	21 (61.8)				
Chronic kidney disease	Yes	6 (20.0)	2 (5.9)	0.133	4.000 (0.74–21.58)	0.361	3.360 (0.25–45.19)
	No	24 (80.0)	32 (94.1)				
HIF-1a	< 8.807 pg/mL	15 (50.0)	17 (50.0)	<0.001	9.333 (2.91–29.87)	0.021	11.116 (1.44–85.75)*
	≥ 8.807 pg/mL	15 (50.0)	17 (50.0)				

*p<0.05. ^{a}OR = *adjusted odds ratio*; † chi-square test

CONCLUSION

In conclusion. HIF-1α has a significant role in predicting amputation in DFU patients. The risk of amputation is 11 times higher in DFU patients with HIF-1α tissue concentration lower than 8.807 pg/mL. Meanwhile, patient characteristics such as age, gender, nutritional status, CKD, hypertension, and smoking have no significant role in determining the amputation risk.

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None.

ETHICAL STATEMENT

This study has been approved by the Institutional Review Board of Dr. Cipto

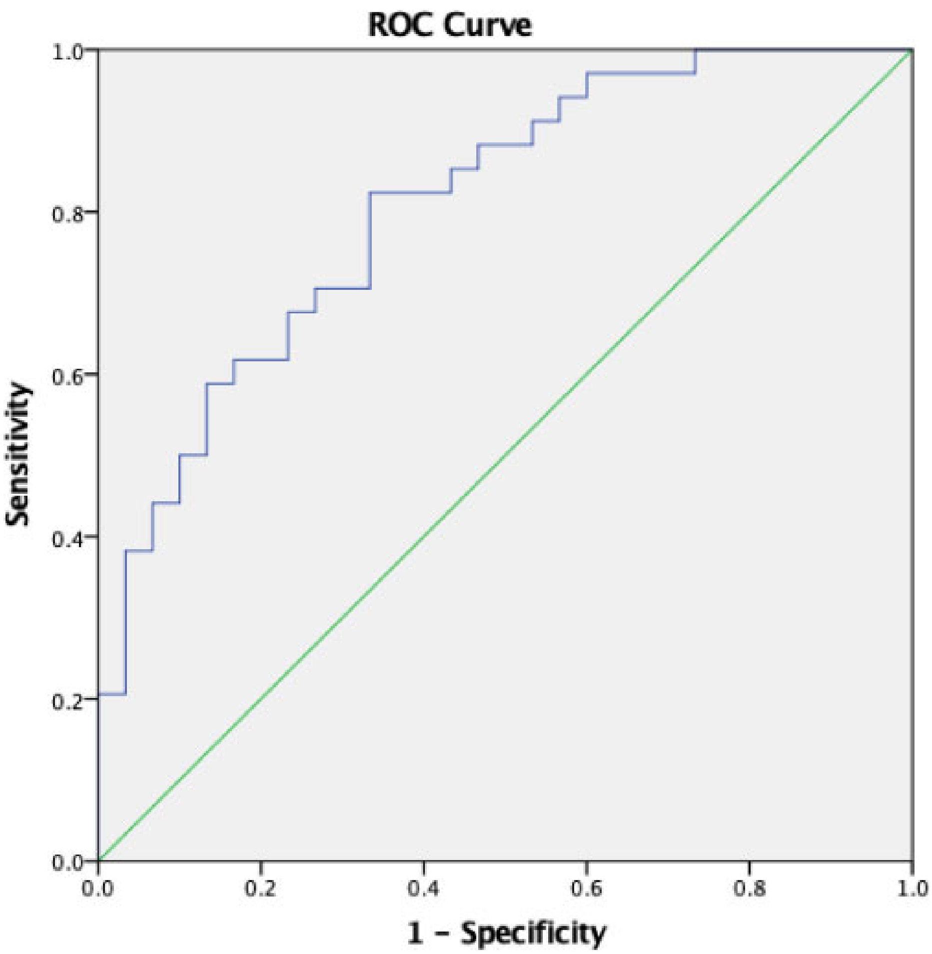

Figure 2. HIF-1α ROC curve.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

PD contributed to all processes of this research. EY contributed to suggesting concepts and intellectual definitions of the research and manuscript editing and review. AK contributed to giving the intellectual definition, conducting the data and statistical analysis, and also contributed to manuscript editing and review. AM, SWJ, and HSY contributed to giving the intellectual definition and manuscript editing and review. SHP contributed to suggesting the design, literature searching, conducting the research, and data analysis.

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