Al-Azhar Med. J. *Vol. 49(2), April, 2020, 491-502*
DOI : 10.12816/amj.2020.82567
https://amj.journals.ekb.eg/article_82567.html

ASSOCIATION BETWEEN NEUTROPHIL TO LYMPHOCYTE RATIO AND INFLAMMATORY MARKERS IN HEMODIALYSIS PATIENTS

By

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ABSTRACT

Back ground: Hemodialysis patients have an inflammatory state which is attributed to their morbidity and mortality compared to the general population.

Objective: To study the association between neutrophil to lymphocyte ratio and other inflammatory markers: (Interleukin-6 (IL-6), high sensitive C reactive protein (hs-CRP), and other inflammatory markers) in hemodialysis patients.

Patients and methods: Forty two hemodialysis patients. Participated in this study. They divided into two groups. Group I: Patients whose hs-CRP > 10 mg. Group II: Patients whose hs-CRP < 10 mg. The association between neutrophil to lymphocyte ration and the other inflammatory markers war studied.

Results: 69% of the patients (29 patient) whose hs-CRP were more than 10 mg and 31% of the patients (13 patients) whose hs-CRP were less than 10 mg. Additionally, there has been a positive correlation between neutrophil to lymphocyte ratio and interleukin-6 along with platelet to lymphocyte ratio. Moreover, interleukin 6 that indicated inflammation in hemodialysis, was more than 30 ng. Further, the value of neutrophil to lymphocyte ratio which represented the inflammation in hemodialysis patients, was equal or more than 1.6.

Conclusion: Cardiovascular condition of hemodialysis patients affects their morbidity and mortality. Consequently, it could be assessed by neutrophil to lymphocyte ratio which has high specificity and sensitivity.

Keywords: CRP, WBCs, ESR, Ulcerative colitis.

INTRODUCTION

Both cardiovascular diseases and infection are linked to inflammation and ESKD has recently been considered a state of chronic inflammation, which is the cornerstone of pathogenesis of atherosclerosis, is increased in ESRD patients compared to normal population. It is thought that early detection of inflammation might improve the quality of the life of those patients and decrease rate of morbidity and mortality *(Dai et al., 2017)*.

Patients on RHD have increased level of inflammatory mediators including C-reactive protein, tumour necrosis factor and IL6, as it plays major role in malnutrition, inflammation and

atherosclerosis as well as overall mortality rate in these patients *(Ahbap et al., 2016)*. Leukocyte is considered among the classic inflammatory markers due to their role in pathogenesis of atherosclerosis and its complications by mediating several biochemichal pathways *(Ahbap et al., 2016)*.

Several studies have revealed that elevated neutrophil count was strongly associated with malnutrition and inflammation and that decreased lymphocyte count had a weaker association. Increased neutrophils and decreased lymphocyte count was also independent predictor of mortality in hemodialysis patients *(Yaprak et al., 2016)*. Neutrophil –to- lymphocyte ratio is considered a novel cheap and available indicator, which reflect the extent of inflammation and atherosclerosis and predicts the clinical outcome and estimate survival in cardiac and non-cardiac including ESRD *(Gürağaç and Demirer, 2016)*. The neutrophil to lymphocyte ratio is obtained by dividing the absolute neutrophil count by the absolute lymphocyte count. It is a marker of poor prognosis in several disorders like malignancies, chronic kidney disease and myocardial function *(Yaprak et al., 2016)*. Based on that, the present study was designed to evaluate the NLR compared with hs-CRP along with IL6 in ESRD patients on regular hemodialysis.

This study aimed to study the association between neutrophil-to-lymphocytic ratio and other inflammatory markers (IL-6, hs-CRP, platelet to lymphocyte ratio (PLR), etc.) in hemodialysis patients.

PATIENTS AND METHODS

This study was designed to assess inflammation in hemodialysis patients and study the association between hs-CRP and the other inflammatory markers in those patients. Initially, forty two patients with end stage renal disease (ESRD), on regular hemodialysis in dialysis unit, Internal Medicine Department, AL-Hussien Hospital, Faculty of Medicine, Al-Azhar University-Egypt, were recruited to participate in our study.

Ethical approval and written informed consent:

An approval of the study was obtained from Al- Azhar University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the operation.

Inclusion criteria:

Patients undergoing HD for more than three months who will agree to be included in this study.

Exclusion criteria:

We are going to exclude patients who have:

- Inflammatory state due to infection,
- Autoimmune diseases,
- Older than 75 year-old,
- Current malignancy or history of malignancy,
- Immunosuppressive therapy.

Study design:

We divided our patients into two groups above and below hs-CRP10 mg/dl, then we studied the correlation between Hs-CRP, NLR, PLR, IL6 and other

predictors of inflammation in the group of CRP >10 mg/dl. After that we detected the cut off values for them.

All subjects in this study were subjected to the following:

1. Full history including age, weight, height, BMI, history of high blood pressure, diabetes, peripheral vascular disease along with cerebrovascular disease.

2. Laboratory investigations:

Venous blood samples will be drawn from all subjects after an overnight fasting period. Sampling was particularly performed in a morning of midweek dialysis session prior to heparinization in HD patients. Then, we measured:

- Serum albumin, ferritin, mean platelet volume (MPV), high sensitive CRP and interleukin-6.
- Calcium.
- Phosphorus.

The white blood cell differentiation will be detected as part of CBC, and then we calculate neutrophil to lymphocyte ratio and platelet to lymphocyte ratio.

Statistical analysis:

Data were analyzed using Statistical Package for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. The following tests were done:

Pearson's correlation coefficient (r) test was used for correlating data.

- P-value <0.05 was considered significant.

RESULTS

The mean age of studied patients was 50.36 ± 10.66 years old with minimum age of 24 years and maximum age of 70 years. There were 28 males (66.6%) and 14 females (33.33%) in studied patients. The mean weight of studied patients was 74.05 ± 13.66 kg with minimum weight of 53 kg and maximum weight of 120 kg. The mean height of studied patients was 1.67 ± 0.09 m with minimum height of 1.5 m and maximum height of 1.84 m. The mean BMI of studied patients was 26.92 ± 5.35 kg/m2 with minimum BMI of 19.11 kg/m2 and maximum BMI of 46.89 kg/m2. The mean dialysis duration of studied patients was 5.81 ± 4.89 years with minimum duration of 0.5 year and maximum duration of 17 years. There were 34 non-diabetic (80.95%) and 8 diabetic (19.05%) in studied patients. There were 10 non-hypertensive (23.81%) and 32 hypertensive (76.19%) in studied patients. There were 39 patients with no cerebrovascular diseases (92.86%) and 3 patients with cerebrovascular diseases (7.14%) in studied patients. There were 22 patients with no cardiovascular diseases (52.38%) and 20 patients with cardiovascular diseases (47.62%) in studied patients. **(Table 1)**.

Table (1): Description of demographic data of studied patients

Variables		Studied patients (N = 42)	
Age (years)	**Mean ±SD**	50.36 ± 10.66	
	Min – Max	24 – 70	
Sex (n, %)	**Male**	28	66.67%
	Female	14	33.33%
Weight (kg)	**Mean ±SD**	74.05 ± 13.66	
	Min – Max	53 – 120	
Height (m)	**Mean ±SD**	1.67 ± 0.09	
	Min – Max	1.5 – 1.84	
BMI (kg/m^2)	**Mean ±SD**	26.92 ± 5.35	
	Min – Max	19.11 – 46.89	
Dialysis duration (years)	**Mean ±SD**	5.81 ± 4.89	
	Min – Max	0.5 – 17	
DM (n, %)	**No**	34	80.95%
	Yes	8	19.05%
HTN (n, %)	**No**	10	23.81%
	Yes	32	76.19%
Cerebrovascular Ds (n, %)	**No**	39	92.86%
	Yes	3	7.14%
Cardiovascular Ds (n, %)	**No**	22	52.38%
	Yes	20	47.62%

Classification of studied patients according to Hs-CRP. Shuwed thet there were 13 patients (31%) < 10 mg/dl CRP with mean of 2.6 ± 1.5 while there were 29 patients (69%) > 10 mg/dl with mean of 81.4 ± 26.1. **(Table 2)**.

Table (2): Classification of studied patients according to Hs-CRP

Variables		Studied patients (n = 42)	
		CRP < 10 mg/dl	**CRP > 10 mg/dl**
Hs-CRP	N, %	13 (31%)	29 (69%)
	Mean ± SD	2.6 ± 1.5	81.4 ± 26.1

Statistical significant (p-value < 0.001) positive correlation was found between hs-CRP vs NLR & NLR vs PLR in patients with CRP > 10 mg/dl group. Also, a statistically significant (p-value < 0.05) positive correlation between hs-CRP vs PLR, hs-CRP vs IL-6, NLR vs IL-6 & PLR vs IL-6 in patients with CRP > 10 mg/dl group **(Table 3)**.

Table (3): Correlation study between Hs-CRP, NLR, PLR & IL-6 in patients with CRP > 10mg/dl group

	Hs-CRP		NLR		PLR		IL-6	
	r	P-value	R	P-value	R	P-value	R	P-value
Hs-CRP	---	---	0.65	< 0.001	0.54	0.002	0.45	0.013
NLR	0.65	< 0.001	---	---	0.89	< 0.001	0.53	0.003
PLR	0.54	0.002	0.89	< 0.001	---	---	0.54	0.002
IL-6	0.45	0.013	0.53	0.003	0.54	0.002	---	---

(r): Pearson correlation coefficient.

Statistical significant (p-value < 0.001) positive correlation occurred between hs-CRP vs NLR in patients with CRP > 10 mg/dl group. There was a statistically significant (p-value < 0.05) positive correlation between hs-CRP vs PLR, hs-CRP vs IL-6 and hs-CRP vs Ferritin as well as statistically significant (p-value < 0.05) negative correlation between hs-CRP vs ALB and hs-CRP vs Hb in patients with CRP > 10 mg/dl group. No statistical significant (p-value > 0.05) correlation between hs-CRP and other studied parameters in patients with CRP > 10 mg/dl group **(Table 4)**.

Table (4): Correlation study between Hs-CRP and other studied parameters in patients with CRP > 10mg/dl group

Variables	Pearson Corr.	P-value	Variables	Pearson Corr.	P-value
Hs-CRP vs T. Ca	0.08	0.67	**Hs-CRP vs Hb**	- 0.55	0.009
Hs-CRP vs Ionized Ca	- 0.22	0.24	**Hs-CRP vs NLR**	**0.65**	**< 0.001**
Hs-CRP vs Ca x Ph	- 0.3	0.1	**Hs-CRP vs PLR**	**0.54**	**0.002**
Hs-CRP vs Ph	- 0.35	0.056	**Hs-CRP vs MPV**	- 0.25	0.17
Hs-CRP vs T. Sat.	0.2	0.27	**Hs-CRP vs IL-6**	**0.45**	**0.013**
Hs-CRP vs TIBC	– 0.39	0.6	**Hs-CRP vs PTH**	- 0.04	0.83
Hs-CRP vs iron	0.057	0.77	**Hs-CRP vs ALB**	- 0.65	0.007
Hs-CRP vs ferritin	**0.76**	**0.008**			

(r): Pearson correlation coefficient.

Statistical significant (p-value < 0.001) positive correlation occurred between NLR vs PLR in patients with CRP > 10 mg/dl group. There was a statistically significant (p-value < 0.05) positive correlation between NLR vs IL-6 and NLR vs ferritin as well as statistically significant (p-value < 0.05) negative correlation between NLR vs MPV, NLR vs Hb and NLR vs ALB in patients with CRP > 10 mg/dl group. No statistical significant (p-value > 0.05) correlation between NLR and other studied parameters in patients with CRP > 10 mg/dl group **(Table 5)**.

Table (5): Correlation study between NLR and other studied parameters in patients with CRP > 10mg/dl group

Variables	Pearson Corr.		Variables	Pearson Corr.	
	r	P-value		r	P-value
NLR vs T. Ca	-0.09	0.63	**NLR vs ferritin**	**0.55**	**0.01**
NLR vs Ionized Ca	-0.035	0.059	**NLR vs Hb**	**-0.65**	**0.009**
NLR vs Ca x Ph	-0.15	0.41	**NLR vs PLR**	0.89	**< 0.001**
NLR vs Ph	-0.15	0.42	**NLR vs MPV**	-0.49	**0.007**
NLR vs T. Sat.	0.1	0.58	**NLR vs IL-6**	0.53	**0.003**
NLR vs TIBC	-0.27	0.15	**NLR vs PTH**	0.14	0.44
NLR vs iron	-0.02	0.9	**NLR vs ALB**	**-0.77**	**0.003**

(r): Pearson correlation coefficient.

Statistical significant (p-value < 0.001) positive correlation was found between PLR vs NLR in patients with CRP > 10 mg/dl group. Also, there was statistically significant (p-value < 0.05) positive correlation between PLR vs IL-6 and PLR vs ferritin as well as statistically significant (p-value < 0.05) negative correlation between PLR vs Hb and PLR vs ALB in patients with CRP > 10 mg/dl group. No statistical significant (p-value > 0.05) correlation between PLR and other studied parameters in patients with CRP > 10 mg/dl group **(Table 6)**.

Table (6): Correlation study between PLR and other studied parameters in patients with CRP > 10mg/dl group

Variables	Pearson Corr.	P-value	Variables	Pearson Corr.	P-value
PLR vs T. Ca	- 0.13	0.47	**PLR vs ferritin**	**0.89**	**0.007**
PLR vs Ionized Ca	- 0.36	0.005	**PLR vs Hb**	**- 0.67**	**0.008**
PLR vs Ca x Ph	-0.27	0.14	**PLR vs NLR**	0.89	**< 0.001**
PLR vs Ph	- 0.22	0.23	**PLR vs MPV**	- 0.49	**0.7**
PLR vs T. Sat.	0.088	0.64	**PLR vs IL-6**	0.54	**0.002**
PLR vs TIBC	- 0.25	0.18	**PLR vs PTH**	0.26	0.16
PLR vs iron	0.05	0.77	**PLR vs ALB**	**- 0.72**	**0.001**

(r): Pearson correlation coefficient.

Statistically significant (p-value < 0.05) positive correlation occurred between NLR vs IL-6 and (IL-6 vs ferritin as well as statistically significant (p-value < 0.05) negative correlation between PLR vs IL-6, IL-6 vs Hb and IL-6 vs ALB in patients with CRP > 10 mg/dl group. No statistical significant (p-value > 0.05) correlation between IL-6 and other studied parameters in patients with CRP > 10 mg/dl group **(Table 7)**.

Table (7): Correlation study between IL-6 and other studied parameters in patients with CRP > 10mg/dl group

Variables	Pearson Corr.		Variables	Pearson Corr.	
	r	p-value		r	P-value
IL-6vs T. Ca	- 0.04	0.8	**IL-6 vs ferritin**	**0.88**	**0.007**
IL-6 vs Ionized Ca	- 0.32	0.08	**IL-6 vs Hb**	**- 0.24**	**0.03**
IL-6 vs Ca x Ph	- 0.008	0.9	**IL-6 vs NLR**	**0.53**	**0.003**
IL-6 vs Ph	- 0.003	0.9	**IL-6 vs MPV**	- 0.31	0.1
IL-6 vs T. Sat.	0.06	0.73	**IL-6 vs PLR**	**0.54**	**0.002**
IL-6 vs TIBC	- 0.35	0.06	**IL-6 vs PTH**	0.07	0.71
IL-6 vs iron	- 0.04	0.8	**IL-6 vs ALB**	**- 0.34**	**0.01**

(r): Pearson correlation coefficient.

Using roc curve, it was shown that NLR can be used to predict cases with inflammation at a cutoff level >1.6, with 93.1% sensitivity, 92.3% specificity, 92.4 % PPV and 93.4 % NPV. Also it was shown that IL-6 can be used to predict cases with inflammation at a cutoff level > 30, with 96.6% sensitivity, 69.2% specificity, 75.8 % PPV and 95.3 % NPV. Additionally it was found that PLR can be used to predict cases with inflammation at a cutoff level >180.2, with 82.8% sensitivity, 92.3% specificity, 91.5% PPV and 84.3 % NPV **(Table 8)**.

Table (8): Diagnostic performance of NLR, Il-6 and PLR to predict cases with inflammation

	Cut off	Area under the curve	Sensitivity	Specificity	PPV	NPV	p-value
NLR	> 1.6	0.92	93.1 %	92.3 %	92.4 %	93.04%	< 0.001
IL-6	> 30	0.77	96.6 %	69.2 %	75.8 %	95.3%	0.004
PLR	>180.2	0.87	82.8 %	92.3 %	91.5 %	84.3%	0.0001

PPV: positive predictive value. NPV: negative predictive value.

DISCUSSION

This was a cross-sectional study designed to study the prevalence of subclinical inflammation in hemodialysis (HD) patients. Additionally, we compared the relation between NLR, IL-6, PLR and in hs-CRP those patients.

Sixty three (69%) patients were found to have a high hs-CRP (>10 mg/L) indicating presence of inflammation, despite absence of any overt signs and symptoms of inflammation. This went in agreement with *Taheri et al. (2017)* who reported that the prevalence of inflammation in HD patients varied between 35% and 65%. Other results went in favor with the chronic sub-inflammatory state present in end stage renal disease (ESRD), where *Dai et al. (2017)* concluded that dialysis-related factors such as use of catheters for vascular access, poor dialyzer membrane biocompatibility, dialysate contamination, exposure to endotoxins, and back-leak of dialysate across the dialysis membrane in hemodialysis (HD) may promote a persistent, low-grade inflammatory response. Besides, other comorbidities,

kidney disease per se, life style factors, genetic predisposition and, in particular, the state of uremia is of major importance as a promoter of a persistent, low-grade inflammatory response in ESRD patients.

Our results showed a negative significant correlation between hs-CRP and albumin along with hemoglobin. These results were consistent with the study of *Ozcicek et al. (2017)* for hemoglobin and albumin. On the other side, our study revealed no statistical significance regarding Calcium, phosphorus, Transferin saturation (TSAT) as well as iron, mean platelet volume (MPV) and parathyroid hormone (PTH).

Further results showed positive correlation with significance between hs-CRP and PLR. These results were consistent with the study of *Ahbap et al. (2016)* where Both NLR and PLR were positively correlated with hs-CRP.

Furthermore, we found a statistically significant positive correlation between NLR and hs-CRP. These results were consistent with the studies of *Pineault et al. (2017)* who showed a positive correlation between hs-CRP and NLR, *Ahbap et al. (2016)* and *Neuen et al. (2016)* found same correlation.

In addition, our results revealed a positive significant correlation between hs-CRP and IL- 6. This study was consistent with *Shelbaya et al. (2012)* who found a positive significant correlation between hs-CRP and IL- 6.

In terms of independent predictors of inflammation in our study, it showed statistically significant in views to ferritin, HB, IL-6 and albumin, whereas, it revealed highly significance regarding NLR and PLR. By using univariate and multiple variate analysis to test for independent predictors of hs -CRP levels as an indicator for inflammation, it was found that NLR can be used as an independent predictor of hs-CRP as an inflammatory marker, with a statistically significant correlation.

In our study, we detected the cut off point for IL-6 that indicated the inflammation in hemodialysis patients. It was 30ng/L with sensitivity of 96.6 and specificity of 69.2%. Furthermore, cut off point regarding PLR in hemodialysis patients which indicated the inflammation, was calculated. It was 180.2 with sensitivity of 82.2% and specificity of 92.3%. In addition to calculating the cut off point for NLR indicator for the presence of inflammation, we found that it was 1.6 with sensitivity of 93.1% and specificity of 92.3%. In contrast to our results, *Ahbap et al. (2016)* found a cutoff point of NLR, which were 2.82 with sensitivity of 65.7% and specificity of 63.3%.

Our reference range regarding hs-CRP that indicated inflammation was ≥ 10 mg/L. While, the American Heart Association (CDC/AHA) suggested use of hs-CRP cut points of low risk (< 1.0 mg/L), average risk (1.0 – 3.0 mg/L) and high risk (> 3.0 mg/L). In this regard, *Ahbap et al. (2016)* data were compared in patients with hs-CRP levels of ≤ 3 mg/L vs. > 3 mg/L in the study.

Other studies determined NLR as a marker and predictor of CVD mortality. *Solak et al. (2013)* and *Abe et al. (2015)* studies reported NLR > 3.76 and 3.72 to be significant and independent of CRP predictors of cardiovascular events in

dialysis-dependent patients, respectively. While *An et al. (2012)*, reported that NLR > 3.5 was associated with an increase in the risk of cardiovascular and all- cause mortality in peritoneal dialysis patients. *Neuen et al. (2016)* reported that NLR > 3.3 was associated with increased cardiovascular mortality in hemodialysis patients.

On further classification of the study group according to previously calculated cut off point for NLR, it was found that 69% of patients were considered positive for inflammation and 31% without inflammation.

CONCLUSION

Most of end stage renal disease patients on regular hemodialysis had established inflammation, which is a major risk for CVDS. Cut off values of IL6, NLR and PLR in our patients had high sensitivity and specificity compared to hs-CRP.

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العلاقة بين نسبة خلايا النيتروفيل (خلايا الدم المتعادلة) إلى الخلايا الليمفاوية ودلالات الإلتهاب فى مرضى الغسيل الكلوى

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خلفية البحث: لدى مرضى الغسيل الكلوى حالة من الالتهاب والتى تؤثر بشكل رئيسى فى التأثير على حياتهم وأعمارهم.

الهدف من البحث: تقييم العلاقة بين نسبة خلايا النيتروفيل ودلالات الالتهاب الأخرى (انترلوكين ٦ والبروتين المتفاعل عالى الحساسية وغيرهم) فى مرضى الغسيل الكلوى.

المرضى وطرق البحث: شارك فى البحث ٤٢ مريض غسيل كلوى وتم تقسيمهم إلى مجموعتين:

المجموعة الأولى: المرضى الذين لديهم البروتين المتفاعل عالى الحساسية أكثر من ١٠ مجم.

المجموعة الثانية: المرضى الذين لديهم البروتين المتفاعل عالى الحساسية أقل من ١٠ مجم.

وقد تم دراسة العلاقة بين نسبة خلايا النيتوفيل إلى الخلايا الليمفاوية مع دلالات الإلتهاب الأخرى.

نتائج البحث: أظهرت نتائج هذه الدراسة أن ٦٩% (٢٩ مريض) معدل البروتين المتفاعل عالى الحساسية أكثر من ١٠ مجم و٣١% (١٣ مريض) أقل من ١٠ مجم، والعلاقة الطردية بين نسبة خلايا النيتروفيل

إلى الخلايا الليمفاوية مع انترولوكين ٦ الدالة على الإلتهاب وكانت > أكثر من ٣٠ نانوجرام وأيضاً نسبة خلايا النيتروفيل إلى الخلايا الليمفاوية الدالة على الإلتهاب في مرضى الغسيل الكلوى أكثر من أو تساوى ١,٦.

الاستنتاج: الحالة القلبية لمرضى الغسيل الكلوى لها دور رئيسى على حياتهم وأعمارهم ويمكن متابعتها بنسبة خلايا النيتروفيل إلى الخلايا الليمفاوية والتى تبين أنها عالية الحساسية والخصوصية.