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Association between monocyte-high density lipoprotein ratio (MHR) and severity level of lower extremity artery disease (LEAD)

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ABSTRACT

The incidence of lower extremity artery disease (LEAD) has increased worldwide in the last decade. Its severity has been associated with increased morbidity and mortality. Atherosclerosis is believed as the main cause of LEAD. Monocytes and low-density lipoprotein (LDL) are the hallmarks of atherosclerosis. High-density lipoprotein (HDL) plays a role in suppressing the activation of monocytes. The monocyte to HDL ratio (MHR) has been reported as a marker of coronary artery disease complexity. However, this marker has not been investigated to assessthe LEAD severity. The study aimed to investigate the association between MHR and LEAD severity. This was an analytic observational study using a cross-sectional design. Patients were selected from the Vascular Disease Registry in Dr.Sardjito General Hospital, Yogyakarta from January 2016 – January 2019. The blood sample was drawn at one day prior, on the day, or one day after duplex ultrasound performed. The duplex ultrasound was then interpreted based on the duplex ultrasound score. Patients were classified into two groups according to the score i.e. severe (score ≥ 8) and nonsevere (score< 8). Where as, the MHR was classified into two groups according to the cut-off point i.e. high (≥ 14.51) and low (< 14.51). The Chi-square test was used for statistical analysis and pvalue <0.05 was considered as statistically significant. A total of 50 patients were involved in this study. There were 21 (42%) patients in the severe group and 29 (58%) in the nonsevere group. The proportion of the high MHR group and the low MHR group with severe levels of LEAD were 12 (57.1%) and 9 (42.9%), respectively. However, it was not statistically significant [p = 0.145; CI95% PR 1.57 (0.81 – 3.03)]. In conclusion, there is no association between MHR and LEAD severity.

ABSTRAK

Insidensi penyakit arteri ekstremitas bawah (*lower extremity artery disease* / LEAD) meningkat di seluruh dunia pada beberapa decade terakhir. Tingkat keparahannya dikaitkan dengan meningkatnya morbiditas dan mortalitas. Aterosklerosis diduga penyebab utama LEAD. Monosit dan lipoprotein densitas rendah (LDL) adalah penanda adanya aterosklerosis. Sedangkan lipoprotein densitas tinggi (HDL) berperan penting menekan aktivasi monosit. Rasio monosit terhadap HDL (MHR) telah dilaporkan sebagai penanda kompleksitas penyakit arteri koroner. Namun demikian, apakah penanda ini dapat menggambarkan keparahan LEAD belum pernah dikaji. Penelitian ini bertujuan untuk mengkaji hubungan antara MHR dan keparahan LEAD. Penelitian ini merupakan penelitian observasi dengan rancangan potong lintang. Subjek penelitian diseleksi dari Register Penyakit Vaskular di RSUP Dr. Sardjito, Yogyakarta dari Januari 2016 - Januari 2019. Sampel darah diambil satu hari sebelum, pada saat dan sesudah *duplex ultra sound* dilakukan. Duplex ultra sound selanjutnya diinterpretasi berdasarkan skor *duplex ultra sound.* Subjek dikelompokan menjadi dua kelompok menurut skorya itu parah (nilai ≥ 8) dan ringan (nilai< 8). Sedangkan MHR dikelompokkan menjadi dua kelompok menurut nilai ambang batas yaitu tinggi (≥ 14,51) dan rendah (< 14,51). Uji Chi-square digunakan untuk analisis statistiknya dan nilai p< 0,05 dianggap berbeda secara nyata. Sebanyak 50 pasien dilibatkan dalam penelitian ini. Terdapat 21 (42%) pasien dalam kelompok parah dan 29 (58%) ringan. Proporsi kelompok MHR tinggi dan rendah dengan tingkat keparahan LEAD berturut-turut adalah 12 (57,1%) dan 9 (42,9%). Namun demikian, hal ini tidak menunjukkan perbedaan nyata secara statistic [p = 0,145; CI95% PR 1,57 (0,81 – 3,03)]. Dapat disimpulkan, tidak ada hubungan antara MHR dan keparahan LEAD.

Keywords:
monocyte;
high density lipoprotein;
lower extremity artery disease;
duplex ultrasound score;
marker;

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INTRODUCTION

Atherosclerosis is a chronic arterial disease characterized by inflammation and oxidation stress that results in the accumulation of lipids in the intima layer, lipid oxidation, foam cell formation, and plaque formation. Lower extremity artery disease (LEAD) is one of the main manifestations of atherosclerosis. The total number of individuals suffering from LEAD is increasing rapidly, with an increase of 23% in the last decade. Both symptomatic and asymptomatic, patients with LEAD have a high risk of experiencing cardio cerebrovascular events until death.[1-7]

Most LEAD could be detected with an ankle brachial index (ABI) ≤ 0.9 or a weakened pulse. Duplex ultrasound (DUS) is the first-line recommended imaging method to confirm LEAD. It is a noninvasive diagnostic modality that is easily accessible with relatively low costs. DUS was said to have sensitivity and specificity to detect lesions ≥ 50% (which were considered hemodynamically significant) at 88 and 96 % when it was compared to digital subtraction angiography, computed tomography angiography (CTA) and magnetic resonance imaging (MRA).[1,3,8] Hiremath *et al.*[9] created a DUS scoring system to assess the severity of LEAD. This DUS scoring system was used in this study to assess the severity of LEAD lesions.

The monocyte to high density lipoprotein ratio (MHR) has been extensively investigated to assess the severity of coronary artery disease.[10,11] However, until now, there has been no study of MHR in LEAD. MHR is based on the principle of the proinflammatory profile of monocytes in the chronic process of atherosclerosis and the antiinflammatory properties of high-density lipoprotein (HDL). These laboratory parameters are widely accessible. The aim of this study was to determine the prevalence ratio between high MHR and the level of severity lesions of LEAD assessed based on DUS scores.

MATERIALS AND METHODS

Design and subjects

This was an observational analytic study using a cross-sectional design which carried out by taking of secondary data of DUS and laboratory examinataions nonrandomly from the Vascular Disease Registry in Dr. Sardjito General Hospital, Yogyakarta from January 2016 – January 2019. Tracking of the clinical data was performed through medical records. If the DUS and laboratory examination data were not taken according to a predetermined time, then DUS examination and the laboratory were repeated. During this period, there were 238 subjects. A total of 120 subjects were excluded. A total of 118 subjects who fulfilled the inclusion and exclusion criteria were subjected to a sampling process. The DUS video of eighteen subjects was not found. The DUS video of 18 subjects was not readable, and 32 subjects were not subjected to laboratory tests when DUS was conducted. The estimated sample size was calculated using a comparison of two proportions formula and resulted 19 subjects of the minimum sample size for each group. FIGURE 1 shows the number of subjects as many as 50 subjects who met the inclusion and exclusion criteria. This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine-Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta.

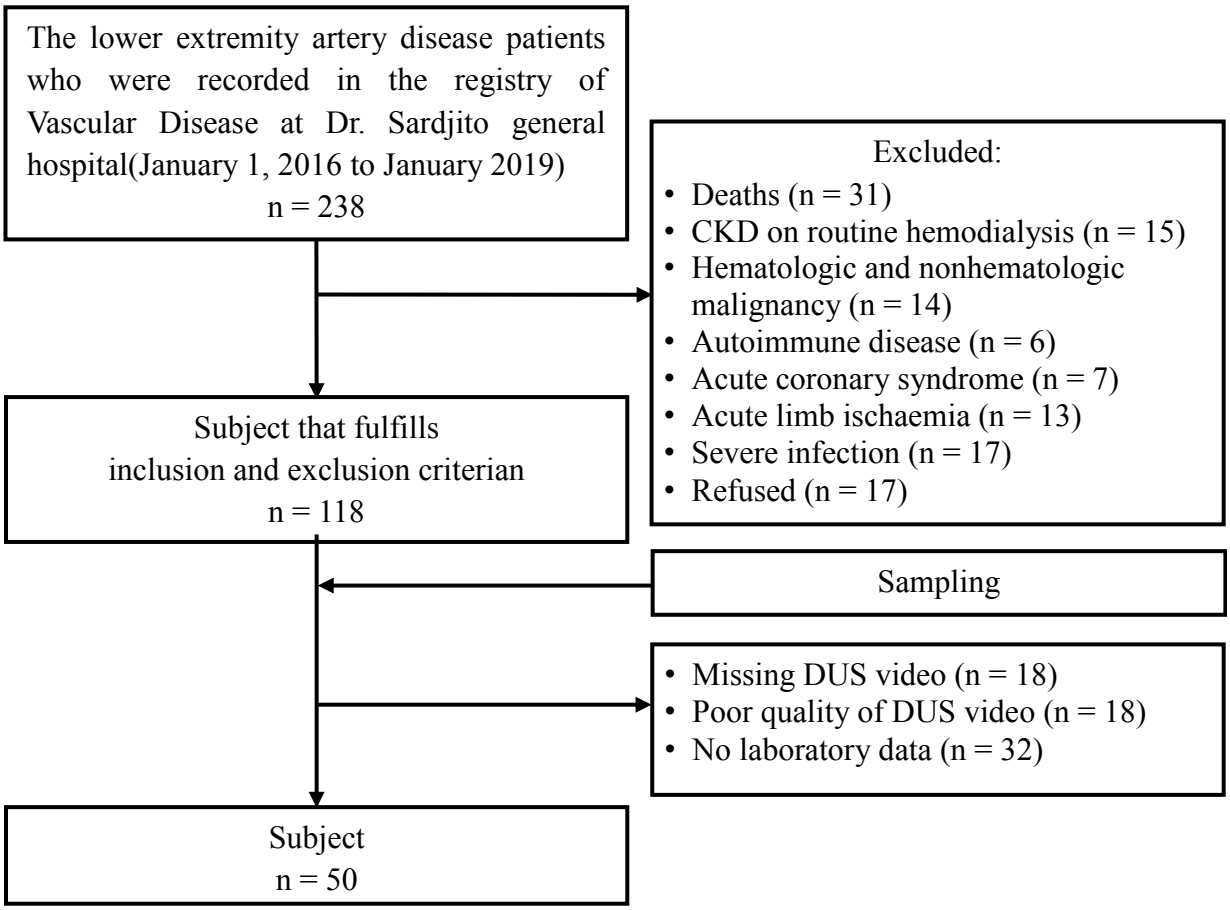

FIGURE 1.Recruitment flowchart of study subjects

Procedure

The independent variable was MHR, whereas the dependent variable was the severity of the lesion based on the DUS score. Confounding variables included age, gender, body mass index (BMI), ankle brachial index (ABI), diabetes mellitus, hypertension, smoking, drugs (statin groups, angiotensin converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB), calcium channel blockers (CCB)), monocyte count, and HDL.

MHR was divided into two groups based on the cutoff point of the receiver operating characteristics (ROC) curve. The first group was the high MHR ratio subjects with the ratio value of ≥ 14.51 and the second group was low MHR ratio subjects with the ratio value of <14.51. Intraobserver reliability was also tested with Cohen's Kappa by a trained sonographer who was blind to the MHR value.

Duplex ultrasound examination was performed using a machine (Philips 1, Philips 2, GE Vivid 7) with a linear transducer 7 - 12 MHz.The examination started from the common femoral artery using B-mode, color Doppler, and pulse wave Doppler. The severity of the lesion was interpreted based on the DUS score. The component of the DUS score system is shown in TABLE 1, which consists of thickening of the intima (point 1). If there was a plaque, it was assessed whether the plaque causes stenosis. The amount of stenosis was measured by pulse or continuous wave Doppler examination in the intra- and proximal stenosis to obtain the PSV ratio value. If there was plaque, one of the criteria for plaque was chosen to determine whether there was no stenosis (point 1), stenosis less than 50% (point 2) or stenosis more than 50%

(point 3). If there was thrombosis, the length of the thrombus was measured. If the thrombus size was less than half of the artery (point 1). If the thrombus was more than half of the artery (point 2). If stenosis or thrombosis was obtained, an assessment of collateral and distal flow must be assessed. If collateral and distal flow were absent, then each has 2points. If collateral and distal flow were present, then each had1 point. The total maximum score was 10.[9,12,13]

The severity level was classified into two groups: severe and nonsevere, as shown in TABLE 2.

TABLE 1. DUS scoring system of lower exremity artery diasease[14]

Severity level	Positive DUS finding	Present	Absent
1	Intimo-medial thickening	1	0
2	Plaques not causing stenosis	1	0
3	Plaques causing <50% stenosis	2	0
4	Plaques causing >50% stenosis	3	0
5	Thrombosis of short segment	1	0
6	Thrombosis of long segment	2	0
In the presence of stenosis or thrombosis			
7	Collaterals	1	2
8	Distal flow	1	2

Note:
Intimo-medial thickening: 1-2 mm; plaque: > 2 mm; stenosis: turbulensi in colour; stenosis < 50%: peak systolic velocity ratio < 2; Stenosis > 50%: peak systolic velocity ratio ≥ 2; short segment: < half of the artery. long segment: > half of the artery. The total maximum score was 10; DUS: duplex ultrasound.9, 12, 13

TABLE 2. Grading of the severity level of lower extremity artery disease based on DUS.

DUS Score	Severity level
1 – 7	Nonsevere
8 -10	Severe

Note. DUS: duplex ultrasound

A blood sample examination was drawn on one day prior, on the day, or one day after the DUS examination. These times of blood examination were based on the 20-40 hour availability of monocytes in circulation.[15] MHR was calculated by dividing the monocyte count by the HDL count.

Statistical analysis

Hypothesis tests of the prevalence of MHR and lesion severity were carried out between 2 categorical variables using Chi square or Fisher's exact test. Bivariate analysis was performed to determine confounding factors which influenced the severity of lesions. Stratification analysis was performed to control for confounding variables. A p value <0.05 was considered statistically significant. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

The characteristics of the subjects are presented in TABLE 3. The age of the subjects ranged from 49 to 88 years with a mean of 66.92 ± 10.51 years, and the majority of subjects aged ≥ 50 years were 46 subjects (92%). Subjects were male and female, respectively, 25 (50%). The BMI range of subjects was 18.37 to 32.42 kg/m^2 with a median of 23.26 kg/m^2. The obesity category, namely, BMI ≥ 25 kg/m^{2,} includedas many as 14 subjects (28%). The median ABI value was 0.87 (0.5 - 0.9), where as median score of DUS was 7 (5 - 8).

Risk factors in the subjects were diabetes mellitus in 40 (80%), hypertension in 42 (84%), and smoking in 13 (26%). The number of subjects who consumed routine treatment in the form of statins was 43 (86%), ACEi/ARB was 44 (88%), and CCB was 23 (46%). The number of subjects with criteria for lesion severity based on DUS examination were grouped into two groups, namely, severe and nonsevere, 21 (42%) and 29 (58%), respectively.

TABLE 3. The characteristics of the study population

Variable	n = 50
Age [years or n (%)]*	66.92 ± 10.51
• ≥ 50	46 (92)
• < 50	4 (8)
Sex[n (%)]	
• Male	25 (50)
• Female	25 (50)
BMI [kg/m^{2}orn (%)]**	23.26 (18.37 – 32.42)
• ≥ 25	14 (28)
• < 25	36 (72)
ABI**	0.87 (0.5 – 0.9)
Risk factors [n (%)]	
• Diabetes mellitus	40 (80)
• Hipertension	42 (84)
• Smoker	23 (46)
Medication [n (%)]	
• Statin	43 (86)
• ACEi/ARB	44 (88)
• CCB	23 (46)
Severity level [n (%)]	
• Severe	21 (42)
• Nonsevere	29 (58)
DUS Score**	7 (5 – 8)
Monocyte count (/µL)**	530 (270 – 1340)
HDL (mg/dL)*	39.1 ± 10.73
MHR [rasio or n (%)]	14.51
• High	23 (46)
• Low	27 (54)

Note:
LEAD: lower extremity artery disease; DUS: duplex ultrasound; BMI: body mass index, ABI: ankle brachial index; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; CCB: calcium channel blockers; HDL: high density lipoprotein; MHR: monosit-high density lipoproteinratio; *Data are given as the mean and standard deviation; **Data are given as median and persentile

The MHR was categorized into two groups, high and low,based on the cut-off point of the ROC curve as shown in FIGURE 2 and was determined using the Youden index value. The area under the curve (AUC) on the ROC curve was 55.3% [p = 0.523; CI95% (38.8 – 71.9)]. The MHR cut-offpoint of 14.51 has a sensitivity and specificity of 57.1% and 62.1%, respectively, to predict the severe level of LEAD.

Hypothesis testing to determine the difference in the prevalence ratio between high and low MHR values to the severity level of LEAD was performed using a 2x2 table test with Chi Square test. Chi-square analysis, as shown in TABLE 4, showed no difference in proportion between high MHR values compared to low MHR values for the occurrence of a severe level of LEAD [p = 0.145; CI95% 1.57 (0.81 - 3.03)].

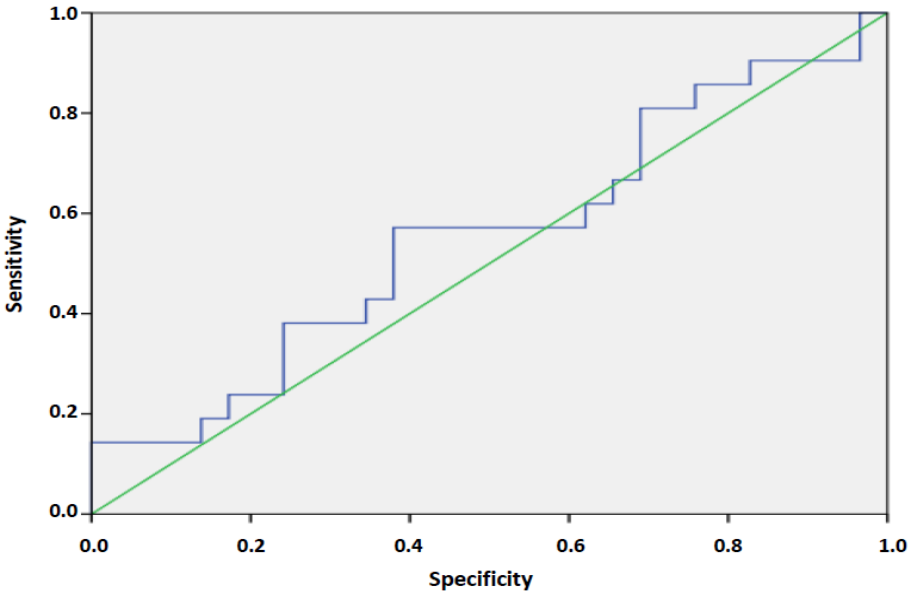

FIGURE 2. ROC curveof MHR to severity level of LEAD; AUC 55.3% [p = 0.523; CI95% (38.8 – 71.9)].

TABLE 4. Hypothetical test prevalence ratio between MHR and LEAD

Variable	Severity level of LEAD		p	PR	CI 95%	
	Severe (n = 21)	Nonsevere (n = 29)			Min	Max
MHR value [n (%)]						
• High	12 (57.1)	11 (37.9)	0.145	1.57	0.81	3.03
• Low	9 (42.9)	18 (62.1)				

Note:
MHR: Monosit-high density lipoprotein ratio; LEAD: lower extremity artery disease; PR: prevalence ratio, CI: confidence interval

Bivariate analysis was performed to determine the relationship between confounding factors and the severity of lesions. The results of the bivariate analysis are shown in TABLE 5. The bivariate test results found significant differences between the ABI values and the incidence of severe degrees of lesions (p = 0.002). The median ABI values with severe and nonsevere lesions were 0.8 (0.5 - 0.9) and 0.89 (0.76 - 0.9), respectively.

TABLE 5. Bivariate analysis of confounding factors to severity level of LEAD

Variable	Severity level of LEAD		p	PR	CI 95% (Min-Max)
	Severe (n = 21)	Nonsevere (n = 29)			
Sex [n (%)]					
• Male	9 (42.9)	16 (55.2)	0.284	0.75	0.39 - 1.46
• Female	12 (57.1)	13 (44.8)			
Age [years or n (%)]*	69.14± 10.98	65.31 ± 10.03	0.103		3.83(-2,18–9.85)
• ≥ 50	20 (95.2)	26 (89.7)	0.425	1.74	0.31- 9.80
• < 50	1 (4.8)	3 (10.3)			
BMI [kg/m^2 or n(%)]**	22.76 (18.37-31.18)	26.41 (19.98–32.42)	0.301		
• ≥ 25	5 (23.8)	9 (31)	0.404	0.80	0.36 - 1.77
• < 25	16 (76.2)	20 (69)			
ABI**	0.80 (0.5 - 0.9)	0.89 (0.76-0.9)	0.002		
DM [n(%)]					
• Yes	17 (81)	23 (79.3)	0.589	1.06	0.46-2.46
• No	4 (19)	6 (20.7)			
Hipertension [n (%)]					
• Yes	17 (81)	25 (86.2)	0.451	0.81	0.37 - 1.77
• Not	4 (19)	4 (13.8)			
Smoker[n(%)]					
• Yes	6 (28.6)	7 (24.1)	0.490	1.14	0.56 - 2.30
• Not	15 (71.4)	22 (75.9)			
Statin [n (%)]					
• No	18 (85.7)	25 (86.2)	0.635	0.98	0.39 - 2.46
• Yes	3 (14.3)	4 (13.8)			
ACEi/ARB [n (%)]					
• No	18 (85.7)	26 (89.7)	0.499	0.82	0.34-1.96
• Yes	3 (14.3)	3 (10.3)			
CCB [n (%)]					
• No	9 (42.9)	14 (48.3)	0.464	0.88	0.45 - 1.71
• Yes	12 (57.1)	15 (51.7)			
Monocyte count (/µL)**	620(340-1340)	500 (270-770)	0.059		
HDL(mg/dL)*	41.38 (13.27)	37.45 (8.29)	0.120		3.93(-2.76-10.62)

Note: LEAD: lower extremity artery disease; DUS: duplex ultrasound; BMI: body mass index, ABI: ankle brachial index;DM: diabetes mellitus; ACEi:angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; CCB: calcium channel blockers; CI: conficence interval; PR: prevalence rate; HDL: high density lipoprotein; *Data are given as the mean and standard deviation; **Data are given as median and persentile

DISCUSSION

The cut-off point of the MHR in this study was 14.51. This cut-off point devided the high MHR group ≥ 14.51 and low MHR <14.51. The number of subjects with high and low MHR was 23 (46%) and 27 (54%), respectively. The results of this study found a greater proportion of high MHR values than low MHR values for the occurrence of severe lesions, which amounted to 12 (57.1%) versus 9 (42.9%). However, the difference in this proportion was not statistically significant [p=0.145; CI95% 1.57 (0.81 - 3.03)].

Some factors might contribute to the results of this study. They included that the lesion severity score system used in this study was practically a novel scoring system, where further research is still needed. This study used this novel scoring system because there was no other severity assessment scoring system that also used DUS examination. DUS itself has several disadvantages, including its inability to describe the whole arteries, and the results obtained were often subjective depending on the operator. It also shows that the gold standard of LEAD lesion assessment still remains digital substration angiography or other imaging modalities such as magnetic resonance angiography or CT angiography.[1,9]

The value of monocytes in this study did not differ between groups of severe and nonsevere lesions (p=0.059). This was different from the study conducted by Wildgruber *et al.*[16] where there was an increase in monocytes in the clinical severity of LEAD based on Rutherford criteria. However, a significant increase in the study did not explain whether the Rutherford III classification was accompanied by acute tissue loss, whereas this MHR study did not involve subjects with acute Rutherford III. It was also interesting to point out that intermediate monocytes (CD14++/CD16+) were the only monocytes that increased in severe peripheral arterial disease.[16]

Monocytes had 3 subpopulations in the form of (a) classical monocytes (CD14++/ CD16-), which were almost 90% of all monocytes, and more proinflammatory CD16+, which were divided into (b) intermediate monocytes (CD14++/CD16+) and (c) nonclassical monocytes (CD14+/ CD16++). Intermediate monocytes (CD14++/CD16+) were said to have more proinflammatory properties because they produced reactive oxygen species (ROS), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Intermediate and nonclassical monocytes could even survive in circulation for 3-5 days.[17]

Monocytes are a type of leukocyte that play an important role in inflammation and atherosclerosis. Activated monocytes in endothelial cells trigger excessive expression of proinflammatory cytokines such as monocyte chemotactic protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Then, monocytes will differentiate into macrophages that engulf low-density lipoprotein (LDL) cholesterol to produce foam cells.[18]

HDL values in the group of severe and nonsevere lesions were not different (p = 0.120). The HDLmean value in the severe lesion group was even greater than that in the nonsevere lesion group, amounting to 41.38 (13.27) mg/dL versus 37.45 (8.29) mg/dL. HDL has an anti-inflammatory effect on arteries through the inhibition of MCP-1, VCAM-1 and ICAM-1 so that it could prevent the process of initiating atherosclerosis characterized by recruitment and infiltration of monocytes into the subendothelial layer.[19] According to the density, HDL particles were divided into two main subclasses: low-density HDL2, which was rich in lipids, and high-density HDL3, which was rich in protein. These two subclasses were divided into

three by size: (a) small HDL (7.3–8.2 nm) including HDL3c and HDL3b, (b) moderate HDL (8.2–8.8 nm) including HDL3a, and (c) large HDL (8.8–13.0 nm) including HDL2a and HDL2b.[20] In a clinical study, it was said that small HDL had a positive correlation with the presence and severity and progression of atherosclerotic disease, in contrast to large HDL.[21] The HDL study in LEAD by Kasko *et al.*[22] showed that there is an HDL subfraction, namely, HDL3, which increased in LEAD. HDL3 is easily subjected to behavioral changes and is actually atherogenic in certain conditions, such as diabetes mellitus. The average age of the subjects in this study was 69.92 ± 10.5 years with the majority of subjects aged s easily subjected to behavioral changes and is actually atherogenic in Aboyans *et al.*[1] that LEAD occurs at the age of> 50 years. The relationship between increasing age and sex in the occurrence of LEAD is the same as that mentioned by Savji *et al.* in a study involving more than 3.6 million subjects aged urrence of[23] Sex differences in the incidence of LEAD are still debated. Lower extremity artery disease tends to be difficult to recognize in women due to the asymptomatic majority.[24,25] The male and female sex in this study were the same. It was in accordance with the ESC guidelines that risk factors that had been shown to be strongly related to LEAD were age, dyslipidemia, hypertension, diabetes mellitus, and smoking. Other risk factors, including gender, are still being studied. In this study, age and sex did not show differences in high and low group MHR values or in the severity level of group lesions. Other risk factors assessed in this study were as much as 42 (84%) hypertension, 40 (80%) diabetes mellitus, 14 (28%) obesity and 13 (26%) smoking. These risk factors did not show significant differences in high and low group MHR or in the severity level of group lesions.

The ABI value showed a statistically significant difference in the severity of LEAD lesions (p=0.002). The median ABI values with severe and nonsevere lesions were 0.8 (0.5 - 0.9) and 0.89 (0.76 - 0.9), respectively. ABI is the first screening modality and diagnosis of LEAD. ABI ≤0.9 has a sensitivity and specificity of 75% and 86%, respectively, to establish a diagnosis of LEAD.[1]

Medical history in the form of statin, ACEi/ARB, and CCB drugs was the same between the two groups of severe and not severe lesions with each p = 0.635; p = 0.499; and p = 0.464. These drugs were commonly used to treat the risk factors of LEAD recommended by the ESC guidelines.[1] These drugs were not different between the high- and low-MHR groups.

This study has several limitations, including the design of a cross-sectional study where monocytes and HDL samples were taken at one moment while the atherosclerotic process was a chronic process.

The assessment of the severity of the lesion using a DUS examination has disadvantages in describing the whole arterial system in the body. The DUS scoring system used in this study was a novel scoring system to assess the severity of LEAD. This DUS scoring system had never been studied to compare its accuracy in detecting lesions with the gold standard method, i.e., the DUS scoring system. digital substration angiography or other imaging modalities, such as magnetic resonance angiography or CT angiography, to assess the severity of LEAD. This DUS scoring system might need to be modified based on further research.

This study neither carried out HDL3, an HDL subfraction examination that easily underwent behavioral changes to be proaterogenic nor intermediate monocytes (CD14 ++/CD16+), the only subclass of monocytes that increased in severe peripheral arterial disease based on a previous study.

CONCLUSION

There is no association between the MHR and theprevalence of LEAD severity.

ACKNOWLEDGMENTS

None.

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