

**Original Research Article****Association of Neutrophil Lymphocyte ratio with Diabetic Nephropathy in type 2 diabetes mellitus**

Authors

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Dr M.K. Uthaya Sankar**Abstract**

Introduction: Diabetic nephropathy (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). DN is clinically manifested as increased albuminuria starting from microalbuminuria to macroalbuminuria and eventually ESRD. 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.

Objective: To assess the association between neutrophil lymphocyte ratio and diabetic nephropathy in type 2 diabetes mellitus.

Materials and Methods: 100 consecutive type 2 diabetes patients were selected for this study and complete blood count blood sugar values, 24-hours urine protein and spot urine dipstick were done and compared.

Results: Most of the patients belong to 41-60 years of age. Of these, 14 patients had overt diabetic nephropathy (24 hrs Urine protein >300mg/dl). Neutrophil lymphocyte ratio (NLR) significantly increased in parallel to albuminuria levels with average of 2.25 in Protein excretion < 300mg/dl group and 3.2 in >300mg/dl group. There was a significant difference between the normal group and DN group with relation to NLR ($p = 0.01$). On comparing with urine dipstick method, there is a significant difference between urine dipstick protein in relation to 24hours urine protein excretion ($P=0.02$) and eGFR (0.04).

Conclusion: Neutrophil lymphocyte ratio was significantly increased in relation to albuminuria levels. When it was compared with urine dipstick protein, there was no significant correlation with NLR, but it was positively correlated with 24 hours urine protein and eGFR. NLR may serve as a cost-effective and readily accessible marker of DN.

Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Type 2 diabetes

mellitus (DM) can have serious socio-economic effects due to its many potential complications, which include microvascular -diabetic nephropathy (DN), neuropathy and retinopathy

and macrovascular complications¹. Diabetic nephropathy (DN) leads to significant problems in 25–40% of diabetic patients and is the major cause of end stage renal failure². The urine microalbumin excretion rate (UMAER) can be used to detect and monitor the progression of DN. DN is a common microangiopathic complication in patients with diabetes. DN is one of the most common causes of end stage renal disease (ESRD). DN is clinically manifested as increased albumin excretion starting from microalbuminuria to macroalbuminuria and eventually ESRD³. 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.

The Asian Indian population has more prevalence of DN as compared to the Caucasian population⁴. The pathogenesis of DN is still not fully elucidated, and induction of inflammation and oxidative stress by the metabolism of hyperglycemia and dyslipidaemia may play a significant role in developing vascular complications including DN. The reduction in microalbuminuria was associated with a slower decline in glomerular filtration rate (GFR) and decreased cardiovascular risk.

Neutrophil to lymphocyte ratio (NLR) rather than other white cell parameters (e.g., total white cell, monocyte count, and absolute neutrophil count) was found to be a useful inflammatory marker that predicts adverse outcomes in many medical and surgical conditions⁴. In recent years, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were introduced as potential markers to determine inflammation in cardiac and non-cardiac disorders. Circulating leukocyte rates are changed during the inflammatory response. Neutrophilia is accompanied by relative lymphopenia.

Additionally, an association between NLR and worsening renal function in diabetic patients has been determined⁵. Once overt DN develops, there is persistent proteinuria, and progression toward ESRD could only be slowed but could not be

stopped. 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.

Material and Methods

Study Setting and Design

This study was done at Sri Manakula Vinayagar Medical College and Hospital from May 2018 to December 2018 for a period of eight months. Sri manakula vinayagar medical college is a tertiary care hospital located at Madagadipet, Puducherry. The study design employed was a cross sectional study.

Study Participants

All diagnosed T2DM patients were included in this study. Type 1 DM patients, patients with infections like urinary tract infection (UTI) and patients with systemic disorder such as coronary artery disease, chronic liver disease and patients having diseases affecting urinary protein excretion as nephrotic syndrome, urolithiasis, renal insufficiency, renal artery stenosis and patients having low glomerular filtration rate (GFR) without microalbuminuria are excluded from this study.

Methodology

All participants had a 24-hour urinary albumin excretion (UAE) record. Age, sex, diabetes duration were recorded in the proforma. Several biochemical and haematological parameters such as haemoglobin, white blood cell (WBC), platelet count, neutrophil count, lymphocyte count, neutrophil lymphocyte ratio (NLR), urea, creatinine, fasting blood sugar (FBS), postprandial blood sugar (PPBS) and glycated haemoglobin (HbA1c) were done. GFR was calculated according to the modification in Diet in Renal disease (MDRD) formula. Incipient nephropathy is the initial presence of low but abnormal amounts of urine albumin, referred to as microalbuminuria (persistent albuminuria at level 30–299 mg/24 hours). Overt nephropathy or macroalbuminuria (persistent albuminuria at level ≥ 300 mg/24 hours) develops after many years in type 1 diabetes but may be present at the time of

diagnosis of type 2 diabetes. Patients were divided into two groups i.e. Patient with overt DN and without overt DN. Patients were also divided in three groups according to the urine dipstick albumin and variables were compared within these groups.

Statistical Analysis

Statistical analysis was done by SPSS version 9.0 (SPSS, Chicago, IL). Data were given as mean ± standard deviation for continuous variables and frequency and percentage for categorical variables. Group comparisons were performed via independent t Test and ANOVA test. p-value less than 0.05 were considered as statistically significant.

Results

A total of 100 diabetic patients were registered. Of these 14 patients had over diabetic nephropathy (24 hrs Urine protein > 300mg/dl) and considered as one group and less than 300mg/dl as another group. Most of the patients belong to 41-60 years of age and there were no significant differences observed regarding gender. Laboratory parameters such as Urea and creatinine and glycemc parameters such as FBS, PPBS and HbA1c were compared in both groups along with Neutrophil Lymphocyte Ratio. There was a significant difference between the normal group and DN group with relation to NLR (P - 0.01). Neutrophil lymphocyte ratio significantly increased in parallel to albuminuria levels with average of 2.25 in Protein excretion < 300mg/dl group and 3.2 in >300mg/dl group. In the present study, renal function tests of all patients were carried out, and estimated GFR (eGFR) was calculated by MDRD formulae. In relation to eGFR, although there was no significant difference between the two groups (P = 0.13), patients with albuminuria had a significantly low eGFR (mean eGFR = 70.16) than the normal group (mean eGFR = 82.64). Similarly, other investigations such as blood serum creatinine had differences in these two groups but not statistically significant. In

reference to glycemc parameters, we did not observe any significant difference with respect to PPBS (P = 0.53), and HbA1c (P = 0.29) in the two groups, i.e., normal diabetic patients and patients with DN. However, the FBS is more controlled in DN group than the other group which may be due to intensive use of insulin therapy in these group of patients. When the variables are compared with Urine dipsticks values divided into three groups as no trace, trace and 1+ albumin and >2+ albumin in urine, there is a significant difference between this three group in relation to 24hours urine protein excretion (P-0.02) and eGFR(0.04). Although urine dipstick is correlated along with 24hours urine protein and eGFR, there was no statistically significant difference noted between these groups and Neutrophil lymphocyte ratio (P-0.36). Apart from 24 hrs Protein excretion and eGFR, statistically significant difference is noted between serum creatinine values (P-0.03).

Table 1: Distribution of age among the study population

| Age category (in years) | Frequency | Percent |
|-------------------------|-----------|---------|
| 30-40 | 9 | 9.0 |
| 41-50 | 23 | 23.0 |
| 51-60 | 41 | 41.0 |
| 61-70 | 20 | 20.0 |
| >70 | 7 | 7.0 |
| Total | 100 | 100.0 |

In our study majority of patients falls under 51-60 years of age (41%).

Table 2: Gender distribution

| Gender | Frequency | Percent |
|--------------|-----------|---------|
| Female | 52 | 52.0 |
| Male | 48 | 48.0 |
| Total | 100 | 100.0 |

In our study majority of the participants were female (52%).

Table 3: Duration of diabetes in years

| Variable | Mean | S.D |
|---------------------------|-------|-----|
| Duration of DM (in years) | 11.79 | 8.6 |

The mean duration of diabetes for the participants is 11.7 years.

Table 4: Distribution of various variables with 24hr urine protein

| Variables | 24 hrs urine protein | | P value |
|--------------------|----------------------|---------------------|---------|
| | < 300 mg/dl N=87 | >301 mg/dl N= 13 | |
| Duration DM | 11.74(8.7) | 12.24(8.2) | 0.87 |
| N-L ratio | 2.25 (0.87) | 3.2 (0.66) | 0.01 |
| eGFR | 82.64 (28.3) | 70.16 (24.6) | 0.13 |
| Hb1Ac | 10.64 (2.4) | 9.87 (2.4) | 0.29 |
| FBS | 200.15 (78.18) | 142.38 (75.06) | 0.02 |
| PPBS | 307.8 (100.0) | 289.00 (98.6) | 0.53 |
| Urea | 30.06 (15.16) | 32.31 (15.52) | 0.61 |
| Creatinine | 0.97 (0.38) | 1.20 (0.65) | 0.06 |

From the above table it is clear that N:L ratio is correlating with 24hours urine protein and it is statistically significant with a p value of 0.01.

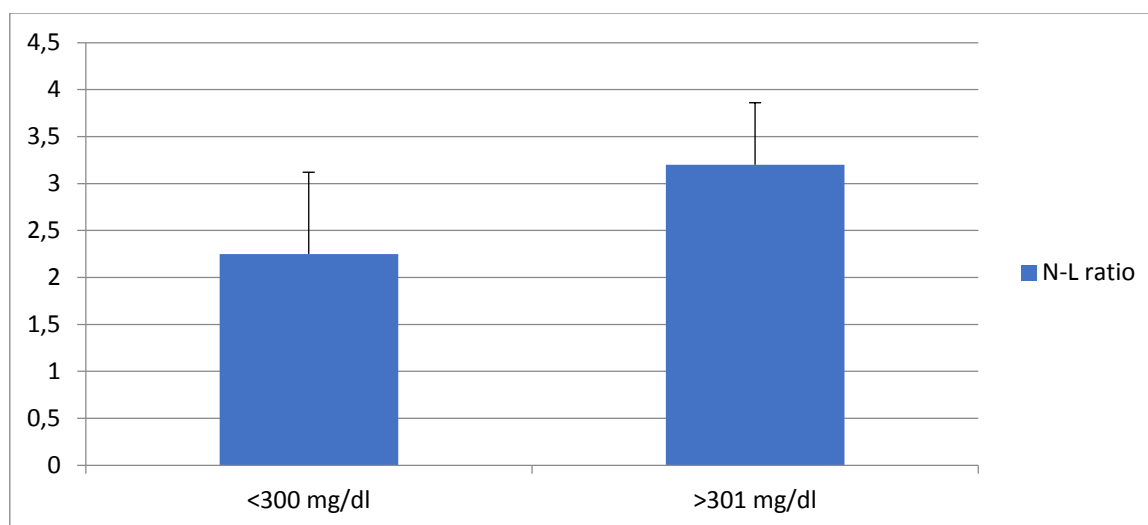


Fig 1: Correlation between N-L ratio and urine albumin.

It is clear from the above figure that N-L ratio is increased with patients having albuminuria of >300mg/dl.

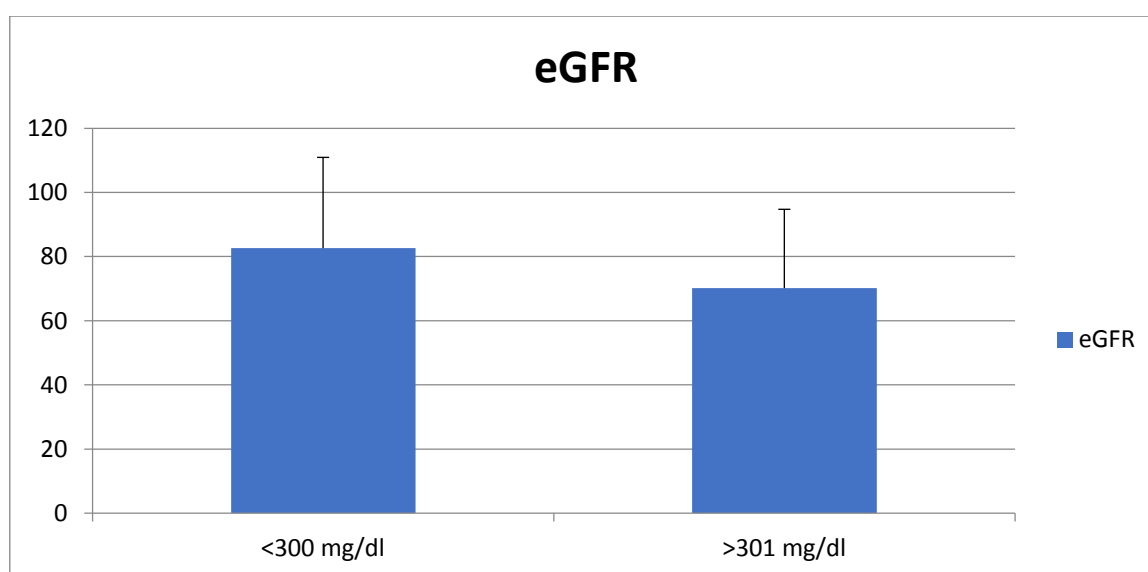


Fig 2: Correlation between eGFR and urine albumin.

From the above figure it is evident that eGFR is low in patients with Uring albumin above 300mg/dl.

Table 5: Correlation between Various variables and urine albumin by dipstick method

| Variables | Urine albumin | | | P value |
|---------------------|-------------------|-------------------|-----------------|---------|
| | No traces N=40 | 1+, trace N=44 | >2+ N=16 | |
| 24 hrs urine | 167.70 (154.38) | 121.18 (68.77) | 308.16 (273.32) | 0.02 |
| eGFR | 88.82 (22.91) | 78.99 (30.43) | 67.63 (28.23) | 0.04 |
| N-L Ratio | 2.26 (0.69) | 2.36 (1.01) | 2.76 (0.87) | 0.36 |
| FBS | 205.2 (78.77) | 190.23 (86.77) | 166.06 (60.30) | 0.26 |
| PPBS | 320.06 (100.82) | 299.68 (107.7) | 280.91 (73.95) | 0.34 |
| Hb1Ac | 10.73 (2.74) | 10.45 (2.35) | 10.30 (1.72) | 0.75 |
| Urea | 26.28 (8.89) | 32.82 (19.16) | 33.75 (13.6) | 0.11 |
| Creatinine | 0.85 (0.20) | 1.04 (0.45) | 1.26 (0.64) | 0.03 |

From the above table it is evident that urine dipstick is correlating with 24 hrs Protein excretion and eGFR and also with creatinine values and it is statistically significant.

Discussion

Diabetic nephropathy-related increase in proteinuria is a part of the cascade of clinical events involving increased blood pressure and progressive decrease in GFR. Glomerular damage gives rise to proteinuria and progressive renal damage in DM. Consequently, fibrosis and inflammation of the collective tubules result in progressive loss of functional nephrons. Furthermore, DM is not only a metabolic disorder. It is now recognized that several molecules associated with inflammation play a major role in development of DM and DM-related complications. Experimental and clinical studies have demonstrated the significant role of inflammatory molecules and endothelial dysfunction in the setting of DN. Moreover, it has also been reported that renal inflammation has a crucial place in development and progression of DN. In a study conducted by Spranger et al, it was suggested that circulating inflammatory cytokines modify the development of type 2 DM; elevation of IL-6 and IL-1 together increased the risk of type 2DM.

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 defence whereas lymphocytes are the regulatory or protective

component of inflammation. Interestingly, NLR has been found to have a positive relation with not only the presence but also the severity of metabolic syndrome. A study done by Imtiaz et al. has suggested that chronic diseases such as hypertension and diabetes have a significant association with systemic inflammation, reflected by NLR. Shiny et al. 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 our study on 100 patients, there was equal distribution among both the sexes, with peak incidence of diabetes in 5th and 6th decade of life. The association between neutrophil lymphocyte ratio and other parameters like 24-hour urine protein, spot urine analysis, FBS, PPBS& HbA1c was analysed. There was a significant relationship between 24 hr urine protein and NLR suggested that NLR can used as an effective marker in determining the presence of a chronic inflammatory state. These findings were similar to studies conducted by Shiny et al. Thus, NLR can be used as a predictive tool in development of diabetic nephropathy. On further evaluation with other parameter like urine dipstick method, though there was no significant correlation with NLR, an important observation was there was a parallel increasing trend in NLR along with increase in spot urinary protein. In our study, though there was no significant correlation with urine dipstick and NLR, there was statistically significant relation between 24 hrs urine protein and eGFR.

The main limitation of our study was, it was a single centred cross-sectional study with relatively smaller sample size and follow up of the patients was not done.

Conclusion

In conclusion, in the present study, the possible associations between albuminuria levels, GFR and NLR were investigated in type 2 diabetic patients. Neutrophil lymphocyte ratio was significantly increased in parallel to albuminuria levels. When it was compared with urine dipstick protein, there was no significant correlation with NLR, but it was positively correlated with 24 hours urine protein and eGFR. These results support the fact that DN involves an inflammatory process and that NLR may represent a marker of this inflammation. Therefore, NLR may serve as a cost-effective and readily accessible marker of DN. The clinical utility of NLR in the case of DN remains to be established in future studies and to address the demerits.

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