Relationship between glomerular filtration rate with uric acid and neutrophil to lymphocyte ratio in diabetic patients

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Abstract
Aim: In this study we aimed to investigate the relationship between uric acid (UA) and neutrophil-lymphocyte ratio (NLR) with glomerular filtration rate (GFR) in diabetic patients. Material and Method: 355 diabetic patients were included in this study. Patients with gout, liver fatigue, coronary artery disease, heart failure, cancer disease, polycythemia, hypothyroidism, sarcoidosis, obesity, rheumatic diseases and active infection were excluded from the study. The biochemistry and complete blood count tests were recorded. GFR were calculated by the formula of Modification of Diet in Renal Disease. 24-hour urine microalbumin and protein levels were measured. Results: The patients with estimated GFR<60 were compared to the patients with e-GFR>60. The patients with e-GFR>60 were older and had higher NLR ratio, UA levels; more proteinuria-microalbuminuria. The age, UA levels and microalbuminuria was determined as e-GFR independent predictors. It was detected that when UA value was taken as >5.70mg/dl, e-GFR<60 mL/min/1.73m² was demonstrated by the sensitivity of 80% and the specificity of 75% (AUC:0.863, 95%CI 0.785-0.941, p<0.001). Discussion: The high level of UA can be used as one of the independent predictors of e-GFR<60 mL/min/1.73m² in patients with DM. Although there is significant negative correlation between e-GFR and NLR, this doesn’t predict e-GFR<60 mL/min/1.73m².

Keywords
Neutrophil Lymphocyte Ratio; Uric Acid; Diabetic Nephropathy
Introduction
Diabetic nephropathy has a growing importance today as the most common cause of end-stage renal failure. It is known that the diabetic nephropathy develops in 20-40% of the patients with diabetes around the world [1].

Uric acid is the end product of purine catabolism in humans. At the same time, it is suggested as a result of the studies performed that the uric acid is a protective antioxidant against the oxidative stress [2,3]. However, while it is expected that the uric acid, which is known to be an anti-oxidant in the diabetic patients, corrects the renal damage, it is said that it increases the renal damage [4-6].

It is acknowledged that the oxidative stress develops in diabetics, and this oxidative stress contributes to the formation of both the disease and the complications [7-11]. It is also showed in the studies performed that the inflammation plays a role in the etiopathogenesis of diabetes and its complications [12,13]. The neutrophil-lymphocyte ratio (NLR) is an easily measurable systemic inflammatory marker and is showed to have a close relationship with morbidity and mortality in numerous diseases [14]. We aimed in this study to investigate the relationship of uric acid (UA) and NLR with the estimated glomerular filtration rate (e-GFR) in diabetic patients, and whether or not they can be used as clinical markers to show the changes in e-GFR.

Material and Method
In this retrospective longitudinal study, our study consisted of a population of non-selected 466 patients, who visited our clinic from January to August 2014. The exclusion criteria were gout disease (n=5), hepatic steatosis (n=20), coronary artery disease (n=21), heart failure (n=11), sarcoidosis (n=1), obesity (n=15), cancer disease (n=5), rheumatic diseases (n=4), inflammation which may cause a systemic infection in the body (n=12), polycythemia (n=1), and hypothyroidism (n=16). Finally, the study population consisted of 355 patients. All participants gave an informed consent and the study was approved by the local ethics committee.

After 12 hours of fasting, the blood samples were taken from all patients, who underwent a detailed physical examination, for the routine biochemical parameters and the complete blood count tests. The blood samples were collected in gel tubes that did not include anticoagulant to measure the fasting glucose, urea, creatinine, albumin, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and uric acid (A). The blood samples were centrifuged at 1800g for 15 minutes, and the plasma and the serum samples were obtained. The fasting glucose, total cholesterol, TG, HDL-C, LDL-C, and UA, urea, creatinine and the albumin levels were measured chromatographically using an Abbott original reagent on Abbott Architect 8000 auto analyzer. They were measured by the method of HbA1cHigh Performance Liquid Chromatography (HPLC) on the device of Automatic Glycohaemoglobin Analyzer ADAMS A1c HA-8160 (Arkay).

The venous blood is routinely collected in a tube containing EDTA for the measurement of hemoglobin, total WBC, neutrophils, lymphocytes were determined using an automated blood cell counter by an Abbott Cell-Dyn 3700 Hematology in all patients.

GFR of the patients were calculated considering the serum creatinine, age, race and gender based on Modification of Diet in Renal Disease (MDRD) formula [14]. For the staging of chronic kidney disease; stage 1 was described as GFR ≥90 mL / min / 1.73 m2, stage 2 as GFR = 60-89 mL / min / 1.73 m2, stage 3 as GFR = 30-59 mL / min / 1.73 m2, stage 4 as GFR = 15-29 mL / min / 1.73 m2, stage 5 as GFR <15 mL / min / 1.73 m2 or as undergoing dialysis.

All patients were explained the 24-hour urine collection method and the samples were collected as described and transported to the laboratory [15]. The microalbumin and the protein levels in the urine collected were studied by the immunoturbidimetric technique on the device of Abbott Architect 8000 auto analyzer. The results were defined as following: normoalbuminuria<30 mg / day, microalbuminuria 30-200 mg / day, macroalbuminuria, ≥300 mg/day, proteinuria> 150 mg / day [1].

Statistical analysis
The quantitative variables are expressed as mean ± SD, and the categorical variables are expressed as percentage. In order to test the differences of the numerical variables between the groups, Student’s t test or Mann-Whitney U test was used. In order to test the differences of the categorical variables between the groups, Chi-square test was used. Pearson or Spearman correlation test was used for the correlation analysis between the variables. Multiple linear regression analysis was used for the detection of the independent predictors of the estimated GFR (what is found significant in the univariate analysis: the age, hemoglobin (HB), red cell distribution width (RDW), neutrophil-lymphocyte ratio (NLR), uric acid (UA), albumin, fasting blood glucose, and microalbuminuria). ROC analysis was used to determine the best predictive value in the estimation of the estimated GFR (e-GFR). A P value of <0.05 was accepted as the limit of significance. SPSS 2.0 (SPSS Inc., Chicago, Illinois) software package was used for all statistical analysis.

Results
A total of 355 diabetic patients were included in the study. The mean age of the patients was 57 ± 10 and 42.9% of them were men. E-GFR was measured as > 90 in 54.5% of the patients, 60-89 in 27%, 30-59 in 6.5%, 15-29 in 1%, and <15 in 11%. When E-GFR was categorized according to 60, it was measured as >60 in 81.6% of the patients, and <60 in 18.4% of the patients. The number of the patients undergoing dialysis in the entire group was 10.8%. The other clinical and the biochemical variables of the entire population are summarized in Table 1. The ones with e-GFR <60, compared to the ones with e-GFR >60, were older, had a higher male gender ratio, lower albumin levels, lower hemoglobin levels, higher RDW and NLR, more proteinuria and microalbuminuria, and higher levels of UA (Table 2).

There was no difference between groups in terms of hypertension (p= 0.58) The age (r = -0.416, p <0.001), HB (r = 0.314, p <0.001), and RDW (r = -0.485, p <0.001), NL (r = -0.316, p <0.001), A (r = -0.515, p <0.001) were observed by e-GFR. A negative correlation was found between both GFR and UA (figure 1) and NLR. A positive correlation was found between
Glomerular filtration rate

In order to detect the independent variables of e-GFR, multiple linear regression analysis was used. Accordingly, the age, UA level and the amount of microalbuminuria were determined as the independent predictors of e-GFR (Table 3). In ROC analysis carried out to predict e-GFR <60, UA level >5.70 is showed with a sensitivity of 80% and a specificity of 75% (AUC: 0.863, 95% CI 0.785 to 0.941, p < 0.001) (Figure 2).

Discussion

In this study, we found that there was a negative correlation between GFR value and UA and NLR; UA was one of the independent predictors of GFR <60 mL/min/1.73m²; and NLR did not predict GFR decline.

RDW: Red Cell Distribution Width, NRL: Neutrophil-Lymphocyte Ratio, GFR: Glomerular Filtration Rate, CRD: Chronic Renal Disease.
Lipid peroxidation, one of the most important causes of cell damage, is initiated by free oxygen radicals (FOR) and formed as a result of the oxidative degradation of unsaturated fatty acids in the cell membrane structure. It accumulates in the body as a result of the increase in the production of FOR occurred physiologically or a defect in the anti-oxidant defense system which eliminates the free radicals. Free oxygen radicals, interacting with lipids, proteins, and nucleic acids may lead to the loss of membrane integrity, the structural or functional changes in proteins and the genetic mutations [16]. It is reported in the studies that free oxygen radicals and lipid peroxidation significantly increases in diabetic patients; and the oxidative stress has an important role in the formation and the progression of diabetes [17]. It is also mentioned that the oxidative stress plays a role in the development of chronic complications of diabetes. The imbalance between the formation rate of free radicals and the antioxidant defense capacity cause to the chronic complications of diabetes meaning that hyper-glycemia leads to the oxidative stress and therefore damages organs [18-21].

It has been reported that uric acid cleans up the toxic reactants at normal levels and is protective against the oxidative stress [2]. It has been demonstrated in the studies that the level of increased serum UA is related to type 2 DM and uric acid is one of the independent predictors of type 2 diabetes. In addition, in certain studies it is reported that hyperuricemia is common in the patients with chronic renal failure and has an important role in the development and progression of chronic renal disease; it is related to persistent macroalbuminuria and microalbuminuria in diabetic patients [4,23], can contribute to the formation of diabetic nephropathy and is one of the independent risk factors of renal dysfunction in diabetic patients [5]. It has also been demonstrated in the studies that a high level of serum uric acid which is reported to be harmful to the kidneys, induce the endothelial dysfunction and leads to glomerular hypertension and renal hypertrophy; and it decreases the renal perfusion by stimulating the vascular smooth muscle cells in arteriolo[5,24,25]. It was found in the studies that UA, which was known as antioxidant, caused renal damage although it was expected to have positive effects on the renal functions. In our study, we also found that the increased serum UA levels has a negative correlation with GFR, and are one of the independent predictors of GFR <60 mL/ min / 1.73 m2. It is mentioned in some studies that the antioxidants may act as the prooxidants in certain cases [25,26]. It is also known that uric acid acts as an antioxidant in the early stages of atherosclerotic process, and is the most powerful predictor of plasma antioxidant capacity [5,26]. However, the case of being antioxidiant in the late stages of atherosclerotic process paradoxically turns into being prooxidant. This paradoxical situation depends on several environmental factors such as the process, tissue and substrate localization, acidity, oxidative environment, the other local antioxidant depletion and the release and presence of oxidants and enzymes to the medium [26]. Diabetic nephropathy and GFR decline are seen in the advanced stages of diabetes. It is also reported in some studies that UA may act as a pro-oxidant if it increases 1/3 or more than the normal levels.

Uric acid may turn into prooxidant in the patients with diabetes in which the complications develop like in the atherosclerotic process [27]. Zapolski et al. also report as a result of their studies that a high level of uric acid is related to the pro-inflammatory markers; and the relation with inflammation may cause to renal dysfunction in the patients with metabolic syndrome and coronary artery disease [28]. There is a need for studies to explain the etiopathogenesis of renal damage uric acid causes in the patients with diabetes.

Many studies show that inflammation plays an important role in the development of diabetic nephropathy. The inflammatory cytokines play an important role at every stage from the development of acute renal failure to the chronic renal disease. The destructive enzymes released as a result of the stimulation of the inflammatory cells (macrophages, natural cellar, neutrophils, etc.) for any reason (ischemia, exposure to sepsis or nephrotoxic agents) lead to the structural and the functional changes in endothelial and tubular epithelium; and the inflammatory pathways have an important role in the progression of renal damage as well as in harming the vascular permeability and the endothelial functions and inducing the acute renal damage [11-13,29]. In a number of studies in recent years, it is detected that NLR is a non-specific marker of the systemic inflammation and related to many cardiac and non-cardiac diseases [30]. Azab et al. suggest that NLR is not only a marker of presence of nephropathy but a factor in the pathogenesis of the disease [31]. In another study, it was found that NLR predicts albuminuria in diabetic patients [32]. On the other hand, it was observed in another study that no correlation existed between the inflammatory marker of CRP levels and diabetic nephropathy (GFR 60 mL/min / 1.73 m2) [31]. In this study, we found that when compared the ones with the estimated GFR <60 with the ones with GFR >60, NLR significantly increased in the ones with the estimated GFR <60, but didn’t independently predict the estimated GFR <60. Considering the fact that the onset and the progression of diabetic nephropathy is related to many mechanisms, we think that there is a reason for NLR was not found to be an independent predictor in our study.

Diabetic nephropathy is one of the major complications of diabetes and is formed with many complex pathophysiological mechanisms. It was demonstrated in the studies that oxidative stress and inflammation can stimulate each other [33,34]. It is difficult to know which pathophysiological mechanisms dominate in which patient. This makes difficult the early prediction of diabetic nephropathy, and therefore the treatment decision. In the view of these results, we believe that the application of treatments with anti-inflammatory and anti-oxide from the properties may be worthy for further investigation in the prevention of early intervention or progression of DM nephropathy.

**Limitations**

This is an observational, single-institution study that had a relatively small sample size and thus was subject to various unaccounted confounders inherent in such an analysis. Additionally, we could not compare NLR with other inflammatory markers, CRP, fibrinogen, or myeloperoxidase, because they were not routinely measured in our study population.
Conclusion
A high level of UA may be used as one of the independent pre-
dictor of diabetic nephropathy in the patients with DM (GFR
<60 ml / min / 1.73 m²). Although there is a significant negative
correlation between GFR and NLR, NLR does not predict GFR
<60 ml / min / 1.73 m². There is need for more studies to ex-
plain the etiopathogenesis of diabetic renal damages.

Scientific Responsibility Statement
The authors declare that they are responsible for the article’s
scientific content including study design, data collection, analy-
ysis and interpretation, writing, some of the main line, or all of
the preparation and scientific review of the contents and ap-
proval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with
the ethical standards of the institutional and/or national re-
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Conflict of interest
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