Caspian Journal of Internal Medicine 2026 (Winter); 17(1): 191-199
DOI: 10.22088/cjim.17.1.191

Original Article

Association of interleukin-8 and neutrophil-to-lymphocyte ratio with clinical indices in diabetic nephropathy

Mahboobeh Freidoon (MD) [1]
Hadi Kazemzadehghadim (MD) [2]
Sara Assadiasl (MD, PhD) [3, 4*]
Naghmeh Sayadi (MD) [2]
Narjes Soleimanifar (PhD) [3]
Maryam Sadr (MSc) [3]
Hanieh Mojtahedi (MSc) [3]
Mohammad Hossein Nicknam (MD, PhD) [3, 5]

1. Department of Nephrology, Shohada-ye-Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Internal Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Iranian Tissue Bank and Research Center, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

*** Correspondence:**
Sara Assadiasl, Molecular Immunology Research Center, No.142, Nosrat Street, Tehran, Iran

E-mail: assadiasl@sina.tums.ac.ir
Tel: +98 2166944508

Received: 1 Dec 2024
Revised: 13 July 2025
Accepted: 19 July 2025
Published: 21 Jan 2026

Abstract

Background: Diabetic nephropathy (DN) is the most prevalent reason for chronic kidney diseases that end up with renal failure if it is not diagnosed and treated at early stages. Therefore, low-cost, non-invasive, and easy-to-use tests are necessary for efficient follow-up and early diagnosis of DN in diabetic patients. Interleukin-8 (IL-8) as the main recruiter of neutrophils may play a role in inflammatory responses that end up with renal damage and dysfunction in DN.

Methods: In a case-control study, serum levels of IL-8 and neutrophil-to-lymphocyte ratios (NLR) were evaluated in correlation with clinical findings in 60 patients with DN, 60 type 2 diabetic patients without renal involvement, and 60 age and sex-matched healthy subjects.

Results: IL-8 levels were significantly higher in diabetic nephropathy patients than in diabetic patients without nephropathy and healthy controls (both p-value<0.0001). However, the frequency of neutrophils in both groups of patients was higher than that of healthy individuals. Despite increased NLRs in diabetic patients, the difference was only significant between DN and healthy groups (P-value: 0.03). A direct correlation was observed between IL-8 levels and NLR (P-value: 0.02), and a negative correlation between IL-8 and eGFR (P-value: 0.01). In addition, UACR was associated with IL-8 concentrations (P-value: 0.04) in DN patients. IL-8 level showed a higher diagnostic value for diabetic nephropathy [AUC: 0.87 (p<0.0001)] than NLR and neutrophil count.

Conclusion: Increased level of IL-8 in DN is associated with elevated NLR and UACR, and reduced eGFR. Therefore, IL-8 levels might be considered a diagnostic or prognostic biomarker for DN.

Keywords: Diabetes, Diabetic nephropathy, Interleukin-8, Neutrophil, Neutrophil to lymphocyte ratio.

Citation:
Freidoon M, Kazemzadehghadim H, Assadiasl S, et al. Association of interleukin-8 and neutrophil-to-lymphocyte ratio with clinical indices in diabetic nephropathy. Caspian J Intern Med 2026; 17(1): 191-199.

Diabetic nephropathy (DN) is a common complication of uncontrolled hyperglycemia and the most common cause of chronic kidney disease (CKD), and end-stage renal disease (ESRD) (1). Besides metabolic, hemodynamic, and environmental factors, recent developments in defining the pathogenesis of diabetic nephropathy have suggested a critical role for inflammatory cells in tubulointerstitial damages. Accordingly, some immune cells, cytokines, chemokines, and cytokine receptors have been considered as potential diagnostic or prognostic biomarkers or therapeutic targets to inhibit disease progression (2). Neutrophils as a major subset of innate immunity have been shown to be involved in pathogenesis of many chronic inflammatory disorders (3). Neutrophils recruitment and activation have been demonstrated in vascular injuries observed in DN patients (4). Therefore, neutrophil count and neutrophil-to-lymphocyte ratio (NLR) were proposed as prognostic markers in diabetic patients with higher risk of vascular complications and comorbidities such as hypertension (5).

 Publisher: Babol University of Medical Sciences

Interleukin-8 (IL-8) or chemokine (C-X-C motif) ligand 8 (CXCL8) is a chemokine produced by various immune and non-immune cells e.g. macrophages, endothelial cells, and epithelial cells in response to danger- or pathogen-associated molecules, leading to neutrophil recruitment (6). Previous studies have reported an enhanced activation of the IL-8-CXCR1/2 axis in diabetic kidney disease. Moreover, an in vitro study showed that podocytes express IL-8 in hyperglycemic condition (7). IL-8 is supposed to be implicated in dysregulated cell proliferation, permeability, angiogenesis, apoptosis, and fibrogenesis in renal tissue in diabetic nephropathy (8). Furthermore, urinary IL-8 levels were elevated in the advanced stages of diabetic nephropathy and significantly correlated with the urinary albuminuria-to-creatinine ratio (UACR), while negatively correlated with estimated glomerular filtration rate (eGFR) (9, 10). One study showed elevating serum levels of IL-8 in line with the deterioration of renal function in diabetic patients, suggesting serum IL-8 monitoring for early diagnosis of diabetic nephropathy (11).

Regarding the increased level of IL-8 in diabetic nephropathy, researchers have investigated IL-8 blockade for reducing disease progression in experimental models of diabetic kidney disease. For instance, Loretelli et al. inhibited IL-8/CXCR1/2 axis with reparixin in mice model of diabetes that after 18 weeks resulted in a significantly lower UACR in treated mice compared to the control group. Mesangial expansion was also reduced in the former group (7). Nonetheless, despite these results, there is not yet sufficient evidence for proposing IL-8 and NLR as prognostic, diagnostic, or therapeutic targets in diabetic nephropathy.

Therefore, we aimed to evaluate serum levels of IL-8 in patients diagnosed with diabetic nephropathy compared to diabetic patients without nephropathy and healthy individuals and to investigate its correlation with neutrophil indices and clinical findings. If a significant association of neutrophil count and ratio or neutrophil-related cytokine, IL-8, levels are established with renal function indices, regular monitoring of IL-8 levels and neutrophil frequency might be suggested for patient follow-up as a noninvasive and low-cost test. Moreover, IL-8 inhibition might be proposed as a potential option for treating patients diagnosed with DN.

Methods

Patients: Peripheral blood samples were taken from 60 type 2 diabetes patients diagnosed with nephropathy according to the clinical (albuminuria>30 and/or reduced eGFR) (12), and/or pathological criteria (interstitial fibrosis and tubular, arteriolar hyalinosis, arteriosclerosis, thickening of the glomerular basement membrane, mesangial expansion, nodular sclerosis, and glomerulosclerosis) (13).

Sixty diabetic patients without albuminuria and normal GFR were also recruited to the study, then 60 age- and sex-matched healthy individuals with A1C of lower than 6.5% and fasting blood glucose lower than 125 mg/dl were included as the control group. The patients were treated either with metformin or empagliflozin for hyperglycemia. Amlodipine, diltiazem, losartan, or valsartan were used to control hypertension, none of which were known to affect Jak genes expression. The participants with a medical history of other autoimmune disorders, allergies, malignancies, active infections, pregnancy, and eGFR<15 ml/min were excluded from the study. Patient selection and sampling took place at Shohada-ye-Tajrish Hospital in Tehran between April 2023 and April 2024. All subjects gave informed consent for inclusion before they participated in the study. The study was conducted under the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1402.342).

Paraclinical variations measurement: The patients' and controls' demographic and paraclinical data, including age, gender, smoking status, disease duration, systolic and diastolic pressure, weight, body mass index (BMI), disease duration, DN duration, blood urea nitrogen (BUN), serum and urine creatinine, calcium, serum and urine albumin, proteinuria (24 hours), fasting blood sugar (FBS), hemoglobulin, complete blood count (CBC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglyceride (TG), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were extracted from the clinical documentations and individual questionnaires.

Neutrophil to Lymphocyte Ratio (NLR), and urine albumin-creatinine ratio (UACR) were calculated with dividing numbers. Estimated glomerular filtration rate (eGFR) was calculated with the Cockcroft and Gault formula: CCr= {((140–age) x weight)/(72xSCr)}x 0.85 (if female) [CCr (creatinine clearance) = mL/minute, SCr (serum creatinine) = mg/Dl].

Interleukin-8 levels measurement: The ELISA tests were performed using a Human IL-8 Kit (MBS9135731, MyBioSource, Inc. USA) with sensitivity of 5.9pg/mL, according to the manufacturer's instructions. All samples were tested in duplicate form. Absorbance was read using the Hiperion MPR4 ++ Microplate Reader (Medizintechnik GmbH & Co.KG Germany). The calibration curve was drawn to determine the concentration of the cytokines.

Statistical analysis: The data were presented as mean±standard deviation (SD) or mean±standard error of means (SEM). The normality of the distribution was evaluated with the Kolmogorov-Smirnov test. The One-Way ANOVA test was used to compare quantitative variables with a normal distribution, and the Kruskal-Wallis test was applied for non-parametric variables.

The comparison between two groups was performed using post HOC test. The correlation between quantitative variables was assessed using Pearson's correlation test presented with R and Correlation Coefficient (R^2). Area under the curve (AUC), a quantitative measure of the model's discriminative ability, was used to evaluate the diagnostic value of variables. P-values lower than 0.05 were considered significant. Data analysis was performed using IBM SPSS 26.0 (IBM Corp., Armonk, NY, USA).

Results

1- Basic characteristics of the studied population: Sixty patients with diabetic nephropathy, 60 patients with type 2 diabetes without nephropathy, and 60 healthy controls were evaluated. In addition to the kidney function tests, the smoking rate, fasting blood sugar (FBS), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin, white blood cell (WBC), neutrophil count, Neutrophil-to-lymphocyte ratio (NLR), and estimated glomerular filtration rate (eGFR) were significantly different within groups. The clinical and paraclinical findings have been presented in table 1.

2- Increased levels of IL-8 in diabetic nephropathy: The serum levels of IL-8 in patients with diabetic nephropathy, diabetic patients, and healthy subjects were 14.7±6.3, 8.4±4.3, 5.7±3.3 pg/ml (mean±SD), respectively. IL-8 levels were significantly higher in nephropathy patients than in healthy controls (p-value<0.0001). Moreover, a significant difference was observed between diabetic patients with and without nephropathy (p-value<0.0001). However, there was no significant difference between healthy controls and diabetic patients (figure 1).

Table 1. Demographic and clinical data of the studied population

	Diabetic nephropathy	Diabetic patients	Healthy controls	P-value (between 3 groups)
Number	60	60	60	1
Age (year) (mean±SD)	56.7±8.03	58.8±4.4	53±8.3	0.1
Gender ratio (M:F)	28/32	28/32	28/32	1
Smoking (yes/no)	18/42	14/46	11/49	0.02*
Disease (month) (mean±SD)	120.2±10.8	96±8.3	-	0.08
DN (month) (mean±SD)	53±41	-	-	-
DN (stage)	Stage 1: 20 Stage 2: 16 Stage 3: 22 Stage 4: 2	-	-	-
Systolic pressure (mean±SD)	130±14	125±12.6	122±10.5	0.05
Diastolic pressure (mean±SD)	77±8	76.4±7	71±6.7	0.08
Weight (kg) (mean±SD)	75±10	72±15	70±15	0.4
BMI (mean±SD)	27.4±3.3	25.3±5.1	23.3±5.1	0.09
BUN (mg/dl) (mean±SD)	58.6±27.6	24±12.7	25.3±3.8	0.001*
Cr (mg/dl) (mean±SD)	1.6±0.7	1±0.2	0.9±0.1	0.001*
Calcium (mean±SD)	8.5±0.7	8.5±0.4	8.9±0.5	0.63
Serum ALB (g/dl) (mean±SD)	3.3±0.5	3.4±0.3	3.5±0.5	0.42
Urine ALB (g/l) (mean±SEM)	115±32	5±2	-	<0.0001*

	Diabetic nephropathy	Diabetic patients	Healthy controls	P-value (between 3 groups)
FBS (mean±SD)	134±26	113±21	105±24	0.04*
HBA1C (mean±SD)	7.3±1.9	6.2±1.7	-	0.3
CRP (mean±SEM)	30±6.5	23±6.3	1.5±1.1	0.002*
ESR (mean±SD)	32±15	23.3±19	20.3±4	0.008*
HB (mean±SD)	10.8±2	11.5±2.2	11.8±2.5	0.03*
WBC (mean±SD)	8370±2036	8442±2010	6713±1973	0.004*
Lymph count (mean±SD)	1754±651	2162±828	1871±678	0.14
Neut count (mean±SD)	5609±1503	5612±1399	4483±1409	0.009*
PLT (mean±SD)	238400±9615	222550±9033	243000±8160	0.6
LDL (mean±SD)	83.1±23.2	83.6±19.5	80±18.5	0.6
HDL (mean±SD)	37±10	38±7	37.7±6	0.4
TG (mean±SD)	128±52	131±41	111±39	0.8
NLR (mean±SD)	3.46±1.19	2.9±1.08	2.51±0.7	0.004*
Urine Cr (mean±SEM)	66±10.8	-	-	
Proteinuria 24 hours (mg) (mean±SEM)	1591±195	-	-	
UACR (mean±SEM)	218±45	-	-	
eGFR (mean±SD)	52.6±20.6	90.8±18.3	92.3±15.3	<0.0001*
Other diseases	HTN (33) CVD (14)	HTN (24) CVD (10)	- -	0.07 0.2

DN: diabetic nephropathy, BMI: body mass index, BUN: blood urea nitrogen, Cr: creatinine, ALB: albumin, FBS: fasting blood sugar, ESR: erythrocyte sedimentation rate CRP: C-reactive protein, HB: hemoglobin, WBC: white blood cell, PLT: platelet, LDL: low-density lipoproteins, HDL: high-density lipoproteins, TG: Triglyceride, NLR: Neutrophil to Lymphocyte Ratio, UACR: urine albumin-creatinine ratio, eGFR: estimated glomerular filtration rate, SD: standard deviation, SEM: standard error of means, HTN: hypertension, CVD: cardiovascular diseases.

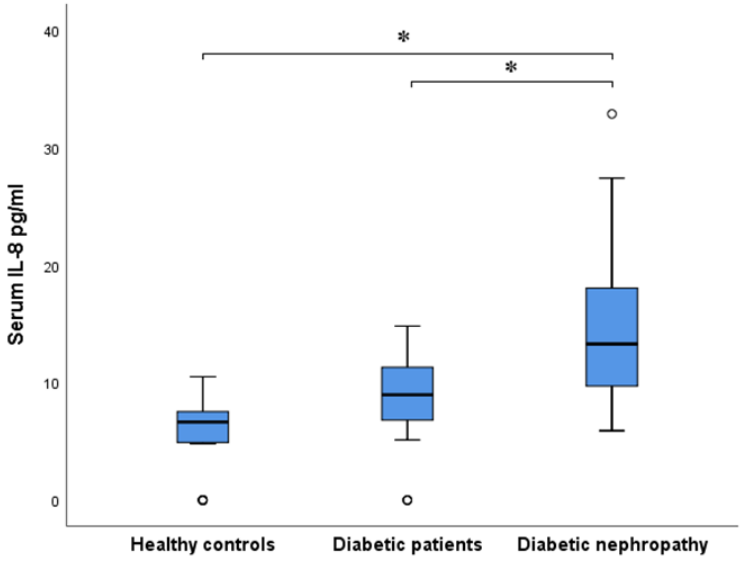

Figure 1. Higher serum levels of IL-8 in diabetic nephropathy compared to diabetic patients and healthy controls (p-value < 0.0001)

3- Increased neutrophil count and NLR in diabetic and diabetic nephropathy patients: The neutrophil count was higher in diabetic nephropathy compared to healthy controls [(5609±1503 vs. 4483±1409 (mean±SD) (P-value: 0.01)]. It was also higher in diabetic patients than in healthy subjects [(5612±1399 vs. 4483±1409 (mean±SD) (P-value: 0.03)], but there was not considerable difference between diabetic patients and diabetic nephropathy. Neutrophil-to-lymphocyte ratios were significantly increased in diabetic nephropathy compared to healthy individuals [(3.46±1.19 vs. 2.51±0.7 (mean±SD) (P-value: 0.003)]; however, despite higher NLR values in nephropathy group, there was no significant difference between diabetic patients with or without nephropathy [(3.46±1.19 vs. 2.9±1.08 (mean±SD) (P-value: NS)] (figure 2).

4- Significant correlation between IL-8 levels and NLR: There was a weak direct correlation between the serum levels of IL-8 and the frequency of neutrophils in the patient groups, including diabetes and diabetic nephropathy, which was not statistically significant [Pearson correlation: 0.12, R2 coefficient of determination: 0.014 (P-value: NS)]. However, analysis showed a significant direct correlation between the serum level of IL-8 and NLR in patients (with and without nephropathy) [Pearson correlation: 0.27, R2 coefficient of determination: 0.072 (P-value: 0.02)] (figure 3).

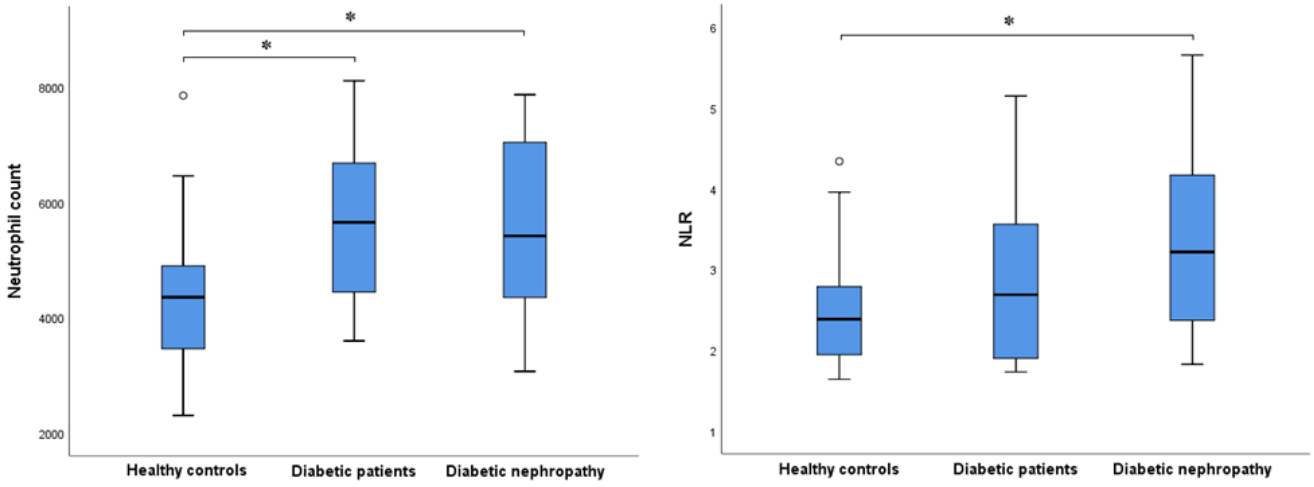

Figure 2. Elevated neutrophil count in diabetic (P-value= 0.03) and diabetic nephropathy (P-value= 0.015) patients compared to healthy subjects and higher neutrophil-to-lymphocyte ratios in nephropathy (P-value= 0.003)

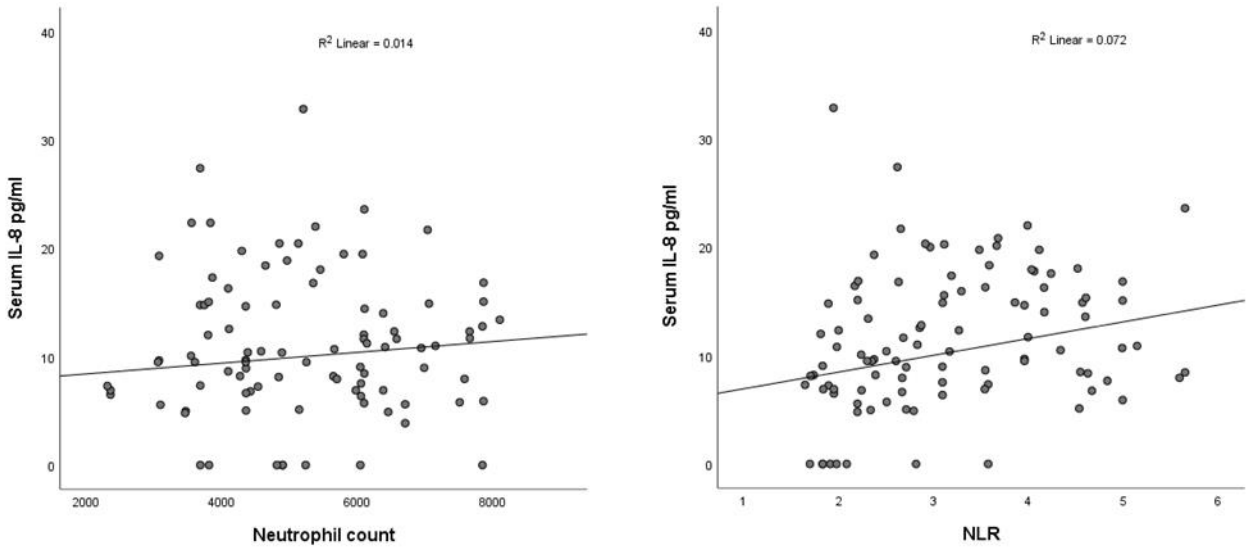

Figure 3. Significant direct correlation between IL-8 levels and neutrophil-to-lymphocyte ratio in diabetic patients with and without nephropathy (P-value: 0.02)

5- Significant correlation between IL-8 levels and clinical indices: Regression analysis showed a negative correlation between IL-8 levels and eGFR in the patients' group (with or without nephropathy) [Pearson correlation: -0.36, R2: 0.13 (P-value: 0.01)]. There was also a significant association between serum levels of IL-8 and urine albumin to creatinine ratio (UACR) in patients with diabetic nephropathy [(Pearson correlation: 0.37, coefficient of determination R2: 0.134 (P-value: 0.04)] (figure 4).

6- Significant correlation between NLR and clinical indices: NLR values were negatively correlated with eGFR in the patient groups (with or without nephropathy) [Pearson correlation: - 0.29, R2: 0.085 (P-value: 0.04)]. There was also a significant association between NLR and UACR in DN patients [(Pearson correlation: 0.39, coefficient of determination R2: 0.158 (P-value: 0.03)] (figure 5).

7- Diagnostic value of IL-8 and NLR in diabetic nephropathy: ROC curve analysis showed a significant specificity and sensitivity for serum IL-8 level in diabetic nephropathy with an AUC of 0.87 [95% confidence interval (CI) 0.79-0.95 (P-value<0.0001)]. NLR had a lower but still considerable AUC value: 0.7 [95% CI 0.58-0.82 (P-value: 0.003)]; but the neutrophil count did not show acceptable diagnostic value for DN [AUC: 0.61(95% CI 0.48-0.75 (P-value: NS)] (figure 6).

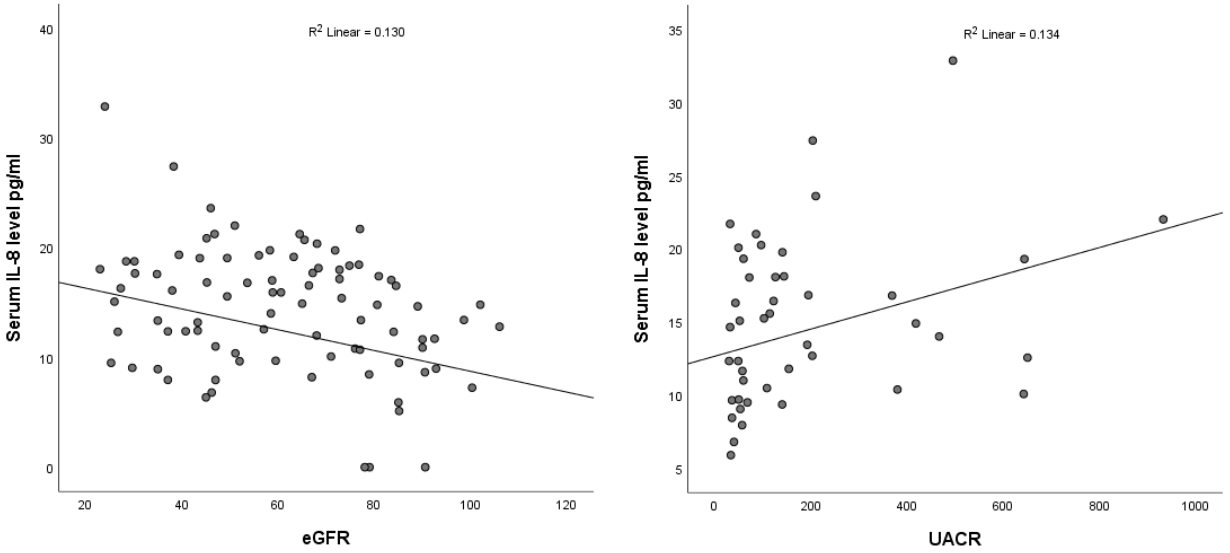

Figure 4. Negative correlation between IL-8 levels and eGFR in patients (diabetic and diabetic nephropathy) (P-value= 0.01); Direct correlation between IL-8 levels and UACR in DN patients (P-value= 0.04)

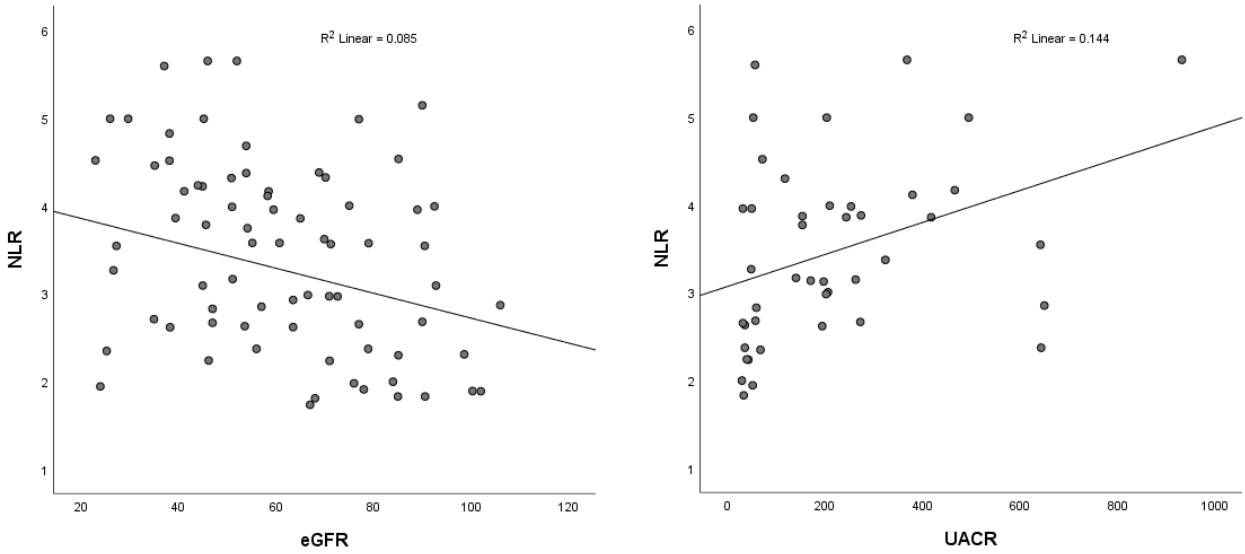

Figure 5. Negative correlation between NLR and eGFR in patients (diabetic and diabetic nephropathy (P-value= 0.04); Direct correlation between NLR and UACR in patients with diabetic nephropathy (P-value= 0.03)

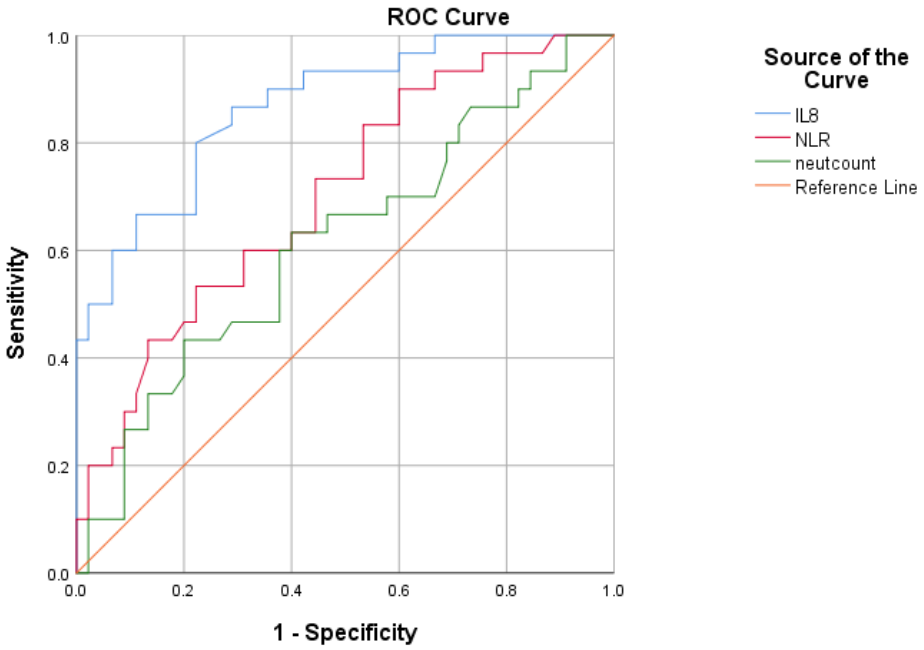

Figure 6. Higher diagnostic value of IL-8 for diabetic nephropathy [AUC= 0.87 (p-value<0.0001)] than neutrophil-to-lymphocyte ratio and neutrophil count

Discussion

The results of the present study showed higher IL-8 serum levels in diabetic nephropathy patients compared to the unaffected diabetic and healthy controls. The IL-8 concentration was slightly elevated in diabetic patients without nephropathy (8.4±4.3 vs. 5. ±3.3 pg/ml (mean±SD), P-value: 0.2) but the difference between the healthy and unaffected diabetic groups was not significant. In line with these findings, it has previously been reported that exposure of human proximal tubular epithelial cells to albumin induced IL-8 gene and protein expression (14). Furthermore, IL-8 secretion from podocytes was increased when cultured in high-glucose medium and its urinary excretion was enhanced in hyperglycemia. Urinary IL-8 further increased in diabetic kidney disease, with the highest levels in patients with the lowest GFR values (7).

Other markers studied in the patients were neutrophil count and neutrophil-to-lymphocyte ratio. Our results showed higher NLRs in patients with diabetic nephropathy compared to the healthy controls with a significant correlation between NLR and UACR in diabetic nephropathy. Moreover, NLR values of the patients' group (diabetic with and without nephropathy) were negatively correlated with eGFR. There was also a significant correlation between NLR and IL-8 levels indicating an inflammatory state and neutrophil recruitment and activation by IL-8. NLR of diabetic nephropathy patients have been investigated in various populations. A Turkish study reported a significant correlation between albuminuria and serum creatinine, eGFR, platelet lymphocyte ratio, and NLR values (15). One investigation on the Syrian population showed an AUC of 0.869 for NLR in the prediction of microalbuminuria in diabetic patients (16). Furthermore, a cross-sectional study of 4813 cases in China showed an increased risk of cardiovascular and diabetic kidney disease in patients in the first quartile of NLR values (17). Another study in Japan demonstrated a direct correlation between NLR and urinary albumin excretion while it was negatively correlated with eGFR (18). Similar results were obtained in a follow-up study conducted by Azab et al. (19) and an observational investigation performed by Huang et al. (20). However, an Indian study found no significant association between NLR and microalbuminuria in diabetic patients but a significant correlation was observed between NLR and microalbuminuria in pre-diabetic subjects with HbA1c between 5.7%−6.4% (21).

Neutrophils infiltration in kidney could induce tissue damage through ROS and NOS production, and recruit other leukocytes via cytokine and chemokine secretion. Neutrophil counts has shown a positive correlation with UACR in type 1 diabetic patients (22). In the present study, neutrophil count was elevated in both groups of patients with and without nephropathy compared to healthy individuals. Despite the small sample size, the findings mentioned above emphasize the importance of innate

immune biomarkers in the early detection of inflammatory responses and tissue damage in the diabetic kidney. Urinary and serum levels of IL-8 might be considered as potential diagnostic or prognostic biomarkers in follow-up of diabetic patients (9). One study suggested the increased urinary IL-8 levels as an independent risk of developing diabetic nephropathy and demonstrated urinary IL-8 alteration in different phases of nephropathy (23). Nonetheless, to establish neutrophil ratio and neutrophil-related cytokines such as IL-8 as routine follow-up biomarkers, large longitudinal studies are warranted to explore the specificity and sensitivity of these markers in different phases of diabetic kidney disease. IL-8 suppression in diabetic experimental models might also give an insight into the significance of this cytokine in disease pathogenesis. Moreover, regular evaluation of NLR changes could be proposed as an affordable monitoring tool for the follow-up of high-risk diabetic patients. In summary, regarding the above-mentioned findings, the serum levels of IL-8 and NLR increased in diabetic nephropathy compared to diabetic patients without nephropathy and healthy subjects. Moreover, IL-8 levels and NLR showed a significant correlation with eGFR and UACR; therefore, they might be considered as prognostic or diagnostic factors for DN. In addition, it is suggested that the beneficial effects of IL-8 inhibition be investigated in treating diabetic nephropathy.

Acknowledgments

We would like to express our appreciation to the patients and healthy volunteers who kindly helped us conduct this project.

Funding: the present study was supported by the Shahid Beheshti University of Medical Sciences (grant number: 43006517).

Ethics approval: All subjects gave informed consent for inclusion before participating in the study. The study was conducted following the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1402.342).

Conflict of interests: the authors declare no conflict of interest.

Authors' contribution: MF, HK, and NS: evaluated the patient and provided clinical data; MS, and HM: performed lab tests; NS: analyzed the results; SA: designed the study and prepared the manuscript; MH: supervised the study. All authors read and approved the final version of the manuscript.

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