

Neutrophil to lymphocyte ratio (NLR) as a surrogate marker of diabetic nephropathy

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International Journal of Frontiers in Medicine and Surgery Research, 2024, 05(02), 001–006

Publication history: Received on 03 February 2024; revised on 25 March 2024; accepted on 28 March 2024

Article DOI: <https://doi.org/10.53294/ijfmsr.2024.5.2.0029>

Abstract

Background and objectives: Diabetic nephropathy and retinopathy are microvascular complications of diabetes. Nephropathy 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 non-cardiac diseases as an inflammatory marker such as acute myocardial infarction, stroke, and heart failure. In this study, the association of neutrophil to lymphocyte ratio (NLR) with diabetic nephropathy and retinopathy.

Methods: This was a prospective observational study performed on patients with diabetic nephropathy +/- retinopathy. A total of 104 subjects diagnosed with type 2 diabetes mellitus registered in this study. NLR was calculated by analyzing differential leukocyte count in complete blood picture. Spot Albuminuria was tested by immune turbidimetry method. Urine creatinine was tested by jaffe's kinetic method by which spot urine microalbumin to creatinine ratio is calculated. ANOVA, Independent t-test, chi-square test, Karl Pearson correlation were applied to find significance. $P < 0.05$ was considered as statistically significant.

Results: Totally 104 diabetic subjects were registered, 52 subjects had DN and 52 had normal urine albumin. Mean \pm SD NLR for a normal group is 2.5 ± 1.1 and in DN group is 3.4 ± 1.4 which was highly significant ($P = 0.001$).

Conclusion: Our study showed that there was a significant relation between NLR and DN; and positive correlation between NLR and urine microalbumin to creatinine ratio. Therefore, NLR may be considered as a novel surrogate marker of DN.

Keywords: Diabetes mellitus; Diabetic nephropathy; Neutrophil to lymphocyte ratio; Urine microalbumin to creatinine ratio

1. Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that result in persistent hyperglycaemia. There are two broad categories of DM, designated type 1 and type 2. Type 1 DM is due to lack of insulin production, whereas Type 2 DM is due to insulin resistance.

The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications (coronary artery disease [CAD], peripheral arterial disease [PAD], cerebrovascular disease). Nonvascular complications include gastro-paresis, infections, skin changes, and hearing loss. [1].

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Diabetic nephropathy [DN] is the leading cause for chronic kidney disease (CKD) and requirement of renal replacement therapy.[2] People with diabetes mellitus is the fastest growing group among renal dialysis and transplant recipients. As a part of comprehensive diabetes care albuminuria should be detected at an early stage when effective therapies can be instituted. Some individuals with type 1 or type 2 DM have a decline in Glomerular filtration rate (GFR) in the absence of albuminuria hence annual measurement of the serum creatinine to estimate GFR should also be performed. [1]

White blood cell (WBC) count is a crude, cost-effective and sensitive indicator of inflammation which can be done easily in laboratory routinely. WBCs are positively associated with inflammation. [7] An increase in the number of neutrophils is associated with thrombus formation and ischemic injury. [8] The neutrophil-lymphocyte ratio (NLR) in complete blood count test is a potential marker for both cardiac and non-cardiac disorders [9–15] and is used to predict the prognosis of diseases such as acute myocardial infarction (MI), stroke, and heart failure. [14]

DN in T2 DM has an inflammatory pathology. [13] Many inflammatory markers have been found to be related to DN such as interleukins (IL1, IL6 and IL8), transforming growth factor beta-1(TGF- β 1), tumour necrosis factor-alpha (TNF- α), and other cytokines. However, their measurement is not used routinely as it is neither feasible nor economical. [12]

To the best of our knowledge, there is only one study on NLR in diabetic nephropathy in India and none in south India.[15]. In this study, the association of neutrophil to lymphocyte ratio (NLR) with diabetic nephropathy and retinopathy.

2. Methodology

This was a retrospective observational study including all the patients diagnosed with diabetic nephropathy and retinopathy previously or on admission. The study included all patients (case records) admitted between June 2021 and June 2023 in the department of Medicine and Endocrinology of a tertiary care institute.

The minimum required sample size in case group and control group is 39 each.

Institutional ethical committee approval was obtained for the study, and informed consent was obtained from all study subjects.

Informed written consent will be taken from all the subjects. A pre-structured case record form will be used to collect the data. Clinical history taking and detailed examination of the subjects will be done.

Urine creatinine is measured by modified Jaffe's method.

All patients in addition to clinical assessment will be subjected for following investigations:

- Total and differential leukocyte count
- Serum Urea and Serum Creatinine
- Urine routine and microscopic examination
- UMACR
- FPG, PPG, HbA1C
- Fundoscopy
- USG imaging of KUB

2.1. Statistical method

Data will be entered in MS EXCEL file, analyzed using SPSS software version 21. All descriptive statistics were represented with percentages, Mean with SD. ANOVA, Independent t-test, chi-square test, Karl Pearson correlation were applied to find significance. $P < 0.05$ was considered as statistically significant.

3. Results

In our study 104 patients with type 2 diabetes, 52 were cases and 52 were controls. The mean age was 56 ± 11.3 years and 50.6 ± 11.8 years in case and control group respectively. 'N' represents the number of subjects.

Among 52 subjects included in the study, 23.1% (n=12) were female and 76.9% (n=40) were male. The mean duration of type 2 DM was 7.4±6.9 years in case, the mean Total leukocyte count (TLC) in case group 8609.6±2046.0 /μL. Mean Absolute neutrophil count is 5774±1569.7 /μL. Mean Absolute lymphocyte count is 1911.5±744.8 /μL.

The minimum NLR in case group was 1.250 while the maximum NLR in case group was 6.7 with a mean NLR for case subjects was 3.4.

Mean urea value in case and control group were 40.8 mg/dl while Mean creatinine value in the study population was 1.5 mg/dl and eGFR mean value for case group was 70.0 ml/min/1.73m².

Table 1 Fundoscopic findings in study participants

Fundoscopy	Frequency	
	N	%
Normal	65	62.5%
Mild	9	8.65%
Moderate	17	12.14%
Severe	6	5.76%
Total	104	100.0%

Fundoscopy examination was done in all patients to look for retinopathy. It was normal in 65 patients, while NPDR was present in 39 patients.

Table 2 Comparison of various variables with fundoscopy

Variable	Fundoscopy	Mean	SD	P-value
NLR	Normal	2.75	1.17	0.17
	Mild	3.05	1.55	
	Moderate	3.49	1.69	
	Severe	3.33	0.76	
eGFR in ml/min/1.73m ²	Normal	94.73	35.24	<0.001
	Mild	83.42	34.01	
	Moderate	66.67	40.11	
	Severe	81.83	37.98	

Mean NLR in subjects with moderate to severe NPDR was >3.0, while <3.0 in normal and mild NPDR. Mean eGFR decreases with increase in severity of NPDR as indicated by significant p value <0.001. UMACR increases as the severity of retinopathy increases (p=0.04).

Table 3 Distribution of NLR in case and control group

NLR	Case	
	N	%
0--1	1	0.9%
1--2	7	6.73%
2--3	35	33.66%
>3	44	42.31%
Total	104	100.0%

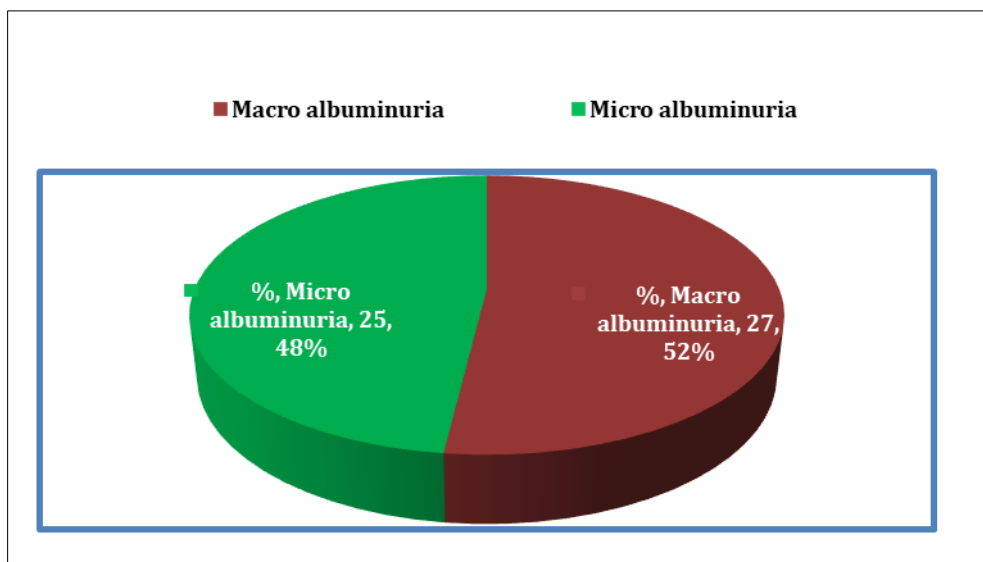


Figure 1 Distribution of macro and microalbuminuria

Among total 52 subjects in case group 51.9% (n=27) had macroalbuminuria and 48.1% (n=25) had microalbuminuria

Among 52 case subjects USG abdomen suggests 43 were normal, 5 had grade I medical renal disease, 3 had grade II medical renal disease, 1 had grade III medical renal disease.

4. Discussion

DM is a most common metabolic disorder associated with DN; it is the leading cause for chronic kidney disease and requirement of renal replacement therapy. [2] India is known as diabetic capital of the world due to its high prevalence 8.7% in the age group of 20 to 70 years. [3] Progression of DN can be arrested by good glycemic control and measures to decrease proteinuria by Angiotensin converting enzyme inhibitors and Angiotensin receptor blockers. Hence early detection and screening of diabetic patients for DN is important to prevent major morbidity and mortality.

NLR is a non-specific marker, but in remote and smaller health care centers facilities for measurement of microalbuminuria are not available there is a need for simple marker for DN. NLR is a novel, simple, cost-effective surrogate marker of renal or glomerular injury, which can be used in such areas. In this respect NLR is a helpful surrogate marker for diabetic nephropathy.

Several studies have shown a good correlation between NLR and diabetic end organ damage. Our study also revealed statistically significant high NLR in subjects with advanced albuminuria in diabetic subjects. We had 52 diabetic subjects with albuminuria > 30mg/g creatinine (cases) and equal number of diabetic subjects with albuminuria < 30 mg/g creatinine (control). Mean age of subjects in 2 groups did not differ much. There was male preponderance in both case and control as shown in table 3. Mean duration of DM was more in cases than control. It is expected as degree of albuminuria is directly related to duration of disease.

Blood sugar levels in both the groups were high without much difference, HbA1C in % being 8.25 ± 1.31 and 8.41 ± 1.41 in cases and controls respectively ($p=0.556$). Serum urea and creatinine were significantly higher in cases as compared to controls with a p value of <0.001 . We observe that renal parameters worsen and NLR increases linearly with degree of albuminuria. The eGFR was calculated by the modification of diet in renal disease (MDRD) equation. Patients with albuminuria had significantly low eGFR (mean eGFR = 70.0 ± 36.4 ml/min/1.73m²) as compared to those patients with normal albumin levels (mean eGFR = 97.2 ± 58.7 ml/min/1.73m²). eGFR is one of the most specific parameters for kidney function.[9] Mean eGFR decreases as disease progresses as shown in table 14, with a significant P value of <0.001 .

Diabetic nephropathy is a common diabetic complication, but its pathogenesis remains unclear. Several studies have reported an association between the presence of diabetic nephropathy and states of chronic inflammation. [10] Few studies examined the relationship between white blood cells (WBC) count and urinary albumin excretion in diabetic patients and demonstrated that higher WBC counts were related to increased urinary albumin excretion rates. [11-13]

There is a need for surrogate marker of inflammation in all such cases to assess the complications. Interestingly, NLR has been found to have a positive relation with not only the presence but also the severity of metabolic syndrome. [10]

LR is a novel marker of chronic inflammation that exhibits a balance of two interdependent components of the immune system. Neutrophils that are the active non specific inflammatory mediator form the first line of defence, whereas lymphocytes are the regulatory or protective component of inflammation. [11] Several studies have found relative increase in ANC and decrease in ALC. NLR appears more sensitive indicator of inflammation less affected by physiological and physical factors.

An observational cross sectional study was done by Khandare SA, et al (2016) in India on 115 type 2 diabetes mellitus patients, 56 patients had diabetic nephropathy 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 ($P = 0.047$) were also significant. [11]

In agreement with our results, Huang W et al, from China, have also found that neutrophil-lymphocyte ratio values were significantly higher in diabetic patients with nephropathy (2.48 ± 0.59) than in diabetic patients without nephropathy (2.20 ± 0.62) and healthy controls (1.80 ± 0.64). [12]

A case control study done by Moursy EY, et al (2015) in Egypt which included 80 healthy volunteers and 200 subjects with type 2 diabetes mellitus among which 108 patients had at least one microvascular complication. Study has shown that mean NLR in patients were 1.41 ± 0.30 , 2.29 ± 1.07 , 2.52 ± 1.21 , for diabetic patients without complications, micro and macroalbuminuria groups respectively ($p < 0.001$). [13]

A study done by Moursy EY, et al in Egypt classified the severity of diabetic retinopathy, into non-proliferative and proliferative retinopathy. The mean NLR was higher in patients with proliferative diabetic retinopathy (2.79 ± 1.45) than in patients with non-proliferative diabetic retinopathy (2.25 ± 0.72) the P value was statistically significant between patients with and without retinopathy. [14]

We also did USG abdomen which suggested that the majority were normal, 5 had grade I medical renal disease, 3 had grade II medical renal disease, 1 had grade III medical renal disease. Vibration sensation tested clinically was decreased, in 65.4% ($n = 34$) of case group, which requires further evaluation for diabetic neuropathy,

The potential correlations between albuminuria levels, GFR, and NLR in diabetic patients were looked at in a study by Kahraman C et al. [13]. Albuminuria levels were strongly connected negatively with neutrophil/lymphocyte ratio, which was considerably correlated positively with CRP and significantly negatively with GFR. These findings confirm the existence of an inflammatory process in DN and suggest that NLR may serve as a marker for this process. In a study by Akbaş et al., it was found that the neutrophil to lymphocyte ratio was higher in the diabetic patient group, whereas there was no significant difference in total lymphocyte count. [15].

5. Conclusion

Our study has shown that there is a significant correlation between NLR and DN

NLR may be considered as a predictor and a prognostic risk marker of DN implying that inflammation could be an integral part of DN

NLR can be taken as a surrogate marker of renal injury and albuminuria.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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