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The Relationship Between Neutrophil/Lymphocyte Ratio, Albuminuria and Renal Dysfunction in Diabetic Nephropathy

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Abstract

Introduction: Diabetes mellitus has become a global public health problem due to treatment costs and attendant complications. Diabetic nephropathy which manifests as albuminuria is the precursor of end-stage renal failure and is an inflammatory process. In the recent past, it has been reported that the neutrophil/lymphocyte ratio (NLR), which is a cost-effective and accessible marker, may be a favourable indicator of the inflammatory status. The aim of this study was to investigate the relationship between the neutrophil/lymphocyte ratio and the presence and severity of diabetic nephropathy (DN).

Material and methods: A total of 200 patients with type-2 DM with nephropathy and 100 patients with normoalbuminuria were enrolled from our internal medicine and nephrology clinics between May 2018 and June 2018. Demographic parameters, biochemical parameters and albuminuria levels were recorded. Patients were divided into three groups according to their level of albuminuria.

Results: Significant differences were detected between the groups in terms of NLR (p = 0.001). A positive correlation was detected between NLR, e GFR, ESR and C-reactive protein.

Conclusions: A high degree of correlation was determined among albuminuria, glomerular filtration rate and NLR levels. These results may suggest the notion that diabetic nephropathy involves an inflammatory process.

Key words: Neutrophil/lymphocyte ratio, inflammation, albuminuria renal dysfunction, diabetic nephropathy.

Introduction

The increasing number of people with diabetes has had a major impact on the prevalence of diabetic nephropathy[1]. Diabetic nephropathy, which occurs in 20% to 40% of all patients with type 2 diabetes mellitus[2] is a metabolic disorder with high morbidity and mortality and is the leading cause of chronic kidney disease[3]. Cardiovascular mortality risk due to atherosclerosis is 10 - 20-fold higher in chronic kidney disease (CKD) patients as compared to general population[2] because of accelerated atherosclerosis[4]. The role of increased leukocyte count in the pathogenesis of atherosclerosis is well understood as it initiates a cascade of inflammatory reaction in the vessel wall. Apart from leukocyte count, inflammatory markers such as interleukin (IL)-1, IL-6, IL-8, and tumour necrosis factor-α have been linked to end organ damage in diabetes but lack of their availability in routine clinical practice is compounded by the associated expense and lack of assay standardisation. The neutrophil-lymphocyte ratio (NLR) (the neutrophil count divided by the lymphocyte count) is easily derived and serves as an indicator of systemic inflammation[5].

Ours, being a resource poor country, becoming global diabetes capital with limited laboratory facilities, we need cheap and effective markers of end organ damage. More importantly, there has been little research on the relation between WBC count and vascular complications of diabetes in our population[6], although a recent report did suggest an association between WBC count and albuminuria in type 2 diabetes[7]. Therefore, we carried-out a cross-sectional analysis to investigate the association of WBC count, a biomarker of inflammation, with severity of albuminuria and renal dysfunction in a consecutive cohort of patients with type 2 diabetes.

Material and methods

In this cross-sectional observational study, 300 consecutive patients with T2DM diagnosed according to the American Diabetes Association criteria, attending the outpatient services of medicine department between April 2018 and June 2018 in a medical college situated in North India were included[8].

The patients with T1DM, patients with current infections or recent history of infections in the past 1 month, pyrexia of unknown origin, parasitic infection, viral infection, tuberculosis, local infection, skin infection, AIDS; patients with known systemic inflammatory disorders, blood disorders, autoimmune disorders, malignancy, poisoning; patients on anti-inflammatory drugs, systemic or topical

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steroids were excluded.

An informed written consent was obtained from all the participants before recruiting them in the study and prior approval for the study was taken from Institutional Ethics Committee.

In all study participants detailed history was obtained and general and systemic examination to assess complications of diabetes was recorded. Blood was collected by venipuncture after an overnight fast for complete blood counts, glucose, HbA1c and lipid profile and was assessed by standard automated techniques. Spot urine was collected for albumin and creatinine. Level of proteinuria (according to American Diabetes Association classification[9]), the albumin/creatinine ratio in spot urine was used to classify proteinuria; no proteinuria: < 30 mg/g creatinine, microalbuminuria: 30 - 300 mg/g creatinine, macroalbuminuria: > 300 mg/g creatinine. The estimated glomerular filtration rate (eGFR) was used for assessment of renal function which was calculated by the Modification of Diet in Renal Disease (MDRD) equation. A simplified MDRD equation was used to estimated GFR (ml/min/1.73 m^2) = 175 x (serum creatinine)$^{-1.154}$ X (age)$^{-0.203}$ X (0.742, if female)

Statistical analysis: Statistical package for the social sciences (SPSS) software version 17.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. For comparison of groups and data, Kolmogorov-Smirnov test was used to check the suitability of data to normal distribution.

For investigation of difference between the parametric data of two groups, Student's *t*-test was used if data was suitable to normal distribution and Mann-Whitney U-test was used in case of nonsuitability of data to normal distribution. For the difference between the categorical data of two groups Chi-square test had been applied. For correlation analysis, Spearman correlation or Pearson's correlation test was used.

Results

In this cross-sectional study, 300 patients of type-2 diabetes were studied, which included 200 patients with nephropathy who had micro or macroalbuminuria and 100 patients had nomoalbuminuria who served as controls. Out of them, 54% were males and 46% were females. The mean age of our patients was 48 ± 4.6 years, and the duration of diabetes was 8.4 ± 5 years. The mean WBC count was 7.2 ± 1.7 x 10^3/mm^3.

Table I summarises the demographic, anthropometric, and metabolic characteristics of the study participants. Patients with macroalbuminuria had significantly longer duration of diabetes and increased prevalence of hypertension.

Table II depicts biochemical parameters and NLR of study participants. The patients with increasing level of albuminuria had significant decline in eGFR and LDL cholesterol. They had significantly higher WBC count and NLR.

Table I: Clinical parameters of study participants.

Parameter	Normoalbuminuria (n = 100)	Microalbuminuria (n = 129)	Macroalbuminuria (n = 71)	P value
Age (years)	48.6 ± 4.2	47.6 ± 5.8	48.2 ± 54.8	0.34
Duration of diabetes (years)	8.4 ± 3.2	10.5 ± 5.6	14 ± 6.8	0.02
Waist circumference (cm)	89.4 ± 3.2	88.6 ± 2.6	89.6±3.2	0.12
BMI (kg/m^2)	24.7 ± 3.4	24.1 ± 4.1	23.2 ± 3.9	0.56
Blood pressure > 140/90 mmHg (%)	32%	45%	82%	0.001

Data is shown as mean ± SD, BMI: Body mass index

Table II: Biochemical parameters of study participants.

Parameter	Normoalbuminuria (n = 100)	Microalbuminuria (n = 129)	Macroalbuminuria (n = 71)	P value
HbA1c (%)	8.0 ± 1.3	7.6 ± 0.7	7.3 ± 0.6	0 - 23
Creatinine (mg/dl)	1.1 ± 0.2	1.1 ± 0.3	1.6 ± 0.5	
HDL-cholesterol (mg/dl)	36.9 ± 4.8	36.4 ± 5.3	36.3 ± 7.1	0.7
Triglyceride (mg/dl)	98.8 ± 13.6	125.6 ± 34.6	122.7 ± 13.2	0.12
LDL-cholesterol (mg/dl)	123.2 ± 20.4	108 ± 58.2	112.0 ± 44.0	0.07
eGFR (ml/min)	118.2 ± 32.4	101 ± 28.2	88.0 ± 34.2	0.01
WBC count (x10^3/mm)	6.5 ± 1.8	8.2 ± 2.3	8.8 ± 2.5	0.001
Neutrophil count (× 10^9/l)	4.2 ± 1.4	4.7 ± 1.5	5.4 ± 1.8	0.012
Lymphocyte count (× 10^9/l)	2.5 ± 0.8	1.8 ± 0.7	1.6 ± 0.6	0.05
NLR	1.6 ± 0.8	2.8 ± 1.0	4.2 ± 1.3	0.001

Data is shown as mean ± SD, HbA1c: Glycosylated haemoglobin, HDL: High density lipoprofein, LDL: Low density lipoprofein, eGFR: estemated Glomerular filtration rate, WBC: White blood cell, NLR: Neutrophil-to-lymphocyte ratio.

Subjects with higher NLR had longer disease duration, higher systolic blood pressure, diastolic blood pressure, BMI, HbA1c, fasting plasma glucose, LDL cholesterol and lower HDL cholesterol.

In correlation analysis as shown in Table III, positive correlation was found between NLR and level of albuminuria (P = 0.002, r = 0.48), CRP (P = 0.01, r = 0.38), and ESR (P = 0.03, r = 0.33), whereas a negative correlation was detected between NLR and eGFR (P = 0.001, r = -0.418).

Table III: Correlation analysis between NLR and other parameters.

Variable	r	P value
albuminuria	0.48	0.002
ESR	0.38	0.01
CRP	0.33	0.03
eGFR	-0.41	0.001

CRP-C Reactive protein,ESR-Erythrocyte sedimentation rate.

Discussion

Cardiovascular disease due to accelerated atherosclerosis is the leading cause of death in patients of chronic kidney disease[10,11]. Inflammation, insulin resistance and endothelial dysfunction are hallmarks of diabetic nephropathy and its progression[12,13]. In the last few years, haematological indices have been proposed as potential markers of inflammation in diabetes[14].

In the present study we assessed WBC counts and NLR in type-2 diabetes patients who had nephropathy. We observed that patients with higher WBC counts and NLR had longer duration of diabetes hypertension, dyslipidaemia, poor glycaemic control and higher level of albuminuria which has been shown in other studies as well[15,16]. The patients with albuminuria who had high NLR also had higher CRP and ESR in our study.

Kocyigit *et al* demonstrated that patients with a high NLR had worse prognosis and significantly faster progression to dialysis compared to those with a low NLR[17].

Solak *et al* reported that NLR was independently related to endothelial dysfunction and could predict composite cardiovascular end-points independent of traditional confounding factors in patients with moderate-to-severe CKD[18]. Another study in newly diagnosed patients with type 2 diabetes mellitus revealed a relationship between NLR and 24 h urine protein excretion[19].

NLR may be associated with poorer prognosis in diabetic nephropathy.

In our study, decline in renal function in form of eGFR was significantly correlated with higher NLR. Huang *et al* have found that NLR was significantly higher in diabetic patients with evidence of nephropathy as compared to those without nephropathy[20]. Akbas *et al*[21] have shown that NLR was significantly increased in patients with increased albuminuria indicating an association between inflammation and endothelial dysfunction in diabetics with nephropathy. Another follow-up study of diabetic patients, showed NLR to be a prognostic indicator for a deterioration in renal function[22]. Other workers have also shown in their study that NLR values were significantly higher in diabetic patients with nephropathy than those of diabetic patients without any microvascular complications and healthy control subjects[23].

The present study shows that low grade chronic inflammation is prevalent in patients with diabetic nephropathy as evident from high NLR, ESR and CRP and may contribute to cardiovascular disease. Therefore, it can be concluded that NLR could be used as an indicator of inflammation and proteinuria in CKD. Chronic inflammation, as indicated by a higher WBC count, may play a linkage role in the development of macro- and micro-vascular complications in diabetes. These inexpensive tests can be used in screening and as markers of inflammation. Our study had many limitations including small sample size and its cross-sectional design. Further research in large number of patients are needed to confirm our findings.

Conclusion

A high degree of correlation was determined among albuminuria, glomerular filtration rate and NLR levels. These results may suggest the notion that diabetic nephropathy involves an inflammatory process. Thus, NLR, which is easy to access and inexpensive, may be a novel biomarker for assessing inflammation associated with diabetic nephropathy.

References

1. de Boer IH, Rue TC, Hall YN *et al*. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* 2011; 305: 2532-9.
2. Tuttle KR, Bakris GL, Bilous RW *et al*. Diabetic kidney disease: a report from an ADA consensus conference. *Diabetes Care* 2014; 37: 2864-83.
3. Collins AJ, Foley RN, Chavers B *et al*. United States Renal Data System 2011 Annual Data Report: atlas of chronic kidney disease and end-stage renal disease in the United States. *Am J Kidney Dis* 2012; 59 (1 Suppl 1): A7, e1-e420.
4. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis* 2000; 35 (4 Suppl 1): S117-31.
5. Balta S, Kurtoglu E, Kucuk U *et al*. Neutrophil-lymphocyte ratio as

an important assessment tool. *Expert Review of Cardiovascular Therapy* 2014; 12: 537-8.

6. Shiny A, Bibin YS, Shanthirani CS *et al*. Association of neutrophil-lymphocyte ratio with glucose intolerance: an indicator of systemic inflammation in patients with type-2 diabetes. *Diabetes Technol Ther* 2014; 16 (8): 524-30.
7. Khandare SA, Chittawar S, Nahar N *et al*. Study of Neutrophil-lymphocyte Ratio as Novel Marker for Diabetic Nephropathy in Type 2 Diabetes. *Indian J Endocrinol Metab* 2017; 21 (3): 387-92.
8. American Diabetes Association. Standards of medical care in diabetes: 2013. *Diabetes Care* 2013; 36 (Suppl 1): S11-S66.
9. American Diabetes Association: clinical practice recommendations 2001: Diabetic nephropathy. *Diabetes Care* 2001; 24 (Suppl 1): S69-S72.
10. Collado S, Coll E, Deulofeu R *et al*. Prevalence of cardiovascular disease in uraemia andrelevance of cardiovascular risk factors. *Nefrologia* 2010; 30: 342-8.
11. Tonelli Tonelli M, Wiebe N, Culleton B *et al*. Chronic kidney disease and mortality risk: A systematic review. J Am Soc Nephrol 2006; 17: 2034-47.
12. Lim AK, Tesch GH. Inflammation in diabetic nephropathy. *Mediators Inflamm* 2012; 2012: 146-54.
13. Astrup AS, Tarnow L, Pietraszek L *et al*. Markers of endothelial dysfunction and inflammation in type 1 diabetic patients with or without diabetic nephropathy followed for 10 years: Association with mortality and decline of glomerular filtration rate. *Diabetes Care* 2008; 31: 1170-6.
14. Twig G, Afek A, Shamiss A *et al*. White blood cells count and incidence of type 2 diabetes in young men. *Diabetes Care* 2013; 36: 276-82.
15. Kahraman C, Kahraman NK, Aras B *et al*. The relationship between neutrophil-to-lymphocyte ratio and albuminuria in type 2 diabetic patients: A pilot study. *Arch Med Sci* 2016; 12: 571-5.
16. Turkmen K, Guney I, Yerlikaya FH *et al*. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. *Renal Failure* 2012; 34: 155-9.
17. Kocyigit I, Eroglu E, Unal A *et al*. Role of neutrophil/lymphocyte ratio in prediction of disease progression in patients with stage-4 chronic kidney disease. *J Nephrol* 2013; 26: 358-65.
18. Solak Y, Yilmaz MI, Sonmez A *et al*. Neutrophil to lymphocyte ratio independently predicts cardiovascular events in patients with chronic kidney disease. *Clin Exp Nephrol* 2013; 17: 532-40.
19. Afsar B. The relationship between neutrophil lymphocyte ratio with urinary protein and albumin excretion in newly diagnosed patients with type 2 diabetes. *Am J Med Sci* 2014; 347: 217-20.
20. Huang W, Huang J, Liu Q *et al*. Neutrophil-lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy. *Clin Endocrinol (Oxf)* 2015; 82: 229-33.
21. Akbas EM, Demirtas L, Ozcicek A *et al*. Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy. *Int J Clin Exp Med* 2014; 7: 1794-801.
22. Azab B, Daoud J, Naeem FB *et al*. Neutrophil-to-lymphocyte ratio as a predictor of worsening renal function in diabetic patients (3-year follow-up study). *Ren Fail* 2012; 34: 571-6.
23. Moursy EY, Megallaa MH, Mouftah RF *et al*. Relationship between neutrophil lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. *Am J Intern Med* 2015; 3: 250-5.