Relationship Between Neutrophil/Lymphocyte Ratio and Albuminuria in Diabetic Patients

Diyabetik Hastalarda Nötrofil/Lenfosit Oranı ile Albüminüri Arasındaki İlişki

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ABSTRACT

OBJECTIVE: We aimed to investigate whether there is a relationship between neutrophil/lymphocyte (N/L) ratio and albuminuria in diabetic patients.

MATERIAL and METHODS: This study included 170 diabetic patients. The patients were divided 3 groups according to urinary albumin creatinine ratio (UACR). The first group included patients with normoalbuminuria (UACR <0.030). The second group included patients with moderately increased albuminuria (0.030-0.300), whereas the third group consisted of patients with severely increased albuminuria (UACR>0.300).

RESULTS: N/L ratio was 1.83, 2.23, and 2.77 in first, second, and third group, respectively. It was meaningfully higher in third group when compared with two groups. Although there was no statistically meaningful difference between the first group and the second group, it was higher in the second group than that in the first group. The hemoglobin concentration was 14.3 ±1.6 g/dl, 13.6 ± 1.8 g/dl, and 12.4 ± 1.8 g/dl in the first, second, and third group, respectively. It was significantly lower in the third group when compared with other groups. However, there was no meaningful difference between the first group and the second group. Presence of anemia increased step-wise from the first group to the third group (8.7%, 21.3%, and 48.1%, respectively).

CONCLUSION: N/L ratio and anemia associate closely with albuminuria in diabetic patients.

KEY WORDS: Albuminuria, Anemia, Diabetes mellitus, Neutrophil/lymphocyte ratio

ÖZ

AMAÇ: Biz diyabetik hastalarda nötrofil/lenfosit (N/L) oranı ve albüminüri arasında bir ilişki olup olmadığını değerlendirmeyi amaçladık.

GEREÇ ve YÖNTEMLER: Yüz yetmiş diyabetik hasta bu çalışmaya alındı. Hastalar idrar albümin/kreatinin oranı (İAKO) değerine göre üç gruba ayrıldı. İlk grupta normoalbüminürik (İAKO <0,030) hastalar vardı. İkinci grubu ılımlı yükselmiş albüminürik (İAKO 0,030-0,300) hastalar oluştururken, üçüncü grubu ağır albüminürik (UACR>0.300) hastalar oluşturuyordu.

BULGULAR: N/L oranı sırasıyla ilk, ikinci ve üçüncü grupta 1,83, 2,23 ve 2,77 idi. Oran üçüncü grupta diğer iki gruba kıyasla anlamlı olarak yüksekti. Birinci ve ikinci grup arasında istatistiksel olarak anlamlı fark olmamasına rağmen, ikinci grupta ilk gruptan daha yüksekti. Hemoglobin konsantrasyonu sırasıyla ilk, ikinci ve üçüncü grupta 14,3 ±1,6 g/dl, 13,6 ± 1,8 g/dl ve 12,4 ± 1,8 g/dl idi. Hemoglobin konsantrasyonu üçüncü grupta diğer iki gruptan anlamlı olarak düşüktü ama ilk ve ikinci gruplar arasında anlamlı fark yoktu. Anemi sıklığı ilk gruptan son gruba doğru basamaklı olarak artıyordu (sırasıyla %8,7, %21,3 ve %48,1).

SONUÇ: Diyabetik hastalarda N/L oranı ve anemi albüminüri ile yakından ilişkilidir.

ANAHTAR SÖZCÜKLER: Albüminüri, Anemi, Diabetes mellitus, Nötrofil/lenfosit oranı

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INTRODUCTION

Diabetic nephropathy is the most common cause of renal disease in patients starting renal replacement therapy (1). Diabetic nephropathy has been classically defined by the presence of proteinuria >0.5 g/24 h. This stage has been referred to as overt nephropathy or macroalbuminuria (2). Moderately increased albuminuria (formerly called microalbuminuria), which is persistent daily urinary albumin excretion between 30 and 300 mg, may be indicative of early diabetic nephropathy in diabetic patients in the absence of coexistent renal disease (3,4).

Inflammatory processes play a key role in chronic diseases including cardiovascular disease, cancer, chronic kidney disease, and diabetes mellitus (5). Previous studies have demonstrated that neutrophil/lymphocyte (N/L) ratio is indicator of systemic inflammation. Furthermore, the N/L ratio has been shown to predict short- and long-term cardiovascular mortality and outcomes in patients with cancer (6,7). In addition, we have recently reported that N/L ratio predict the progression rate of stage 4 chronic kidney disease to dialysis (8).

Prevalence and degree of anemia are significantly higher in diabetic patients compared to those without diabetes. Anemia can occur in diabetic patients without underlying severe chronic renal disease. The cause of this non-renal diabetic anemia remains largely unknown (9).

In this study, we aimed to investigate whether there is a relationship between neutrophil/lymphocyte (N/L) ratio and albuminuria in diabetic patients.

MATERIAL and METHODS

This study was performed on 170 (98 female, 72 male) patients with diabetes mellitus of overall mean age of 58 ± 13 years. We recorded biochemical parameters at their last visit. We also noted demographic characteristics such as age and sex.

Dipotassium ethylene diamine tetra acetic acid (K2EDTA)-based anticoagulated blood samples were drawn each patient and assessed by a Sysmex K-1000 auto analyzer. Hemoglobin, hematocrit, platelets, white blood cell, differential counts (neutrophil, lymphocyte, eosinophil, basophil, and monocyte) and percentages were determined using a blood counter Sysmex K-1000 (Block Scientific, USA). The N/L ratio was calculated in all patients.

The Modification of Diet in Renal Disease (MDRD) formula was used to calculate estimated glomerular filtration rate (eGFR) (10). Urinary albumin creatinine ratio (UACR) was used to asses daily urinary albumin excretion. The patients were divided into 3 groups according to UACR value. The first group included patients with normoalbuminuria, which was defined as UACR <0.030. The second group included patients with moderately increased albuminuria, which was defined as UACR of 0.030-0.300, whereas the third group consisted of patients with severely increased albuminuria, which was defined as UACR >0.300 (4).

Anemia was defined by World Health Organization (WHO) criteria (hemoglobin <13 g/dL for men and <12 g/dL for women) (11).

Statistical analysis was performed using the SPSS 15.0 software (SPSSFW; SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used for normality analysis. Continuous variables with normal distribution are presented as mean ± SD. Statistical analysis for the parametric variables among three groups was performed by one-way ANOVA with Scheffe post-hoc test. Median values were used where normal distribution was absent. The Kruskal-Wallis test was used to compare nonparametric variables among three groups. Then, the Mann-Whitney U-test with Bonferroni correction was used to assess differences among three groups. Qualitative variables were given as percent and the correlation between categorical variables was investigated using the chi-square test. The correlation analysis was evaluated by Spearman correlation test. A P value of <0.05 was considered significant.

RESULTS

Table I shows comparison of demographic and biochemical parameters among three groups. Median N/L ratio was 1.83, 2.23, and 2.77 in first, second, and third group, respectively. It was meaningfully higher in third group when compared with other groups. Although we did not find statistically significant difference between the first group and the second group, it was higher in the second group than that in the first group.

UACR value increased step-wise from the first group to the third group (0.011, 0.120, and 1.30, respectively). Similarly, presence of anemia increased step-wise from the first group to the third group (8.7%, 21.3%, and 48.1%, respectively). On the other hand, eGFR values decreased step-wise from the first group to the third group (107 ± 24 ml/min, 75 ± 25 ml/min, and 42 ± 22 ml/min, respectively).

Age was meaningfully lower in patients with the first group than in the second and third groups (48 ± 15 years, 59 ± 9 years, and 64 ± 11 years, respectively). However, we did not find any significant difference between the second group and third group in terms of age. Hemoglobin concentration was 14.3 ±1.6 g/dl, 13.6 ± 1.8 g/dl, and 12.4 ± 1.8 g/dl in the first, second, and third group, respectively. It was meaningfully lower in the third group when compared with other groups. However, we did not find any significant difference between the first group and second group in terms of hemoglobin concentration. HbA1c value was 6.67 ± 1.43%, 7.17 ± 2.01%, and 8.32 ± 2.36% in the first, second, and third group, respectively. It was significantly lower in the first group compared to the third group. On the other hand, we did not find any significant difference between second group and other two groups in terms of HbA1c value. Low-density lipoprotein was 101 ± 31 mg/dl, 124 ± 31 mg/dl, and 108 ± 52 mg/dl in the first, second, and third group, respectively. It was significantly lower in fist group compared to second group. However, there

Table I: Comparison of demographic and biochemical parameters among three groups.

	First group (n: 46)	Second group (n: 47)	Third group (n:77)	p value
Age (year)	48 ± 15*¶	59 ± 9	64 ± 11	<0.001
Gender				0.653
Male (%)	17 (37.0)	20 (42.6)	35 (45.5)	
Female (%)	29 (63.0)	27 (57.4)	42 (54.5)	
UACR	0.011 (0.001-0.029)*¶	0.120 (0.031-0.300)†	1.30 (0.33-12.00)	<0.001
N/L ratio	1.83 (1.09-4.54)¶	2.23 (1.00-9.58)†	2.77 (1.16-22.68)	<0.001
Hemoglobin (g/dL)	14.3 ± 1.6¶	13.6 ± 1.8†	12.4 ± 1.8	<0.001
Presence of anemia (%)	4 (8.7)	10 (21.3)	37 (48.1)	<0.001
HbA1c (%)	6.67 ± 1.43¶	7.17± 2.01	8.32 ± 2.36	0.005
Glucose (mg/dL)	128 (60-341)	142 (90-467)	158 (41-449)	0.252
Triglyceride (mg/dL)	140 (63-460)	148 (58-958)	149 (69-1341)	0.141
TC (mg/dL)	178 ± 38	199 ± 37	194 ± 71	0.187
LDL (mg/dL)	101 ± 31*	124 ± 31	108 ± 52	0.029
HDL (mg/dL)	44 (27-69)	40 (26-40)	40 (19-102)	0.082
eGFR (ml/min)	107 ± 24*¶	75 ± 25†	42 ± 22	<0.001

UACR: Urinary albumin creatinine ratio, **N/L ratio:** Neutrophil/lymphocyte ratio, **TC:** Total cholesterol, **LDL:** Low-density lipoprotein, **HDL:** High- density lipoprotein, **eGFR:** Estimated glomerular filtration rate

* p <0.05 normoalbuminuric group compared to moderately increased albuminuric group

¶ p < 0.05 normoalbuminuric group compared to severely increased albuminuric group

†p < 0.05 moderately increased albuminuric group compared to severely increased albuminuric group

was no significant difference between the third group and other two groups with regard to low-density lipoprotein. There was no significant difference among the three groups in terms of gender, levels of serum glucose, triglyceride, total cholesterol, and high-density lipoprotein (p > 0.05).

There was significant difference between patients with anemia and those without anemia regarding N/L ratio. The ratio was significantly higher anemic patients compared to non-anemic patients [2.93 (1.31-22.7) vs. 2.07 (1.00-8.13), respectively, p: <0.001] (data not shown). In addition, hemoglobin concentration negatively correlated with N/L ratio (r: -0.358, p: <0.001).

The UACR value positively correlated with N/L ratio (r: 0.416, p: <0.001), age (r: 0.390, p: <0.001), serum glucose level (r: 0.187, p: 0.016), and HbA1c value (r: 0.324, p: 0.002), and negatively correlated with eGFR (r: -0.770, p: <0.001) and hemoglobin concentration (r: -0.460, p: <0.001). Relationships between UACR level and several biochemical and demographic findings including N/L ratio, age, hemoglobin concentration, and eGFR are shown in Figure 1.

DISCUSSION

In the present study, N/L ratio was meaningfully higher in diabetic patients with severely increased albuminuria when compared with diabetic patients with normoalbuminuria or moderately increased albuminuria. Similarly, although the difference was statistically significant, the ratio was higher in diabetic patients with moderately increased albuminuria when compared with normoalbuminuric diabetic patients. In addition, there was a strong and significant correlation between N/L ratio and albuminuria.

Chronic inflammation appears to underlie most of the chronic diseases, including cardiovascular disease, type 2 diabetes mellitus, chronic kidney disease, Alzheimer's disease, and cancer (5). Previous studies have demonstrated that the total white blood cell count and its subtypes, N/L ratio, can be used as an indicator of systemic inflammation (12). Moreover, the N/L ratio has been revealed to predict cardiovascular mortality and survival in malignancies (6,7). Regardless of whether renal injury begins in the glomeruli or in the tubulointerstitium, tubulointerstitial damage is common feature of all chronic progressive kidney disease and is though to be final common

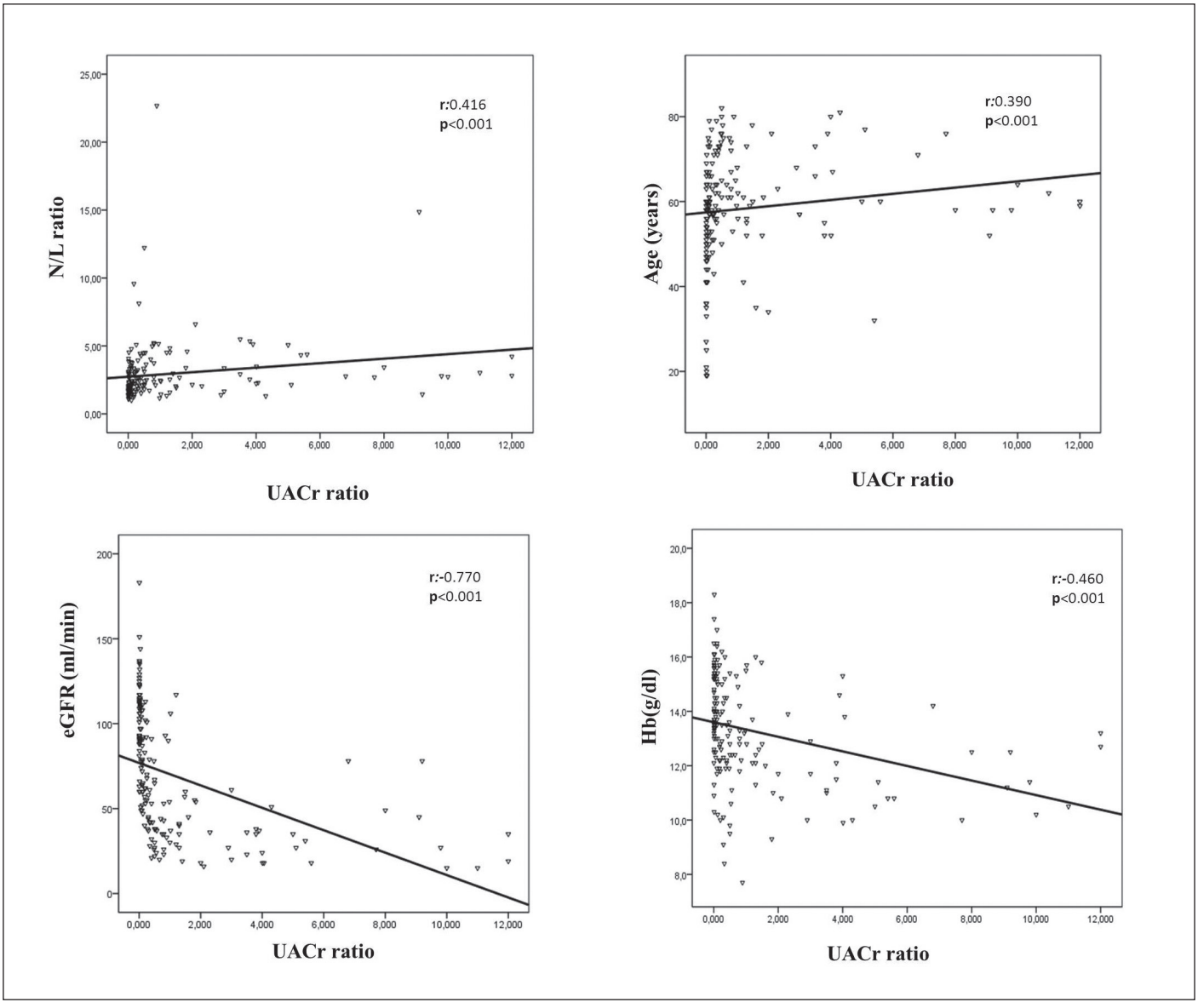

Figure 1: *Correlations between UACR level and N/L ratio, age, hemoglobin concentration, and eGFR.*

pathway in progression to end-stage kidney disease (5). Inflammation is critical mechanism in the damage. Leukocytes play an important role in the initiation and progression of kidney disease via inflammatory mechanisms independent of infection (13).

Leukocytes in diabetic patients may be activated by advanced end products or reactive oxygen radicals or cytokines (14-16). Activated leukocytes secrete many kinds of cytokines and transcription factors having a crucial role in inflammation and thereby contribute to glomerulosclerosis (13,17). In addition, they can release superoxide radicals and proteases, all of which promote oxidative stress (8). Hyperglycemia-induced oxidative stress has an important role in the pathogenesis of diabetic nephropathy (18). Increased urinary protein excretion is the earliest clinical manifestation of diabetic nephropathy (2). In the present study, we observed that step-wise increase of N/L ratio from normoalbuminuric group to macroalbuminuric group and a strong and significant correlation between N/L ratio and degree of proteinuria. Therefore, we thought that chronic inflammation, as indicated by N/L ratio, may play a role in the development and progression of diabetic nephropathy.

One of the most striking findings of this study was that the prevalence of anemia was very high in both moderately increased albuminuria group and severely increased albuminuria group despite the relatively good preservation of renal function. The form of anemia most common in chronic kidney disease is a

normocytic, normochromic anemia with insufficient production of erythrocytes, of which the major cause is erythropoietin (EPO) deficiency (19). There is the pronounced breakdown of EPO production in response to anemia when creatinine clearance is less than 40 mL/min. Therefore, clinically relevant anemia becomes common only with severe renal insufficiency (19). In the present study, we observed that anemia prevalence was 21.3% in moderately increased albuminuria group with 75 mL/min of mean eGFR whereas this prevalence was 48.1% in severely increased albuminuria group with 42 mL/min of mean eGFR. Diabetes mellitus may be linked to premature development of anemia in patients with moderate kidney insufficiency (20). For example, anemia prevalence was observed 8.7%, 7.5%, 22.2%, and 52.4% in renal function categories >89 mL/min, 60-89 mL/min, 30-59 ml/min, and <30 mL/min, respectively, in patients with diabetes mellitus in The Kidney Early Evaluation Program (KEEP). On the other hand, the prevalence was 6.9%, 5.0%, 7.9%, and 50% in same renal function categories, respectively, in those without diabetes mellitus (20). In our study, although mean creatinine clearance values were higher than 40 mL/min in both moderately increased albuminuria and severely increased albuminuria, frequency of anemia was very high (21.2% and 48.1, respectively).

There seems to be several possible mechanisms underlying the link between diabetes mellitus and anemia. Firstly, diabetic autonomic neuropathy can reduce splanchnic sympathetic stimulation of EPO production (20,21). Secondly, diabetes mellitus can damage peritubular and interstitial structures of the kidney, which are sites of EPO production, even without overt diabetic nephropathy (20,21). Thus, EPO deficiency seems to occur early in diabetic renal disease (21). EPO deficiency and anemia are not generally developed in early stages of non-diabetic renal disease except nephrotic syndrome. In patients with nephrotic patients, severe proteinuria has been shown to result in reduction in EPO production and an excessive loss of EPO in the urine (22,23). Furthermore, it has been reported that successful treatment of anemia of nephrotic syndrome with recombinant human EPO (24). The findings of our study may suggest that tubulointerstitial injury of diabetic renal disease causes EPO deficiency and this EPO deficiency may starts even before there is no significant deterioration in excretory function of the kidney in patients with diabetes mellitus. Furthermore, strong and significant relationship between severity of proteinuria and hemoglobin concentration appears to a reflection of an excessive loss of EPO in the urine resulted from severe proteinuria. On the other hand, in this study, we did not measure serum and urine EPO levels. In future studies, this hypothesis can be explored by measuring levels of EPO in diabetic patients with nephrotic syndrome. Finally, EPO-resistance and diminished iron availability caused by inflammation contribute to development of anemia in the relatively early stages of diabetic nephropathy (25). Really, in the present study, we found that N/L ratio and presence of anemia increased step-wise from the first group to the third group. N/L ratio was significantly higher anemic patients compared to non-anemic patients and hemoglobin concentration negatively correlated with N/L ratio.

Our study has several limitations. Firstly, the design of the study was cross-sectional. Therefore, the potential causality among N/L ratio and anemia and albuminuria cannot be concluded. Secondly, other inflammation indicators and oxidative stress markers were not assessed and we did not measure urine and plasma EPO levels. Thirdly, number of patients in the study was relatively low.

In conclusion, our study showed that N/L ratio is associated with diabetic proteinuria. Anemia prevalence was very high in both patients with moderately increased albuminuria and those with severely increased albuminuria despite the relatively good preservation of renal function.

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