AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

Diagnosis of Peripheral Artery Disease by Neutrophil Gelatinase-Associated lipocalin (NGAL), Ankle Brachial Index and Lipid Profile

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Keywords:
Peripheral artery disease, Intermittent and Critical , NGAL, ankle brachial index and lipid profile.

ABSTRACT

Peripheral arterial disease (PAD) is a progressive disorder characterized by stenosis and/or occlusion of large and medium-sized arteries. Systemic atherosclerosis remains the number one cause of morbidity and mortality in the western world and is the major cause of peripheral arterial disease (PAD), causing obstructions in blood flow in one or more of the major limb arteries. A case-control study was performed on 30 Intermittent and 30 Critical patients and 60 healthy controls during the period from February to May 2022.Focus on the estimation of ankle brachial index and lipid profile. detecting serum concentration of NGAL by using sandwich ELISA. The results of the statistical analysis showed a significant difference ($P \leq 0.01$) between Intermittent & Critical patients groups and control group. The data showed an increase in NGAL levels in patients groups (intermittent& critical) compared with a control group where a significant difference ($p<0.001$), Observed the highest level in the intermittent PAD group (4.27±1.06 ng /ml), and the lowest value of NGAL was recorded in the control group (0.63±0.08 ng/mL). This study found that the highest value of the ankle-brachial index (ABI) was recorded control group (1.08±0.23) in the was highly significant with different groups. On the other hand, results showed that the highest level of TC, and TG in the critical group (276.65±78.13 mg/dL,225.34±62.96mg/dL, respectively), while the lowest level in the control group (207.39±74.97 mg/dL, 144.07±57.64mg/dL). The present data suggest a role for NGAL in the diagnosis as a biomarker for early detection of and prognostic factor in patients with peripheral arterial disease.

1. Introduction

Peripheral arterial disease (PAD) is a progressive disorder characterized by stenosis and/or occlusion of

large and medium-sized arteries, other than those that supply the heart (coronary artery disease, CAD) or the brain (cerebrovascular disease) [1].

Systemic atherosclerosis remains the number one cause of morbidity and mortality in the western world and is the major cause of peripheral arterial disease (PAD), causing obstructions in blood flow in one or more of the major limb arteries [2].

Human neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa glycosylated protein from the lipocalin family. The lipocalins' common secondary and tertiary structure corresponds to a single, eight-stranded antiparallel β-barrel around a central pocket that is capable of binding low-molecular-weight ligands [3]. The ankle brachial index, or ABI, is a simple test that compares the blood pressure in the upper and lower limbs. Health care providers calculate ABI by dividing the blood pressure in an artery of the ankle by the blood pressure in an artery of the arm. The result is the ABI. If this ratio is less than 0.9, it may mean that a person has peripheral artery disease (PAD) in the blood vessels in his or her legs [4].

Lipids and lipoproteins are risk factors for CHD. It has been demonstrated that high levels of serum total cholesterol (TC), triglycerides (TG), LDL cholesterol, very-low-density lipoprotein (VLDL), low concentration of HDL cholesterol, and increased body mass index (BMI) are significantly associated with CHD [5].

2. Materials and Methods

2.1 Patients and control

The current study was conducted on (120) individuals (both gender) (patients and control groups) with aged (30-85 years old). Sixty patients were collected from the cardiovascular unit of AL-Emamain AL-Kademyain teaching medical city and Ghazi Harirri Teaching Hospital, during the period from February to May 2022.They were subdivided into 3 groups. Group I: consist of 30 patients with Intermittent claudicating according to Rutherford classification grades, group II: consist of 30 patients with Critical limb ischemia according to Rutherford classification grades and group III: 30 healthy control subjects of comparable age and sex.

2.2 Blood sample collection

Six millilitres of blood samples were obtained from patients and control as follow:

1. The blood samples will be left for 20 minutes at room temperature. After coagulation, sera will be separated by centrifugation at 3000 rpm for 10 min.
2. Then sera were aspirated and divided into small aliquots for: Immediate measurement of lipid profile will be done using appropriate enzymatic colorimetric method. The rest will be stored at -20 until assayed for serum of neutrophil gelatinase associated lipocalin (NGAL).

2.3 Determination of lipid profile

Used clinical chemistry analyzer from Beckman Coulter's for measurement lipid profile

2.4 Determination of neutrophil gelatinase associated lipocalin (NGAL) serum level

Serum level NGAL was determined using commercial ELISA kits for human NGAL (Al-shkairate establishment for medical supply, USA) and the instructions of the manufacturer were followed. Absorbance was measured at a wavelength of 450 nm using a micro-plate reader (Huma Reader HS, Germany). Using an EXCEL sheet, a standard curve (measured absorbance against the concentration of

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

serially diluted standards) was plotted.

2.5 Statistical analysis

The Statistical Analysis values of study variables were presented in terms of ± standard deviation (SD), and the differences between means were assessed using the computer program SPSS version 20 and to compare continuous variables, the analysis of variance (ANOVA) by least significant difference (LSD) test was utilized. The area under the curve (AUC), sensitivity, specificity, and cut-off value were calculated using the receiver operating characteristic (ROC). A probability (p) of ≤ 0.01was regarded as significant.

2.6 Demographic characteristics of the Study Population

In terms of age and gender, in the Patients with Critical PAD, Intermittent PAD, and controls there were no significant differences between the three groups (Table 1).

Table 1: Demographic data of the study population

Variable	Controls (n = 60)	Degree of PAD		P-value
		Intermittent (n = 30)	Critical (n = 30)	
Age, years *Mean±SD* *Range*	57.63 ±9.66 33-75	58.23 ±9.86 33-71	60.37 ±9.65 33-75	0.376
Gender				
Males	36(60%)	24(80%)	20(66.67%)	0.165
Females	24(40%)	6(20%)	10(33.33%)	

Different small letters indicate significant differences
PAD = peripheral artery disease

2.7 Evaluation of serum low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and total cholesterol (TC) in study groups

The data appear increase in low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and total cholesterol (TC) concentration showing an association with PAD as in table 2.

The function of blood lipid markers in the development of atherosclerotic PAD is critical. Epidemiological studies have demonstrated that dyslipidemia alone is sufficient for the development of atherosclerosis, even in the absence of additional risk factors [6].

In [7], [8], [6] this study, indicated that the TG index was an independent predictor of the complexity of the peripheral vascular disease.

Table 2: Biochemical data of the study population

Variable	**Controls (n = 60)**	**Degree of PAD**		**P-value**
		Intermittent (n = 30)	**Critical (n = 30)**	

TC level, mg/dL *Mean±SD* *Median* *Range*	 207.39±74.97 207.3 [a] 100-421	 248.48±76.9 248.4 [b] 140-421	 276.65±78.13 276.6 [b] 164-500	**<0.001**
TG level, mg/dL *Mean±SD* *Median* *Range*	 144.07±57.64 144.07 [a] 80-350	 217.90±73.94 217.9 [b] 111-410	 225.34±62.96 218.7 [b] 107-370	**<0.001**
LDL level, mg/dL *Mean±SD* *Median* *Range*	 110.64±36.99 110.6 13-203	 126.80±40.50 126.8 33-208	 133.29±45.32 133.2 70-261	0.027
Different small letters indicate significant differences *PAD = peripheral artery disease; TC = total cholesterol; TG = triglyceride; LDL = low-density lipoprotein*				

Importantly, an increasing number of studies have shown that altered atypical lipid profiles can lead to the development and progression of atherosclerosis. The triglyceride (TG)/HDL-C ratio may be a good predictor of cardiovascular disease, and several studies have demonstrated that the total cholesterol (TC)/HDL-C ratio is also associated to cardiovascular disease [9].

In the present study of lipid profile agrees with the result reported by [10] we found significant associations for increased low-density lipoprotein cholesterol (LDL-C) concentrations, triglycerides, and increased total cholesterol (TC).

2.8 Evaluation of ankle-brachial index (ABI) in study groups

The mean the ankle-brachial index (ABI) in the control group (1.08±0.23) was greater than in the intermittent and critical PAD groups (0.74±0.23 and 0.70±0.23, respectively) with highly significant differences. However, there is no significant difference between the two groups of patients (Figure 1) and (Table 3).

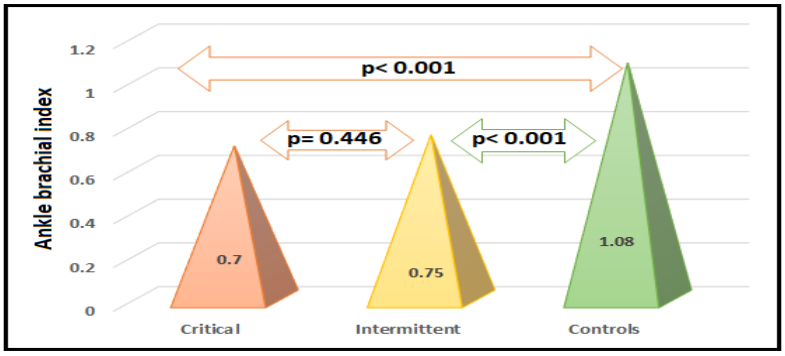

Figure 1: Mean ABI in patients and controls

The ankle-brachial index is useful in screening of peripheral artery disease in at-risk patients and detecting the condition in patients who come with lower extremity clinical signs of the disease. The majority of healthy persons have a score higher than 1.0. A score of less than 0.91 suggests severe peripheral artery disease [11].

ISSN: 0005-2523
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In the present study of ABI agrees with the results of [12], [13] patients were found to have ABI > 1. These results to agree with the result reported by [14].

2.9 Evaluation of serum NGAL in study groups

The mean serum level of NGAL in the control group (2.1±0.38µg/mL) was substantially lower than in the intermittent and critical PAD groups (4.27±1.06, 3.74±0.88 ng/mL, respectively) with highly significant differences. Furthermore patients with moderate PAD had a greater NGAL level than patients with critical PAD with a significant difference (Figure 2) (Table 3).

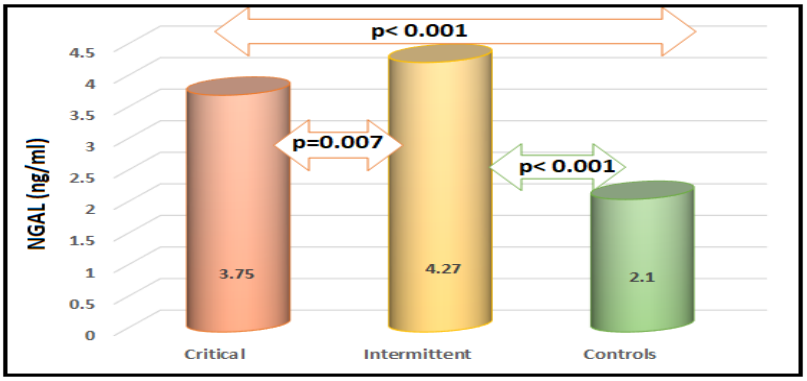

Figure 2: Mean serum level of NGAL in patients and controls

The mean serum level of NGAL in the control group is substantially lower than in the intermittent and critical PAD groups with highly significant differences. Additionally, patients with mild PAD had a significantly higher NGAL level than those with critical PAD. NGAL was discovered to be an inflammatory signal generated by neutrophils in response to bacterial infection. NGAL is abundantly expressed in kidney tubule cells and its levels in urine rise prior to changes in serum creatinine levels, making it a marker of acute kidney damage [15].

The personal results agree with the results of [16] there showed that NGAL levels are related to the progression and consequences of cardiovascular disease. Smooth muscle cells, cardiomyocytes, macrophages and endothelial cells in atherosclerotic plaques express NGAL.

It is thought that elevated NGAL expression in atherosclerotic disease is due to its interaction with metal metalloproteinase 9. (MMP-9). MMP-9 levels and activity have been shown to be elevated in arterial plaque architecture. NGAL binds to MMP-9, blocking its breakdown and resulting in increased and extended activity. The ensuing tissue remodeling contributes to plaque instability, which eventually leads to acute coronary syndrome. MMP-9 levels in the blood have also been found to be higher in PAD patients with intermittent claudication and severe ischemia [17- 19].

Table 3: Serum level of ABI, calprotectin and NGAL in patients with PAD and controls

Variable	**Controls (n = 60)**	**Degree of PAD**		**P-value**
		Intermittent (n = 30)	**Critical (n = 30)**	
ABI *Mean±SD* *Range*	1.08±0.23[a] 0.40- 1.60	0.74±0.23 [b] 0.29- 1.10	0.70±0.23 [b] 0.29 -1.20	**<0.001**

NGAL ng/mL						
Mean±SD	2.10	±0.38[a]	4.26	±1.06[b]	3.74	±0.88[c]	**<0.001**
Range	1.12	-2.90	3.05	-6.95	2.52	-6.36	
Different small letters indicate significant differences *PAD = peripheral artery disease*							

2.10 In the Context of Discrimination between Patients with Intermittent PAD and Controls

The cutoff value of NGAL that obtained from ROC curve explain high levels of ANGLE between groups compared with controls, the test's sensitivity and specificity were 100% for both at a cut-off value of NAGL= 2.92. This difference is useful to diagnose patients with PAD (Figure 3).

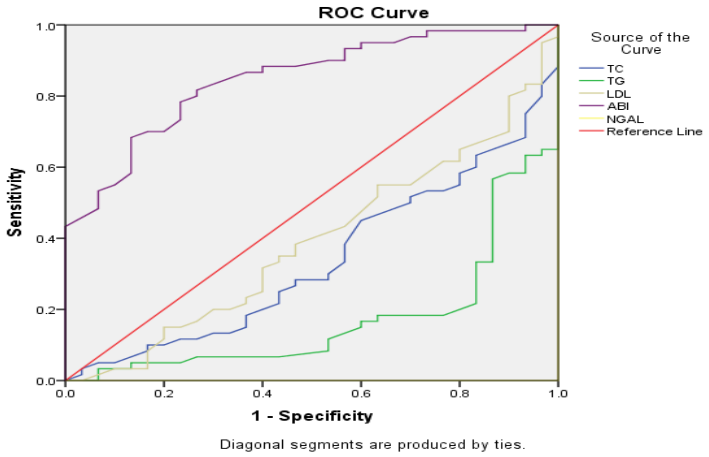

Figure 3: Receiver operating characteristic curve of TC, TG, LDL, ABI and NGAL level in the context of discrimination between Intermittent PAD and controls

2.11 In the Context of Discrimination between Patients with Critical PAD and Controls

The area under the curve (AUC) for ABI was 0.805, 95% CI= 0.715-0.896, p<0.001. The test's sensitivity and specificity were 78% and 69%, respectively, at a cut-off value of ABI= 0.93.

The AUC for NGAL was 1.00, 95% CI= 1.0-1.0, p <0.001. The test's sensitivity and specificity were 100% for both at a cut-off value of NAGL= 2.92 (Figure 1-4).

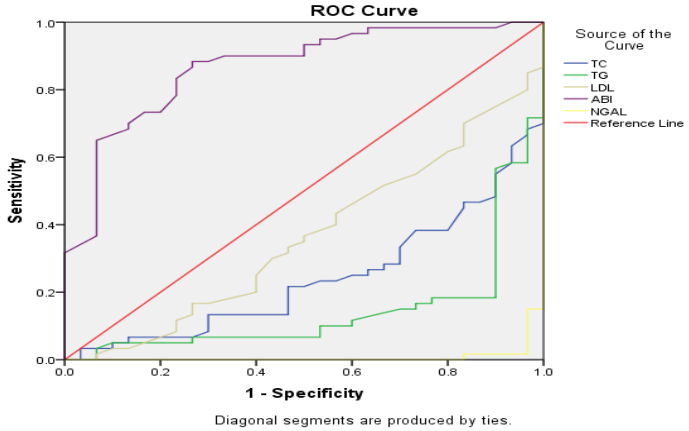

Figure 4: Receiver operating characteristic curve of TC, TG, LDL, ABI and NGAL level in the context of

ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

discrimination between Intermittent PAD and controls

2.12 In the Context of Discrimination between Patients with Critical and Intermittent PAD

The AUC for ABI was 0.840, 95% CI= 0.754-0.925, p <0.001. The test's sensitivity and specificity were 82% and 76%, respectively, at a cut-off value of ABI= 0.92.

The AUC for NGAL was 0.958, 95% CI= 0.918-0.998, p<0.001. The test's sensitivity and specificity were 95% and 82%, respectively, at a cut-off value of NAGL= 2.49 ng/ml

3. Conclusion

- The neutrophil gelatinase-associated lipocalin (NGAL) would be used in the diagnosis as a biomarker for early detection of and prognostic factor in patients with peripheral arterial disease via the level of ankle-brachial index, lipid profile, and detecting serum concentration of NGAL, there was a significant difference ($P \leq 0.01$) between the Intermittent & Critical patient groups and the control group.
- Statistical analysis shows a significant positive correlation between ABI and NGAL for PAD (intermittent, critical) and controls.
- One important takeaway from our results is that NGAL has the potential to be a useful biomarker for predicting PAD-related problems, this is clinically significant because NGAL levels have been linked to atherosclerotic disease.
- Based on these findings, it is therefore conceivable to imply that NGAL may be used to risk stratifies individuals with PAD at an early stage of disease however, bigger cohort studies are needed to substantiate this idea.

4. Acknowledgment

The authors would like to thank the Department of chemistry & biochemistry Clinical Biochemistry College of Medicine/ Al-Nahrain University/Baghdad, Iraq.

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ISSN: 0005-2523
Volume 62, Issue 07, September, 2022

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