

Neutrophil Lymphocyte Ratio as a Marker of Diabetic Nephropathy

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ABSTRACT

BACKGROUND

Diabetic Nephropathy is a potentially serious complication of diabetic mellitus which can lead to renal failure. This is manifested as increased urinary albumin excretion ranging from microalbuminuria to overt macroalbuminuria and eventually end stage renal failure. Most of the current tests can identify the condition only after the damage to the kidney has occurred. Thus, there is a need for early predictors of diabetic nephropathy which are cheap and easily accessible to the population in a resource constrained country like India. Neutrophil Lymphocyte Ratio (NLR) is one such test which was evaluated for its identification of diabetic nephropathy.

METHODS

127 diabetic patients attending a tertiary care center were enrolled in this cross sectional prospective observational study. Glycaemic indices and neutrophil lymphocyte ratio were estimated, and statistical analysis was done.

RESULTS

The mean eGFR of the study population was 91.6 ± 48.7 mL/min. The mean protein creatinine ratio (PCR) was 0.8 ± 0.4 . Correlation analysis revealed a strong positive correlation between the urinary PCR and NLR which was also statistically significant ($r = 0.813$, $p < 0.0001$). NLR cut off of 7 had a sensitivity and specificity of 88.89% and 94.9% respectively with a positive likelihood ratio of 17.481 and a negative likelihood ratio of 0.117.

CONCLUSIONS

NLR is a simple, easily available and cost effective investigation which can be used as a screening tool to detect diabetic nephropathy at an earlier stage.

KEYWORDS

Diabetic Nephropathy, Inflammation, Lymphocyte, NLR, Neutrophil

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BACKGROUND

According to the International Diabetes Federation, in the year 2019, 463 million adults around the world were suffering from diabetes mellitus (DM) and it is estimated that the disease burden will reach around 700 million by the year 2045. Currently, India stands at the second position after China, with an estimated 77 million persons affected by diabetes.¹ Diabetes is the leading cause of Chronic Kidney Disease (CKD) among adults in India and with the prevalence of diabetes expected to increase in the coming years, the magnitude of both CKD and End Stage Renal Disease (ESRD) is also likely to increase and contribute to the increase in morbidity and mortality.² In the Global Burden of Disease study, CKD was ranked globally 16th and 8th in India among the causes of deaths.³

It has been estimated that more than 40% of diabetics will develop CKD and a significant number will develop ESRD requiring renal replacement therapy (RRT). Diabetic Nephropathy (DN) is a microvascular complication of DM and is known to be the leading cause of ESRD in India.⁴ Diabetic nephropathy usually manifests first as microalbuminuria and later progressing to macroalbuminuria. Microalbuminuria is considered to be an early marker of DN. The progression of DN from overt proteinuria to renal failure is usually irreversible.⁵ Therefore, detection of micro albuminuria as early as possible in the course of the disease becomes very important. The American Diabetes Association (ADA) specifies that there must be a regular screening for diabetic nephropathy.⁶

Inflammatory processes play a crucial role in the pathogenesis of complications of type 2 diabetes. They also precede the onset of the disease substantially and may have a role as an early marker. Subclinical inflammation contributes to further worsening of the metabolic disturbances and development of vascular complications.^{7,8} There is also evidence to suggest that chronic inflammation can contribute to both development and acceleration of micro and macro angiopathy in diabetic patients.⁹

The current gold standard marker for early detection of DN is detection of microalbuminuria but it is not a sufficiently accurate predictor of DN due to some of its own limitations.¹⁰ Many biomarkers of diabetic nephropathy (classified as glomerular markers, tubular markers, oxidative stress markers, inflammation and miscellaneous markers) have been identified which can precede the detection of microalbuminuria but still lack the clinical utility in identifying the condition.¹¹ Thus it is possible that the renal damage could have started long before the detection of microalbuminuria and thus potential interventions may be delayed in such patients. So there is an acute need for early predictors of diabetic nephropathy which are reliable. Biomarkers of inflammation such as TNF- α and IL-1 β , which are involved in the onset and progression of DN, have been shown to have a predictive role in the early identification of DN.¹² But these are not routinely used in India as they are quite expensive, not easily available and unapproachable in the primary care setting in India. Hence there is a need for

an inexpensive and readily available investigation which can act as a marker for the inflammatory process.

One such investigation, which has been evaluated by this study is the Neutrophil – Lymphocyte ratio (NLR), which can be calculated through the easily available blood counts. NLR may be considered as a marker of chronic inflammation. It represents a combination of two markers; neutrophils, which represent the active nonspecific mediator initiating the first line of defense and lymphocytes, representing the regulatory or protective component of inflammation.¹³ NLR has been demonstrated to be a simple, cost effective investigation among Egyptian patients.¹⁴ Hence, in this study, the predictive ability of NLR in the diagnosis of diabetic nephropathy among Indian patients was evaluated.

METHODS

Institutional Ethics Committee clearance was obtained. Sample size calculation was calculated (assuming a prevalence of diabetic nephropathy as 27% from previous study, α error of 5% and β error of 20%) as 127.¹⁵ The study was carried out as a single center, cross sectional observational study over a period of 3 months. Patients were accepted to have diabetes mellitus if they had an HbA1c reading $\geq 6.5\%$ at some point of time and were eligible to be enrolled in the study. Patients with established type 1 diabetes, gestational diabetes, secondary diabetes or other forms of monogenic diabetes were excluded from the study. Also, patients suffering from established coronary artery disease, heart failure, acute or chronic infections, acute cerebrovascular diseases, acute and chronic poisonings, malignancies, haematological diseases and chronic inflammatory diseases were excluded. Pregnant women and patients suffering from other diseases that affect urinary protein excretion like nephritic / nephrotic syndrome, urolithiasis, chronic kidney disease due to causes other than diabetes, urinary tract infections, diseases affecting renal blood flow and patients who were on medications known to alter the white blood counts and urinary protein excretion were also excluded from the study.

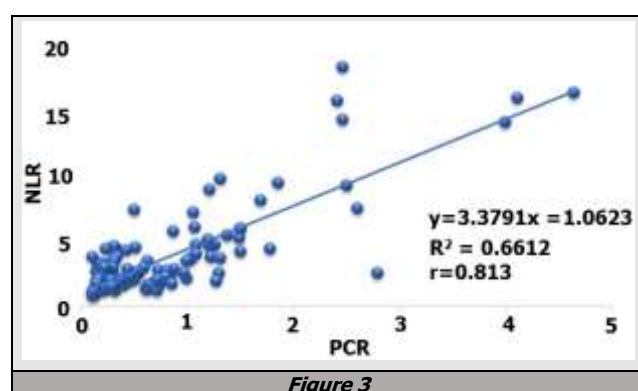
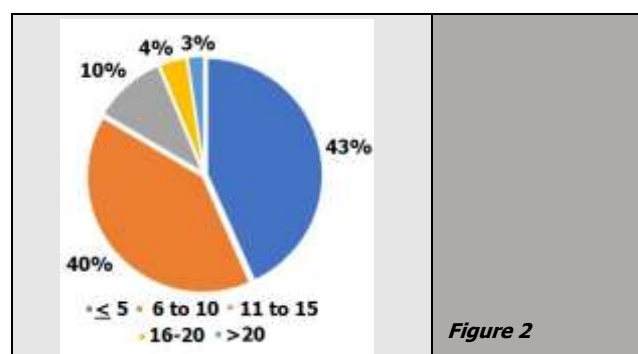
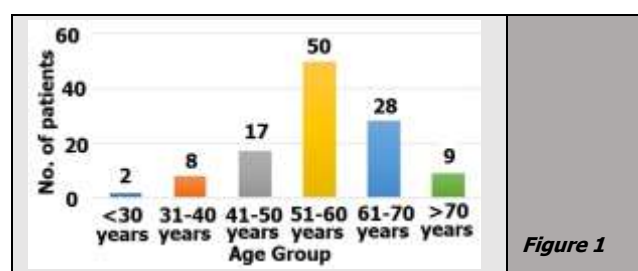
Detailed history and clinical examination was done and blood samples were collected for complete blood counts with differential counts, blood urea, serum creatinine and HbA1c estimation. Urine PCR (Protein Creatinine Ratio) was estimated in a spot urine sample using the TPUC GEN 3, Cobas C, Integra kit. NLR was calculated from the blood counts. EGFR (estimated Glomerular Filtration Rate) was calculated using MDRD equation where $GFR \text{ (in mL/min/1.73 m}^2\text{)} = 175 \times (\text{Serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female})$.

The data obtained was entered into Microsoft Excel spreadsheet and data was analysed using SPSS for Windows version 20. The categorical data was expressed in terms of rates, ratios and percentages and the continuous data was expressed in terms of Mean $\pm$ Standard Deviation. The association between the NLR and diabetic nephropathy as determined by urine PCR was tested using Pearson

Correlation Coefficient. NLR cut-off point was made using the receiver operating characteristic curve (ROC curve). The accuracy of NLR in predicting complications was expressed in terms of sensitivity, specificity, positive and negative likelihood ratio. A probability (p) value of ≤ 0.05 was considered as statistically significant.

RESULTS

The proportion of females in the study population was 47% (Figure 1). Most of the patients were in the age group of 51-60 years. (Figure 1) The mean age of the study population was 57.39 ± 10.6 years. Majority of the patients had been suffering from diabetes between 6-15 years (Figure 2). 83% of the study population had diabetes mellitus for less than 10 years duration. The mean duration of diabetes was 7.9 ± 5.1 years. The mean BMI of the patients was 25.8 ± 4.7 kg/m². The mean serum creatinine of the patients was 1.1 ± 1.0 mg/dL.



The mean eGFR of the study population was 91.6 ± 48.7 mL/min. The mean protein creatinine ratio (PCR) was 0.8 ± 0.4 . 66% of the patients were on oral hypoglycaemic drugs and 18% were on both insulin and oral drugs. Scatter plot for the Urine PCR and NLR is shown in Figure 3. Correlation

analysis revealed a strong positive correlation between the urinary PCR and NLR which was also statistically significant ($r=0.813$, $p<0.0001$). The association between eGFR and NLR was very weak but statistically significant ($r = -0.214$, $p = 0.006$). Using the ROC curve, it was found that an NLR with 7 cut off had a sensitivity and specificity of 88.89% and 94.9% respectively in predicting diabetic nephropathy (AUC = 93.7%) with a positive likelihood ratio of 17.481 and a negative likelihood ratio of 0.117

DISCUSSION

The mean age of the study participants was 57.3 ± 10.6 years. Most of the patients were in the age group of 51-60 years which was similar to the study conducted by Srinivas et al.¹⁶ The proportion of females in the study population was 47%.

The mean NLR of the patients in this study was 3.7 ± 3.3 which was similar to the findings of the study conducted by Fawwad et al.¹⁷ In this study, NLR had a strong positive correlation with diabetic nephropathy ($r=0.813$). Huang et al 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).¹⁸ A study by Imtiaz et al¹⁹ has suggested that chronic diseases such as hypertension and diabetes have a significant association with systemic inflammation, reflected by NLR.

Numerous studies have shown that elevated NLR was associated with the presence of DN and macroalbuminuria.^{20,21} Ulu et al²² demonstrated NLR to be a quick and reliable prognostic marker for diabetic retinopathy and its severity. Shiny et al²³ 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 microvascular complications in patients with glucose intolerance.

In a cross-sectional analysis of 200 patients with type 2 diabetes, elevated neutrophil-lymphocyte ratio was significantly associated with micro albuminuria, macro albuminuria, and reduced estimated glomerular filtration rate.²⁴ In a meta-analysis done by Liu et al,²⁵ NLR level was significantly higher in patients with DN as compared to those without DN.

In order to define a cut off for NLR in this study population, all of the NLR values were plotted under a ROC curve. At NLR cut-off of 7 to predict diabetic nephropathy, the sensitivity was 88.88% and specificity was 94.92%. In a previous study done in south India,¹⁶ NLR cut off obtained was 3.85 which had a sensitivity and specificity of 71% and 75% respectively. Using the same cut-off point in this study, the sensitivity and specificity obtained were 88.89% and 78.81% respectively. The ROC curve showed greater area under the curve (0.937) at NLR of 7 and with a higher sensitivity and specificity compared to the previous studies.^{16,26,27}

The mechanisms responsible for the renal injury leading to albuminuria in diabetic nephropathy is being researched and the exact mechanisms are still unclear. Advanced Glycation End products (AGEs) and its receptor (RAGE) have been implicated in causing an increase in the proinflammatory state associated with diabetic nephropathy.²⁸ Also there is evidence to show an increase in the spontaneous adherence of the neutrophils to the endothelial cell, which may be also implicated in the development of albuminuria.²⁹ In this situation, NLR acts as a marker for the pattern of leucocyte distribution noted in these chronic inflammatory states (increased neutrophils and decreased lymphocytes). Prior studies have shown a role for individual neutrophil and lymphocyte counts in predicting the adverse outcomes in diabetic individuals^{19,28} and have demonstrated the superiority of NLR to the total leukocyte count in such individuals.³⁰

CONCLUSIONS

NLR is a simple, easily available and cost effective investigation which can be used as a screening tool to detect diabetic nephropathy at an earlier stage. In a resource constrained setup with limited laboratory facilities, NLR is a simple test which can be a potential alternative for other costlier inflammatory markers such as interleukins, Tumour Necrosis Factor, cytokines, and high-sensitivity C-Reactive Protein.

Limitations

First this is a cross sectional study and hence any conclusive causal associations between NLR and diabetic nephropathy could not be investigated. Second the sample size was relatively small. Also, the role of a single point estimation of NLR in predicting a condition which may take many years to develop may have to be investigated in further longitudinal studies with a larger sample size.

Compliance with Ethical Standards

The research was carried out in accordance with the National Ethical Guidelines for Biomedical and Health Research. Involving Human Participants. Informed written consent was obtained from every participant of the study.

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