Original Article

Study of Neutrophil-lymphocyte Ratio as Novel Marker for Diabetic Nephropathy in Type 2 Diabetes

Sagar Ashokrao Khandare, Sachin Chittawar, Nitin Nahar, T. N. Dubey, Zahraan Qureshi[1]

Department of Medicine, Gandhi Medical College and Hamidia Hospital, Bhopal, Madhya Pradesh, [1]Medical Affairs, Zydus Discovery, Ahmedabad, Gujarat, India

Abstract

Introduction: Diabetic nephropathy (DN) is a microvascular complication of diabetes. DN is clinically manifested as an increase in urine albumin excretion. Total white blood cell count is a crude but sensitive indicator of inflammation and studied in many cardiac and noncardiac diseases as an inflammatory marker such as acute myocardial infarction, stroke, and heart failure. In this study, the association of neutrophil-lymphocyte ratio (NLR) with DN is studied. **Patients and Methods:** It is an observational cross-sectional study. Totally 115 diagnosed type 2 diabetes mellitus patients were registered in this study. NLR was calculated by analyzing differential leukocyte count in complete blood picture. Albuminuria was tested by MICRAL-II TEST strips by dipstick method. **Results:** Totally 115 diabetic patients were registered. About 56 patients had DN and 59 had normal urine albumin. Mean NLR for a normal group is 1.94 ± 0.65 and in DN group is 2.83 ± 0.85 which was highly significant ($P < 0.001$). Estimated glomerular filtration rate ($P = 0.047$) and serum glutamate pyruvate transaminase ($P < 0.001$) were also significant. **Conclusion:** The results of our study show that there was a significant relation between NLR and DN. Therefore, NLR may be considered as a novel surrogate marker of DN in early stages.

Keywords: Diabetic nephropathy, inflammation, microvascular, neutrophil-lymphocyte ratio, urine albumin

Introduction

Diabetes mellitus is a systemic disease having serious microvascular and macrovascular complications. Microvascular complications include diabetic nephropathy (DN), diabetic retinopathy, and diabetic neuropathy while macrovascular complications include stroke, cardiovascular diseases (CVDs), and peripheral vascular diseases.[1]

DN is a common micro-angiopathic complication in patients with diabetes. DN is one of the most common causes of end-stage renal disease (ESRD).[2] DN is clinically manifested as increased albumin urea excretion starting from microalbuminuria to macroalbuminuria and eventually ESRD.[3] However, the degree of albuminuria is not necessarily linked to disease progression in patients with DN associated with either type 1 or type 2 diabetes mellitus (T2DM).[4,5] In type 1 diabetes, once overt DN develops, there is persistent proteinuria, and progression toward ESRD could only be slowed but could not be stopped.[6,7] Due to this, there is a need of early predictors of DN by which we can predict the disease and can halt the progression of the disease. The Asian Indian population has more prevalence of DN as compared to the Caucasian population.[8]

Several studies that have explored the relationship between systemic inflammation and vascular disease indicated that chronic inflammation promotes the development and acceleration of micro- and macro-angiopathic complications in patients with diabetes. Total white blood cell (TWBC) count is a crude but sensitive indicator of inflammation which can be done easily in laboratory routinely. It is a cost-effective investigation. Increase in the neutrophil count is seen in thrombus formation and ischemic diseases. The neutrophil-lymphocyte ratio (NLR) in complete blood count is studied in many cardiac and noncardiac diseases as an inflammatory marker and is used to predict the prognosis of diseases such as acute myocardial infarction (MI), stroke, and heart failure.[9,10]

Address for correspondence: Dr. Sachin Chittawar, Department of Medicine, Gandhi Medical College and Hamidia Hospital, Bhopal, Madhya Pradesh, India.
E mail: chittawarsachin@gmail.com

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DN in T2DM has an inflammatory pathology. Many inflammatory markers have been found to be related to DN, such as interleukin-1 (IL1), IL6, IL8, transforming growth factor beta 1, tumor necrosis factor-alpha (TNF-α), and cytokines. However, their measurement is not used routinely as it is not easy to do it.[11,12] In this respect, NLR has emerged as a novel surrogate marker.

In the present study, the association of NLR with DN in Indian patients is studied, whether or not NLR can be used as a surrogate marker of DN in this population.

Patients and Methods

An observational cross-sectional study was done from March 2015 to March 2016 in patients referred to the endocrine outpatient department. All diagnosed T2DM patients were included in this study. Patients with type 1 DM; patients with infections, for example, urinary tract infection (UTI), upper respiratory tract infection, lower respiratory tract infections, gastrointestinal infection, otitis media, viral hepatitis, pyrexia of unknown origin, parasitic infection, viral infection, tuberculosis, local infection, skin infection, AIDS; patients with systemic disorder such as CVD, chronic kidney disease (CKD), chronic liver disease, blood disorders, autoimmune disorders, malignancy, poisoning; patients on anti-inflammatory drugs, systemic or topical steroids, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, alcohol; patients with uncontrolled blood pressure (BP); patients having diseases affecting urinary protein excretion as nephritic syndrome, urolithiasis, renal insufficiency, renal artery stenosis, dehydration state, and UTI; and patients having low glomerular filtration rate (GFR) without microalbuminuria were excluded from the study.

Diagnosed T2DM patients were screened. Information about duration of DM, treatment history, age, sex, smoking, alcohol intake history, family history, and other chronic illness was collected. Complete physical examination was done including height, weight, waist to hip ratio (WHR), body mass index (BMI), pulse rate, BP, cyanosis, clubbing, pallor, icterus, jugular venous pressure, acanthosis, and other abnormal signs were noted such as dehydration and pyrexia.

Routine blood investigations such as complete blood picture, liver function tests (LFTs), kidney function tests, urine routine microscopy, urine microscopy, lipid profile, fasting blood sugar (FBS)/postprandial blood sugar (PPBS), chest X-ray, glycated hemoglobin (HbA1c), and ultrasonography of the abdomen with kidney size were done. Fundus examination was done to assess diabetic retinopathy; nerve conduction velocity of all limbs was done to assess for diabetic neuropathy.

Albuminuria was tested by MICRAL-II TEST strips by dipstick method. Urinary albumin excretion (UAE) of 20–200 mg/L was assessed as microalbuminuria. It is a semi-quantitative method of urine albumin analysis. Test was repeated within 3 months of interval.

Evaluation of DN was done by examining urine for albuminuria. According to the American Diabetes Association and Mogensen DN diagnostic criteria, DM patients with UAE ratio reaching 20 mcg/min to 200 mcg/min or 30 mg/day to 300 mg/day are considered to have early stage of DN.[13-15] GFR was calculated using CKD-Epidemiology Collaboration (CKD-EPI) formula. SPSS statistical software (SPSS for Windows, version 20.0; SPSS, Inc., Chicago, IL, USA) was used for statistical calculation. Ethical approval for this study was provided by the Instructional Ethics Committee, Gandhi Medical College, Bhopal.

Results

In this study, diagnosed T2DM patients were screened for DN.

A total of 115 diabetic patients were registered. Of these, 56 patients had DN and 59 had normal urine albumin. All these patients were similar in their age distribution, dietary habits, smoking and alcohol consumption, and other profiles. These groups were compared for various variables such as age, BMI, WHR, BP, total leukocyte count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), NLR, serum creatinine, blood urea, serum glutamate pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), FBS, PPBS level, total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very LDL (VLDL), and HbA1c.

In the present study, the mean age of patients of normal group and DN group was 52.29 ± 11.45 years and 50.05 ± 11.29 years, respectively. Both groups had similar distribution of age ($P = 0.294$). In addition, there was no sex-related variability (in normal group, male = 26, female = 33, and in DN group, male = 25, female = 31) ($P = 0.951$). Metabolic and laboratory parameters such as BMI, lipid profile (total cholesterol, TG, HDL, LDL) and glycemic parameters (blood sugar level [BSL] fraction unbound in the plasma FBS, BSL PPBS, HbA1c) were compared in both groups and are shown in Table 1. There was a significant difference between the normal group and DN group with relation to NLR ($P < 0.001$), but individually, the TWBC count did not differ in the two groups [Table 2]. There was also a significant difference between normal group and DN group with relation to ANC ($P < 0.001$) and ALC ($P < 0.001$) [Table 2]. In the present study, renal function tests of all patients were carried out, and estimated GFR (eGFR) was calculated by CKD-EPI formulae. In relation to eGFR, there was a significant difference between the two groups ($P = 0.047$) [Table 3]. Patients with albuminuria had a significantly low eGFR (mean eGFR = 85.71 ± 27.72) than the normal group (mean eGFR = 96.2 ± 28.23). However, other investigations such as blood urea and serum creatinine had no difference in these two groups [Table 3]. LFTs for all patients were carried out, and SGPT (mean SGPT = 40.89 ± 36.62) was found to be significantly raised in DN patients' group as compared to normal patients' group (mean SGPT = 9.74 ± 13.03), which

Table 1: Comparison of demographic and laboratory parameters of diabetic patients

Variable	Patients with albuminuria (*n*=56)	Patients without albuminuria (*n*=59)	*P*
Age	52.29±11.45	50.05±11.29	0.2942
Gender			
Female	31	33	0.951
Male	25	26	
BMI	26.25±4.41	26.07±4.11	0.8208
Waist	90.7±7.11	90.98±6.74	0.8247
Hip	98.27±6.7	97.2±7.3	0.4177
WHR	0.93±0.08	0.94±0.1	0.3243
Hb (g%)	11.99±1.23	12.46±1.68	0.0987
FBS	173.45±41.99	160.14±41.99	0.092
PPBS	205.13±46.56	197.17±42.93	0.3425
Total cholesterol	178.34±41.44	182.53±57.59	0.7423
TG	159.63±56.19	175.57±112.87	0.48
HDL	37.44±7.97	41.47±12.82	0.1397
LDL	94.33±43.49	95.89±48.76	0.8944
VLDL	39.72±20.16	38.03±23.87	0.7635
BSL FUP FBS	148.17±23.1	140.4±22.93	0.0769
BSL PPBS	179.27±20.69	177.1±19.72	0.5674
HbA1c	8.49±1.59	7.79±1.16	0.0611

Statistically significant (P<0.05). BMI: Body mass index, WHR: Waist-to-hip ratio, Hb: Hemoglobin, FBS: Fasting blood sugar, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HbA1c: Glycated hemoglobin, PPBS: Postprandial blood sugar, BSL: Blood sugar level, FUP: Fraction unbound in the plasma

Table 2: Neutrophil-lymphocyte ratio and other laboratory parameters of diabetic patients

Variable	With nephropathy	Without nephropathy	*P*
NLR	2.83±0.85	1.94±0.65	0.00001
TLC	7580.36±1883.46	7384.75±1445.42	0.5321
ANC	5293.91±1479	4653.51±1185.93	0.0115
ALC	1947.93±580.73	2449.93±580.18	<0.001

Statistically significant (P<0.05). NLR: Neutrophil-lymphocyte ratio, TLC: Total leukocyte count, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count

was highly statistically significant (P < 0.001) [Table 4]. In reference to glycemic parameters, we did not observe any significant difference with respect to FBS (P = 0.0769), PPBS (P = 0.5674), and HbA1c (P = 0.06) in the two groups, i.e., normal diabetic patients and patients with DN. In our study, by applying linear regression analysis, we found HBA1c as a risk factor for DN.

Discussion

The key finding of this study was that NLR levels were found to be significantly associated (P = 0.001) with patients who were diagnosed with early-stage DN as compared to those with normal albumin levels. This study is one of the first in India to assess the relationship between NLR and DN.

Table 3: Renal function test of diabetic patients

Variable	With nephropathy	Without nephropathy	*P*
Serum urea	29.46±14.83	25.39±11.84	0.1053
Serum creatinine	0.89±0.34	0.79±0.26	0.0801
GFR	85.71±27.72	96.2±28.23	0.047

Statistically significant (P<0.05). GFR: Glomerular filtration rate

Table 4: Liver function test of diabetic patients

Variable	With nephropathy	Without nephropathy	*P*
Bilirubin	4.92±17.83	0.63±0.2	0.0674
SGPT	40.89±17.08	29.74±13.03	<0.001
SGOT	41.25±36.62	32.16±12.93	0.0826

Statistically significant (P<0.05). SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamic oxaloacetic transaminase

Over the past decade, multiple studies have shed light on the role and importance of inflammatory molecules (such as adipokines, chemokines, adhesion molecules, and cytokines) and endothelial dysfunction in the development of insulin resistance, diabetes, and its various complications.[16-21] The exact pathogenesis of DN is unknown. However, a cascade of pathological events (with glomerular damage being an early sign, which gives rise to proteinuria, followed by progressive renal damage, fibrosis, inflammation, and finally loss of functional nephrons) is known to play an important role in the development and progression of DN.[21-25] Renal inflammation in the setting of DN is known to play a critical role.[21] WBC count and its subtypes are among the readily available and inexpensive classic inflammatory markers.[26] Multiple studies have established that inflammatory markers such as neutrophilia and relative lymphocytopenia are independent markers of many diseases, especially complications of DM, such as DN.[10-12,20,21,27] However, establishing a diagnosis individually based on WBC, neutrophil, or lymphocyte counts has its own biases, unlike NLR, which is a dynamic parameter that has a higher prognostic value.[11,12,28]

NLR is a novel marker of chronic inflammation that exhibits a balance of two interdependent components of the immune system; neutrophils that are the active nonspecific inflammatory mediator forms the first line of defense whereas lymphocytes are the regulatory or protective component of inflammation.[29] Interestingly, NLR has been found to have a positive relation with not only the presence but also the severity of metabolic syndrome.[30] A study by Imtiaz *et al*.[31] has suggested that chronic diseases such as hypertension and diabetes have a significant association with systemic inflammation, reflected by NLR. Shiny *et al*.[32] have shown that NLR is correlated with increasing severity of glucose intolerance and insulin resistance and can be used as a prognostic marker for macro- and micro-vascular complications in patients with glucose intolerance. Initially, NLR was recognized as a predictive marker in multiple types of cancer that might assist in patient stratification and individual risk assessment.[33-35] But recently,

multiple other studies have indicated that NLR might be a predictive marker for vascular diseases also. Tsai *et al*.[36] had shown that NLR correlated strongly with the risk of ischemic CVDs. Other studies have shown NLR to be an independent predictor of major adverse cardiac events in patients with MI,[37] and NLR was also associated with poor survival rates after coronary artery bypass grafting.[12]

Recently, several studies have suggested that NLR could play a predictive role for assessing the development of microvascular complications of diabetes. In a study, Ulu *et al*.[38] demonstrated NLR to be a quick and reliable prognostic marker for diabetic retinopathy and its severity. Another recent study by Ulu *et al*.[27] concluded that NLR can be considered as a predictive and prognostic marker for sensorineural hearing loss in diabetic patients. A study conducted in geriatric population also suggested that increased NLR levels were in itself an independent predictor for microvascular complications of DM.[39]

In CKD patients, NLR has shown to be an easy and inexpensive laboratory parameter that provides significant information regarding inflammation.[40] Moreover, in a 3-year follow-up study of diabetic patients, NLR served as a predictor of worsening renal function.[41] Afsar has shown that NLR could be related to DN and is also correlated as an indicator of ESRD.[42] In another study, Akbas *et al*.[43] have shown that NLR was significantly elevated in patients with increased albuminuria pointing toward a relationship between inflammation and endothelial dysfunction in diabetics with nephropathy.

Similarly in our study, the mean NLR among diabetic patients with albuminuria (2.83 ± 0.85) was significantly higher than among those without albuminuria (1.94 ± 0.65). In addition, ANC and ALC levels were also found to be significantly correlated with patients with albuminuria. In concordance with our results, Huang *et al*.[44] have also found that NLR values were significantly higher in diabetic patients with evidence of nephropathy (2.48 ± 0.59) than in diabetic patients without nephropathy (2.20 ± 0.62) and healthy controls (1.80 ± 0.64). ANC and ALC levels were also found to correlate with DN in their study. Moreover, a recent study in Egyptian patients has shown that NLR values were significantly higher in diabetic patients with retinopathy ($P < 0.001$), neuropathy ($P = 0.025$), and nephropathy ($P < 0.001$) than those of diabetic patients without any microvascular complications and healthy controls.[45] Another recently published study in Turkish patients has also shown that NLR levels significantly increased in parallel to albuminuria levels in diabetic patients.[46]

There was no significant correlation between normal and DN groups in relation to age, sex, BMI, WHR, Hb, total cholesterol, LDL, TG, HDL, and VLDL as observed in the present study although there was significant difference among the two groups in respect to eGFR values ($P = 0.047$). Patients with albuminuria had significantly low eGFR (mean eGFR = 85.71 ± 27.72) as compared to those patients with normal albumin levels (mean eGFR = 96.2 ± 28.23). eGFR is one of the most specific parameters for kidney function.[47] In case of DN, eGFR decreases as disease progresses. There were no significant intergroup differences for either blood urea or serum creatinine levels.

Among the LFTs, SGPT (mean SGPT = 40.89 ± 36.62) was significantly raised in patients with albuminuria as compared to patients without DN (mean SGPT = 9.74 ± 13.03) ($P = 0.0001$). In T2DM, there is loss of direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver. This in turn causes an increase in hepatic glucose production. In T2DM, hyperinsulinemia in combination with high free fatty acids and hyperglycemia is known to upregulate lipogenic transcription factors. The fatty acids overload the hepatic mitochondrial oxidation system, leading to accumulation of fatty acids in the liver. These mechanisms finally lead to nonalcoholic fatty liver disease (NAFLD) in T2DM patients. In the majority of cases, NAFLD causes asymptomatic abnormality of liver enzyme levels which include SGPT, SGOT, and ALP. Of these liver enzymes, SGPT is most closely related to liver fat accumulation and consequently SGPT has been used as a marker of NAFLD. Other studies have also identified that hyperglycemia may lead to oxidative stress and glycation reactions resulting in advanced glycation end products. Oxidative stress is also one of the factors which alter liver enzymes (SGPT, SGOT, and ALP).[48]

In reference to glycemic parameters, there were no significant differences between the two groups, in relation to FBS, PPBS, or HbA1c though other studies have shown HbA1c to be an independent risk factor for DN.[44,46] On applying linear regression analysis in the present study, HbA1c was also found to be a predictor for DN.

One of the limitations of this study is that this was a cross-sectional analysis and the sample size was relatively small. Since this was not a prospective controlled study, any conclusive causal associations between NLR and DN could not be investigated.

Conclusion

The results of our study have shown that there was a significant correlation between NLR and DN, implying that inflammation and endothelial dysfunction could be an integral part of DN. NLR was significantly and independently raised in patients with type 2 DM having increased albuminuria. Therefore, NLR may be considered as a predictor and a prognostic risk marker of DN. NLR is an easy to calculate parameter in the laboratory by observing the differential leukocyte count. This test is simple, inexpensive, and done routinely. In a setup with limited laboratory facilities, NLR is a simple test which can be an alternative for other costlier inflammatory markers such as ILs, TNF, cytokines, and high-sensitivity C-reactive protein. Further research with a prospective design and multiple NLR measurements will shed more light on the role of NLR as a marker of inflammation and a probable risk factor for DN.

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Conflicts of interest
There are no conflicts of interest.

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