NEUTROPHIL TO LYMPHOCYTE RATIO AS A PREDICTOR OF PERIPHERAL ARTERIAL DISEASES AMONG REGULAR HEMODIALYSIS PATIENTS

By

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ABSTRACT

Background: Peripheral arterial disease (PAD) is common in patients with end-stage renal disease on regular hemodialysis. Neutrophil to lymphocyte ratio (NLR) is considered an indicator of the severity and extent of systemic inflammation and atherosclerosis in patients with renal and cardiovascular disorders.

Objective: To assess neutrophil to lymphocyte ratio as a predictor of peripheral arterial diseases in regular hemodialysis patients.

Patients and Methods: This cross-sectional study was carried out at Hemodialysis Unit of Al-Hussein University Hospital over a period of six months from January to July 2020, and conducted on 40 hemodialysis patients. Demographic data and clinical information were recorded. Laboratory data including CBC, calcium, phosphate, albumin, cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, iron profile were evaluated. The ankle-brachial index was measured in all patients. PAD was diagnosed if ankle-brachial index (ABI) ≤ 0.9. Duplex ultrasound for arterial system of both lower limbs was done for patient with ankle-brachial index≤ 0.9.

Results: There were 29 males (72.5%) and 11 females (27.5%). The mean age of all patients was 43.2 ± 10.3 years. There were 7 diabetic (17.5%) patients, and 27 hypertensive (67.5%) patients. Regarding CBC, the mean of white blood cells (WBCs), neutrophils, lymphocytes, NLR, hemoglobin (Hb) and platelets (PLT) were 6.5 ± 1.7 (x10³/ul), 4.3 ± 1.5 (x10³/ul), 1.74 ± 0.53 (x10³/ul), 2.73 ± 1.3, 10.8 ± 2.3 (g/dl), and 234.7 ± 95.9 (x10³/ul) respectively. Regarding lipid profile, the mean of total cholesterol, high density lipoprotein (HDL) and triglycerides were 214.7 mg/dL, 41.9 mg/dL, 172.2 mg/dL, respectively. Regarding serum albumin, corrected serum calcium (Ca), serum phosphorus (PO4), serum Ca X PO4 product, and parathyroid hormone (PTH), the means were 3.99 ± 0.5 (g/dl), 8.3 ± 0.6 (mg/dl), 5.22 ± 1.1 mg/dl, 43.7 ± 11.2 and 722.1 ± 477.7 (ug/ml) respectively. Regarding to ABI, there were 13 patients (32.5%) ≤ 0.9, while there were 27 patients (67.5%) > 0.9.

Conclusion: Neutrophil-lymphocyte ratio (NLR) could be considered as a predictor of peripheral arterial diseases (PADs) among regular haemodialysis patients.

Key words: Peripheral Arterial Disease among Hemodialysis Patients, Ankle Brachial Index, The Neutrophil-Lymphocyte Ratio.

INTRODUCTION

The NLR has been widely studied as marker of inflammation *(Kim et al., 2019)*. Peripheral blood NLR is widely reported to be associated with inflammatory response and reflect the inflammatory status of many diseases *(Liu et al., 2019)*. Patients with chronic kidney disease (CKD) are closely associated with all-cause mortality and poor prognoses of cardiovascular disorders *(Valga et al., 2019)*. *Ouellet et al. (2016)* found that NLR was associated with all-cause mortality in hemodialysis (HD) patients. NLR measurements were shown to correlate well with vascular calcification in end stage renal disease (ESRD) population *(Turkmen et al., 2014)*. PADs include all arterial diseases including carotid artery disease, mesenteric artery disease, renal artery disease and lower extremity artery disease (LEAD), and other than coronary arteries and the aorta *(Aboyans et al., 2018)*. CKD itself is strongly and independently associated with PAD. In both the general population and patients with CKD, the risk of PAD increases as GFR values decrease *(Chen et al., 2012)*.

The present work aimed to study the neutrophil to lymphocyte ratio (NLR) as a predictor of peripheral arterial disease in patients maintained on regular hemodialysis.

PATIENTS AND METHODS

This cross sectional study included 40 hemodialysis patients which were conducted at the Nephrology Unit, at Al Hussein University Hospital from January to July 2020. Written informed consent obtained from every patient for all procedures that performed. All procedures followed Al-Azhar University Ethical Committee Regulation. All patients were receiving 3 hemodialysis sessions weekly.

Inclusion Criteria:

1. Patient`s age between 18 and 60 years and then Sub grouped into two groups above and below 40 years.

2. Duration of hemodialysis more than 6 months.

3. Uses of native arteriovenous fistula in all patients.

Exclusion Criteria:

1. Patients less than 18 years or more 60 years

2. Duration of hemodialysis less than 6 months

3. Uncontrolled hypertension or diabetes mellitus.

4. Recent inflammation.

5. Known to be collagen disease.

Diagnosis of PAD based on ankle/ brachial index.

All Patients in this study had been subjected to the following:

1. History and clinical examination stressing on ischemic symptoms.

2. Laboratory Investigations (Serum Creatinine – Calcium – phosphorus – intact parathyroid hormone – serum iron – serum ferritin – transferrin saturation - lipid profile(cholesterol-triglycerides-LDL-HDL).

3. Ankle/ brachial index were measured by Doppler ultrasound.

4. Duplex ultrasound for arterial system of both lower limb for patient with ankle-brachial index>0.9.

Statistical analysis of data was done by using Statistical Package for the Social Sciences (SPSS) version 24. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance or Mann Whitney U test was used when comparing between two means. Chi-square test was used when comparing between non-parametric data. Probability (P-value): P-value < 0.05 was considered significant.

RESULTS

The mean age of all studied patients was 45.3 ± 8.8 years with a minimum age of 24 years and a maximum age of 62 years. There were 43 males (53.8%) and 37 females (46.3%), 35 patients (43.7%) were diabetic and 48 patients (60%) were hypertensive (Table 1).

Table (1): Demographic data of studied patients

Variables		Age criteria of the studied patients (N = 40)	
Age (years)	Mean ±SD	43.2 ± 10.3	
	Min – Max	24 – 59	
Sex	Male	29	72.5%
	Female	11	27.5%
DM	No	33	82.5%
	Yes	7	17.5%
HTN	No	13	32.5%
	Yes	27	67.5%

According to laboratory profile, the mean of WBCs, neutrophils, lymphocytes, NLR, Hb and platelets were 6.5 ± 1.7 (x10³/ul), 4.3 ± 1.5 (x10³/ul), 1.74 ± 0.53 (x10³/ul), 2.73 ± 1.3, 10.8 ± 2.3 (g/dl) and 234.7 ± 95.9 (x10³/ul) respectively. Regarding lipid profile, the mean of total cholesterol, HDL and triglycerides were 214.7 mg/dL, 41.9 mg/dL, 172.2 mg/dL, respectively. Regarding corrected serum Ca, serum PO4, serum Ca X PO4 product, and PTH the mean were 3.99 ± 0.5 (g/dl), 8.3 ± 0.6 (mg/dl), 5.22 ± 1.1 mg/dl, 43.7 ± 11.2 and 722.1 ± 477.7 (ug/ml) respectively (Table 2).

Table (2):   laboratory profiles in studied patients

Variables		Studied patients (N = 40)	Normal level
white blood cells (x10³/ul)	Mean ±SD	6.5 ± 1.7	
	Min – Max	2.9 – 10.8	4-11
Neutrophil (x10³/ul)	Mean ±SD	4.3 ± 1.5	
	Min – Max	1.3 – 8.6	3-7
Lymphocytes (x10³/ul)	Mean ±SD	1.74 ± 0.53	1.5-3.5
	Min – Max	0.7 – 3	
NLR	Mean ±SD	2.73 ± 1.3	
	Min – Max	0.72 – 7	.78-3.53
hemoglobin (g/dl)	Mean ±SD	10.8 ± 2.3	Target is 11-12
	Min – Max	6 – 15.9	
Platelets (x10³/ul)	Mean ±SD	234.7 ± 95.9	150-400
	Min – Max	94 – 644	
T. cholesterol (mg/dl)	Mean ±SD	197.4 ± 26.7	
	Min – Max	154 – 260	<200
Triglycerides (mg/dl)	Mean ±SD	153.1 ± 24.01	<150
	Min – Max	123 – 254	
Low density lipoprotein (mg/dl)	Mean ±SD	144.4 ± 17.2	<100
	Min – Max	119 – 188	
High density lipoprotein (mg/dl)	Mean ±SD	41.8 ± 6.3	>40
	Min – Max	30 – 56	
Corrected calcium (mg/dl)	Mean ±SD	8.3 ± 0.6	
	Min – Max	7.4 – 9.6	8.5-10.5
Phosphorus (mg/ml)	Mean ±SD	5.22 ± 1.1	2.4-5.1
	Min – Max	3.5 – 7.2	
Ca X PO4	Mean ±SD	43.7 ± 11.2	
	Min – Max	26.6 – 64.6	<55
Parathyroid hormone (ug/ml)	Mean ±SD	722.1 ± 477.7	14-72
	Min – Max	168 – 2000	

Regarding to ABI, there were 13 patients (32.5%) $\leq$ 0.9, while there were 27 patients (67.5%) > 0.9 **(Table 3)**.

Table (3):   Ankle brachial index in all studied patients.

Ankle brachial index / Studied Patients (N=80)	Index $\leq$ 0.9	Index > 0.9
Number of Positive and Negative patients (%)	13 (32.5%)	27 (67.5%)
Mean ±SD	0.85 ± 0.06	1.13 ± 0.09
Min – Max	0.7 – 0.9	1 – 1.3

There were statistically significant (p-value < 0.05) relationships between ankle brachial index $\leq$ 0.9 and (age, diabetes), and no statistically significant relation (p-value > 0.05) between ankle brachial index and sex & HTN **(Table 4)**.

Table (4): **Relationship between results of Ankle brachial index and demographic data**

Ankle brachial index / Parameters		≤ 0.9 (n = 13)		> 0.9 (n = 27)		P-value
Age (years)	**Mean**	48.5		40.7		**0.023**
	±SD	7.4		10.6		
Sex	**Male**	12	92.3%	17	63%	0.052
	Female	1	7.7%	10	37%	
DM	**No**	8	61.5%	25	92.6%	**0.015**
	Yes	5	38.5%	2	7.4%	
HTN	**No**	2	15.4%	11	40.7%	0.109
	Yes	11	84.6%	16	59.3%	
	±SD	4.1		2.9		

There were high statistically significant (p-value < 0.001) relationships between ankle brachial index ≤ 0.9 and NLR, total cholesterol, LDL, PO4 and Ca X PO4 product, and statistically significant (p-value < 0.05) relationships between ankle brachial index ≤ 0.9 and Hb, TG, lower HDL and corrected Ca. No statistically significant (p-value > 0.05) relationships between ankle brachial index and WBCs& PLTs **(Table 5)**.

Table (5): **Relationship between Ankle brachial index and laboratory results**

Ankle brachial index / Parameters		≤ 0.9 (n = 13)	> 0.9 (n = 27)	P-value
White blood cells (x10³/ul)	**Mean**	7.3	6.2	0.056
	±SD	1.7	1.6	
NLR	**Mean**	4.0	2.1	**< 0.001**
	±SD	1.4	0.7	
Hemoglobin (g/dl)	**Mean**	11.8	10.3	**0.044**
	±SD	2.3	2.1	
Platelets (x10³/ul)	**Mean**	252.8	225.9	0.413
	±SD	128.6	76.9	
T. Cholesterol (mg/dl)	**Mean**	226.8	183.3	**< 0.001**
	±SD	21.1	14.9	
Triglycerides (mg/dl)	**Mean**	169.2	145.3	**0.002**
	±SD	31.8	14.4	
Low density lipoprotein (mg/dl)	**Mean**	160.2	136.8	**< 0.001**
	±SD	15.2	12.5	
High density lipoprotein (mg/dl)	**Mean**	38.1	43.5	**0.009**
	±SD	6.2	5.6	
Corrected calcium (mg/dl)	**Mean**	8.7	8.1	**0.001**
	±SD	0.5	0.5	
Phosphorus (mg/ml)	**Mean**	6.5	4.6	**< 0.001**
	±SD	0.6	0.7	
Ca X PO4	**Mean**	56.2	37.7	**< 0.001**
	±SD	6.8	7.2	
Parathyroid hormone (ug/ml)	**Mean**	1050.8	563.9	**0.002**
	±SD	541.5	356.2	

DISCUSSION

This study was carried out at Al-Hussein University Hospital, hemodialysis unit over a period of six months from January 2020 to July 2020, and conducted on 40 hemodialysis patients. There were 72.5% males and 27.5% females. Other comorbidities were in some patients, 17.5% were diabetic and 67.5% were hypertensive. Regarding pretreatment laboratory profile, results showed that the mean WBCs, and NLR was (6.5 ± 1.7 (x10³/ul)), (4.3 ± 1.5 (x10³/ul)), (1.74 ± 0.53 (x10³/ul)), and (2.73 ± 1.3) respectively. Regarding lipid profile, the mean cholesterol, TG, LDL, and HDL was (197.4 ± 26.7 (mg/dl)), (153.1 ± 24.01 mg/dl), (144.4 ± 17.2 (mg/dl)), and (41.8 ± 6.3 (mg/dl)) respectively. Regarding results of serum Ca, PO4, Ca X PO4 product and PTH the mean was (8.3 ± 0.6 (mg/dl)), (5.22 ± 1.1 mg/dl), (43.7 ± 11.2) and (722.1 ± 477.7 (ug/ml)). The results of current study showed a high statistically significant (p-value < 0.001) correlation between ankle brachial index ≤ 0.9 and NLR, total cholesterol, LDL, Ca X PO4 product, and PO4. Furthermore, statistically significant (p-value < 0.05) correlation between ankle brachial index ≤ 0.9 and TG, corrected Ca, and PTH. There was inversely significant (p-value < 0.05) correlation between ankle brachial index ≤ 0.9 and HDL. *Arroyo et al. (2017)* observed in his large multicenter cohort study that the mean age of the CKD population was 57.9 6 12.8 years, and 61.7% were male patients. Prevalence of hypertension, dyslipidemia and diabetes were 89.3, 64.9 and 25.7%, respectively. CKD patients had a higher prevalence of subclinical PAD than non-CKD controls (28.0% versus 12.3%). In support to these results, *Shah et al. (2017)* found that there is a significant relationship between NLR and PAD. Also, these results came in agreement with *Kuo et al. (2017)* who found that increased neutrophil counts reflected oxidative stress, and that lower lymphocyte counts reflected a deterioration of nutritional status. In support to current study, *Criqui and Aboynas (2015)* observed that the high cholesterol is a significant contributor to PADs. In most studies, total cholesterol is associated with prevalent PADs in multivariable analyses. Also, in support to current study *Criqui and Aboynas (2015)* observed that triglycerides seem to be associated with PADs. In agreement results of current study *Garimella et al. (2014)* observed that hyperphosphatemia is associated with PAD in patients with end-stage renal disease.

CONCLUSION

The neutrophil-lymphocyte ratio (NLR) was a highly sensitive predictor of peripheral arterial diseases (PADs) among regular hemodialysis patients.

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العلاقة النسبية بين الخلايا المتعادلة البيضاء إلى الخلايا الليمفاوية كمؤشر لأمراض الشرايين الطرفية فى مرضى الاستصفاء الدموى المتكرر

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خلفيـــة البحـــث: مــرض الشــرايين الطرفيــه مــرض شــائع فــي مرضــى الكلــي المعاشــين علــي الاستصــفاء الـدموي، وتعتبــر العلاقــة النســبية بــين الخلايــا المتعادلــة البيضــاء إلــى الخلايــا الليمفاويــة مؤشــر علــى شــدة ومــدى التهــاب وتصــلب الشــرايين فــي المرضــى الذين يعانون من قصور الكلى والقلب والأوعية الدموية.

الهـدف مـن البحـث: تقيـيم العلاقـة النسـبية بـين الخلايـا المتعادلـة البيضـاء إلـى الخلايـا الليمفاويــة كمؤشــر لأمــراض الشــرايين الطرفيــة فــى مرضــى الاستصــفاء الــدموى المتكرر.

المرضـى وطـرق البحـث: أجريـت هـذه الدراسـة المقطعيـة فـي وحـدة غسـيل الكلـى بمستشـفى الحسـين الجـامعي علـى مـدى سـتة أشـهر مـن ينـاير إلـى يوليـو 2020 علـى 40 مريضـا مــن مرضــى الغسـيل الكلـوى. وقـد تــم تسـجيل البيانـات الديموغرافيـة والمعلومــات السـريرية والبيانـات المختبريـة بمـا فـي ذلـك صـورة الـدم الكاملـة والكالسـيوم والفوسـفات والألبـومين والكوليسـترول والـدهون الثلاثيـة والبـروتين الـدهني عـالي الكثافـة والبـروتين الـدهني مـنخفض الكثافـة وملـف الحديـد كمـا تـم قيـاس مؤشــر الكاحـل والعضـد فـي جميـع المرضـى و تشـخيص اعـتلال الشـرايين المحيطيـة إذا كـان مؤشـر الكاحـل إلـى العضـد أقـل مـن أو يسـاوى 0.9. كمـا تـم إجـراء الموجـات فـوق الصـوتية المزدوجـة للجهـاز الشـرياني لكـلا الطـرفين السـفليين للمرضـى إذا كـان مؤشر الكاحل إلى العضد أقل من أو يساوى 0.9.

نتـائج البحـث: كـان هنـاك 29 ذكـورا٬ (72.5٪) و 11 إناثـا٬ (27.5٪). وكـان متوسـط العمـر لجميـع المرضـى الخاضـعين للدراسـة 43.2 ± 10.3 سـنة. كمـا كـان هنـاك 7 مـن مرضـى السـكري (17.5٪) و 27 مـن مرضـى إرتفـاع ضـغط الـدم

(67.5٪). وفيما يتعلق بصورة الـدم الكاملـة، كـان متوسـط كـرات الـدم البيضـاء، والخلايــا المتعادلــة، والخلايــا الليمفاويــة، والنسـبة بـين الخلايـا المتعادلـة الـى الخلايـا الليمفاويــــــه، والهيموجلـــــــوبين والصـــــفائح الدمويـــــــة (6.5 ± 1.7) ×10³ خلايــــا/ ميكرولتـــــر، (4.3 ± 1,) ×10³ خلايـــــا/ ميكرولتـــــر ، (1.75± 53) ×10³ خلايـــا/ ميكرولتـــــــر، (2.73 ± 1.3) ،(10.8± 2.3) (جـــــم / ديســـــيلتر) و (234.7 ± 95.9) ×10³ خلايـــا/ ميكرولتـــر علــى التــوالي. وفيمــا يتعلــق بملـف الـدهون، كـان متوسـط الكوليسـترول الكلـي و البـروتين الـدهنى عـالى الكثافـة و الـدهون الثلاثيـة 214,7 مجـم / ديســيلتر، 41.9 مجــم / ديســيلتر، 172.2 مجــم / ديسـيلتر، علــى التــوالي. أمــا فيمــا يتعلـــق بــألبومين المصــل، ومصــحح الكالسـيوم، ومصـل الفسـفور، ونـاتج ضـرب الكالسـيوم فـى الفسـفور و هرمـون الغـدة الجـار درقيـة فقـد كـان المتوسـط 3.99 ± 0.5 (جـــم / ديســـيلتر)، 8.3 ± 0.6 (مجـــم / ديســـيلتر)، 5.22 ± 1.1 مجــم / ديســيلتر، 43.7 ± 11.2 و 722.1 ± 477.7 (ميكروغـــرام / مـــل) علـــى التــوالي. أمـــا عـــن مؤشـــر الكاحـل إلـى العضـد، فقـد كـان هنـاك 13 مريضًـا (32.5٪) ≤0.9 بينمـا كـان هناك 27 مريضًا (67.5٪) 0.9>.

الاسـتنتاج: العلاقـة النسـبية بـين الخلايـا المتعادلـة البيضـاء إلـى الخلايـا الليمفاويـة مؤشر لأمراض الشرايين الطرفية فى مرضى الاستصفاء الدموى المتكرر.

الكلمـات الدالـة: مــرض الشـرايين الطرفيـة فـي المرضـى المعاشـين علـى الاستصـفاء الـدموي المتكـرر، مؤشـر الكاحـل إلـى العضـد، العلاقـة النسـبية بـين الخلايـا المتعادلـة البيضاء إلى الخلايا الليمفاوية.