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Relationship between Calprotectin, glycosylated Haemoglobin (HbA1C), Haemoglobin (Hb), and D-Dimer of Diagnosis Peripheral Artery Disease

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ABSTRACT— Peripheral arterial disease (PAD) is a progressive disorder characterized by stenosis and/or occlusion of large and medium-sized arteries, causing obstructions in blood flow in one or more of the major limb arteries. This study was carried out evaluation the Calprotectin activity to Diagnosis of Peripheral artery disease. The samples were collected (120) individuals (both gender) (patients and control groups) with aged (30-85) years old. Sixty patients (30 Intermittent, 30 Critical) and 60 healthy controls from (February to May 2022). The present study examined levels of Calprotectin, glycosylated Haemoglobin (HbA1C), Haemoglobin (Hb), and D-Dimer. The results depicted that Hb level was significantly higher in PAD of intermittent and critical patients compared to control (12.72± 2.11mg/dL, 14.23± 2.11mg/dL and 14.84± 1.69mg/dL respectively, $p < 0.001$. The results showed of the HbA1c level in PAD of intermittent, critical, and controls groups were (7.37 ± 1.72%, 11.16± 1.62%, and 6.68±1.87%) respectively. The patients with the critical peripheral arterial disease (PAD) had a considerably higher HbA1c level. The present study showed of D-dimer in PAD for intermittent, critical, and controls groups that significant differences ($p<0.001$), recorded the highest value recorded in the critical PAD (783.96±403.94mg/mL).The present study appeared found a significant difference ($p<0.001$) in different groups that calprotectin were recorded in the intermittent PAD group (1.56± 0.42 ng/ml) & the critical PAD group (1.41±0.33 ng/mL), Where they were recorded highest values in the intermittent PAD group and the lowest value in the control group (0.63± 0.08mg/mL). The data showed Elevated serum calprotectin levels in patients groups (intermittent & critical) and it correlation with HbA1C, Hb, and D-Dimer. Where suggest a role for calprotectin to the diagnosis as a biomarker for early detection of peripheral arterial disease.

KEYWORDS: Peripheral artery disease, Calprotectin, (HbA1C), (Hb), D-Dimer

1. INTRODUCTION

Peripheral arterial disease (PAD) is marked by stenosis and/or occlusion of medium- and large-sized arteries other than those supplying the brain (cerebrovascular disease) or the heart (coronary artery disease) [1].

Systemic atherosclerosis is the leading cause of illness and mortality in the world, and it is the leading cause of PAD, which causes blood flow blockages in any or all of the major limb arteries [2].

Calprotectin is a heterodimer composed of 2 proteins in the cytoplasm, S100A8, and S100A9, which are

expressed by white blood cells, particularly neutrophils and monocytes. Calprotectin, also known as myeloid-related protein-8 & -14 (MRP8/14), is a validated indicator of disease activity in bowel inflammation and rheumatic diseases [3].

Calprotectin would be a 36kDa calcium and zinc-binding protein that is prevalent in neutrophil cytoplasm. It slows development by competing for zinc and may be microbicidal at high dosages [4].

2. Materials and Methods

2.1 Patients and control

The current study was conducted on (120) individuals (both gender) (60 patients and 60 control groups) with aged (30-85 years old). Sixty patients (30 intermittent patients and Critical patients) were collected from the cardiovascular unit of Ghazi Harirri Teaching Hospital and AL-Emamain AL-Kademyain teaching medical city, during the period from February - May 2022.

2.2 Blood sample collection

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

1. The blood samples were separated into 3 vials: a/ 1vial; approximately two milliliters were collected into EDTA-containing tubes and delivered to a hospital laboratory the Hb and HbA1c measurements.
2. b/ 2vial; about two milliliters will be collected into Sodium Citrate containing tube and sent to the hospital laboratory for measuring D-Dimer.
3. C/Part 3; -For twenty minutes, the blood samples will be stored at room temperature. After coagulation, serum will be extracted by centrifugation 10 minutes at 3000 rpm.2-The sera were then aspirated and separated into small vials for the following purposes: The remainder will be kept at -20 until the serum of calprotectin is tested.

2.3 Determination of Haemoglobin (Hb)

Use complete blood count from Beckman Coulter's for measurement Haemoglobin (Hb).

2.4 Determination of glycosylated Haemoglobin (HbA_{1C}%) and D-Dimer

Used clinical chemistry analyzer from Beckman Coulter'sfor measurement glycosylated Haemoglobin (HbA_{1C}%) and D-Dimer

2.5 Determination of Calprotectin serum level

Serum level Calprotectinwas determined using commercial ELISA kits for human Calprotectin (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 (HumaReader HS, Germany). Using an EXCEL sheet, a standard curve (measured absorbance against the concentration of serially diluted standards) was plotted.

2.6 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 $\leq 0.01$was regarded as significant.

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2.7 Demographic characteristics of the Study Population

The Age was recorded for the controls group (57.63± 9.66), the Intermittent PAD group (58.23± 9.86)), and the Critical PAD group (60.37± 9.65). While the obtained results showed Gender in the controls group [36(60%) Males, 24(40%) Females] Intermittent PAD group [24(80%) Males, 6(20%) Females] Critical PAD group [20(66.67%) Males, 10(33.33%) Females]. There were no significant differences between the three groups in both age and gender.

2.8 Evaluation of Hb level in Study groups

The results that are shown in table 3-2 of Hb level in PAD of intermittent, critical and controls groups were 12.72± 2.11mg/dL, 14.23± 2.11mg/dL and 14.84±1.69mg/dL respectively, respectively. There was significant differences between the three groups, as a result, the mechanism behind the link between hemoglobin and arterial stiffness or atherosclerosis is yet unknown. The primary cause could be an increase in blood viscosity as a result of elevated hemoglobin content [5]. Another mechanism could be an increase in blood pressure caused by a higher hemoglobin concentration, which would raise the risk of thrombosis [4].

2.9 Evaluation of Hematological Parameter (HbA1c level) of the Study Population

Results showed that patients with critical peripheral arterial disease (PAD) (Table1-2). had a considerably higher HbA1c level (11.16± 1.62 %) than controls (6.68± 1.87%), Inflammation and oxidation have been linked to hyperglycemic pulses (Xia and Yin, 2019). The oxidative stress caused by glucose fluctuations may be linked to endothelial dysfunction, although various researchers have examined the effect of HbA1c variability on mortality and CVD [6]. [7] reported that the Increased HbA1c is significantly associated with an increased risk of peripheral arterial disease [7].

The current study is agreeing with the results with [8] when the results explain of HbA1c an association with PAD. The current results appeared high value for HbA1c and indicate an association between HbA1c and high-risk PAD the results agree with [9].

Table 1: Hematological parameters in patients with PAD and controls

Variable	**Controls (n = 60)**	**Degree of PAD**		**P-value**
		Intermittent (n = 30)	**Critical (n = 30)**	
Hb level, mg/dL ***Mean±SD Range***	**12.72 ±2.11[a]** **8.1-16.7**	**14.23 ± 2.11 [b]** **10.7-18.6**	**14.84 ±1.69 [b]** **11.6-18.7**	**<0.001**
HbA1c, % ***Mean±SD Range***	**6.68±1.87 [a]** **4.1-12.0**	**7.37 ± 1.72 [a]** **7.9-12.0**	**11.16± 1.62 [b]** **54-13.8**	**<0.001**
Different small letters indicate significant differences *PAD = peripheral artery disease*				

2.10 Evaluation of D-dimer in Study Population

The present study explained that significant differences ($P \leq 0.01$) were observed in the D-dimer, the highest value recorded in the critical PAD (Table1-2).

Elevated D-dimer levels indicate activation of the fibrin production, coagulation system, and fibrin breakdown by plasmin [10]. D-dimer is a particular metabolite of cross-linked fibrin produced by plasma

fibrinolytic enzyme [11].

According to prior research, people with PAD had greater levels of hemostatic biomarkers. Furthermore, the data show that these biomarkers can predict the commencement of future arterial events reliably over a two-year period, which is consistent with prior research that found D-dimer to be a marker of PAD worsening and subsequent thrombotic events [12].

They discovered that greater D-dimer levels were related to poorer lower extremity functioning in men and women with and without PAD [13]. D-dimer is inversely and independently related to the degree of functional impairment in a cohort of older men and women with and without PAD [14], [15].

Table 2: Biochemical data of the study population

Variable	**Controls (n = 60)**	**Degree of PAD**		**P-value**
		Intermittent (n = 30)	**Critical (n = 30)**	
D-Dimer level, ng/mL ***Mean±SDMedian Range***	431.55±165.90 431.2 [a] 110-970	606.66±289.07 606.6 [b] 210-1450	783.96±403.94 783.9 [b] 300-1877	**<0.001**
CALPng/mL ***Mean±SD Range***	0.63 ±0.08 [a] 0.48 -0.79	1.56±0.41[b] 1.03 -2.37	1.41±0.33[b] 0.99-2.50	**<0.001**
Different small letters indicate significant differences *PAD = peripheral artery disease*				

Where the results appeared that the D-dimer was also higher in patients with PAD as compared to controls. The results agreed with the results of researcher [12] and this agreed with the results of the study conducted by [14] were found levels are elevated in patients with peripheral artery disease (PAD).

2.11 Evaluation of serum calprotectin in study groups

The mean CALP levels in the control group (0.63 ±0 .08µg/mL) were substantially lower than those in the intermittent and critical PAD groups (1.56±0.41µg/ml and 1.41±0.33µg/mL, respectively) with highly significant differences. Although patients with intermittent PAD had a higher level of calprotectin than those with critical PAD, the difference was not significant (Figure 1) (Table1 2).

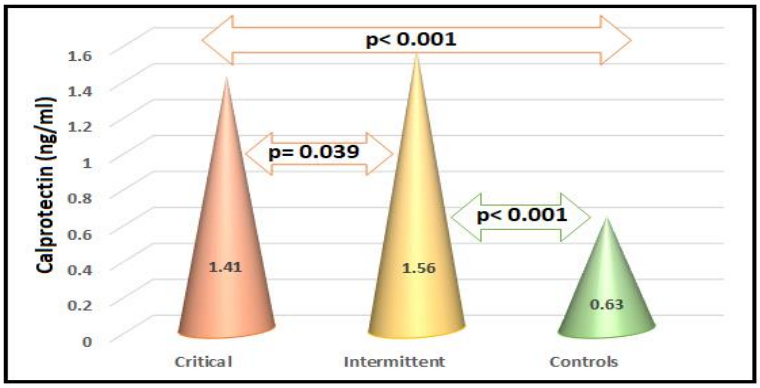

Figure 1: Mean serum level of calprotectin in patients and controls

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Serum calprotectin CLP levels were considerably greater in PAD patients, and intermittent PAD had a higher amount of calprotectin. Serum markers are frequently utilized in PAD to assess disease activity, evaluate treatment response, and as diagnostic techniques. CLP can be a helpful and dependable indicator of PAD activity and severity. Serum CLP levels were considerably greater in PAD patients. Recent studies have found an elevated CLP level of PAD patients [16], [17].

Calprotectin induces reactive oxygen and nitrogen species, as well as thrombogenic and proinflammatory phenotypes in microvascular endothelial cells, as seen by increased vascular permeability and the production of chemokines, cytokines, and adhesion molecules [18]. Calprotectin is also a powerful activator of neutrophils, boosting degranulation and phagocytosis [19].

In the present study of calprotectinagrees agreed with the result that reported by [20] which shows a significant associations for increased calprotectin.

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

The AUC for CALP 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 CALP= 0.81(Figure 2).

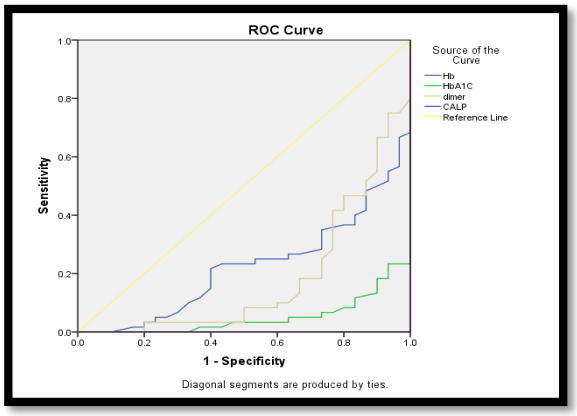

Figure 2: Receiver operating characteristic curve of D-Dimer, HbA1c, Hb, and CALP level in the context of discrimination between Critical PAD and controls

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

The AUC for CALP was 0.995, 95%CI= 0.923-1.0, p<0.001. The test's sensitivity and specificity were 93% and 100%, respectively, at a cut-off value of CALP= 0.85µg/ml.

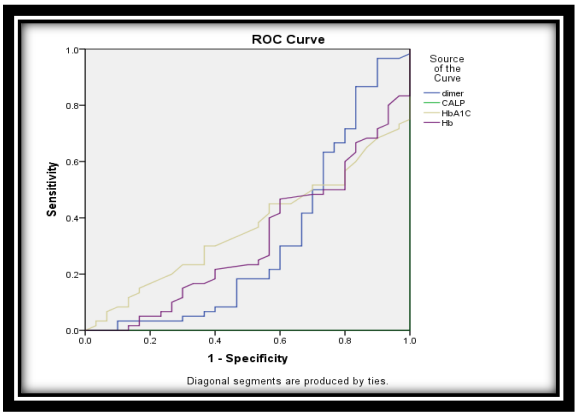

Figure 3: Receiver operating characteristic curve of D-Dimer, HbA1c, Hb, and CALP level in the context of discrimination between Intermittent PAD and controls

3. Conclusion

1. Calprotectin (CLP) can be used in the diagnosis as a biomarker for early detection of and prognostic factor in patients with peripheral arterial disease.
2. The statistical analysis of the results of the level of D-Dimer, HbA1c, Hb, and CALP shows a significant difference ($P \leq 0.01$) between the Intermittent & Critical patient groups and the control group.
3. Statistical analysis shows a significant positive correlation between D-dimer, HbA1c, Hb, and CALP level for PAD (intermittent, critical) and controls.
4. According to these results, Serum CLP is a promising biomarker for evaluating disease activity in PAD patients.
5. One important takeaway from our findings is that calprotectin has the potential to be a useful biomarker for predicting PAD.
6. Serum calprotectin has the potential to be a useful and accurate biomarker of PAD activity and severity.
7. Calprotectin levels in the blood were considerably greater in PAD patients.

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