Correlation Between Neutrophil-Lymphocyte Ratio and Diabetic Nephropathy in Type 2 Diabetes Mellitus Patients

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Abstract

Diabetic nephropathy (DN) is manifested as increased albumin urea excretion starting from microalbuminuria to macroalbuminuria, and is now a leading cause of end stage renal disease.It was reported from the various studies that neutrophil lymphocyte ratio (NLR) is used as an inflammatory marker and to predict the prognosis of many cardiac and non-cardiac diseases.The present cross-sectional study was conducted at tertiary care hospital, Yashoda Hospitals, Malakpet, Hyderabad, from May 2019 to April 2020 with the aim to evaluate the relationship between diabetic nephropathy and inflammation using NLR and estimate whether NLR can be used as a predictive and reliable marker.In the present study the majority of the study subjects were in the age group of 61-70 years and there was male predominance and male female ratio was 1.7:1. It was reported that, out of total 120 cases, 38 cases (31.67%) had albuminuria, ranging from 1+ to 3+, and was categorised as diabetic nephropathy (DN) group. Around 82 cases (68.33%) had nil or trace urine albumin and were in normal group.SGPT had a positive correlation with NLR and negatively with eGFR, but statistically it was not significant.The present study concluded that microalbuminuria was found to be one of the earliest markers for diabetic nephropathy.

Keywords: Diabetes mellitus, Type 2 DM, Neutrophil-Lymphocyte ratio, Nephropathy and albuminuria.

Introduction

Diabetes mellitus (DM) is a progressive chronic disease emerging as a global epidemic.Diabetic patients commonly develop various microvascular complications (nephropathy, etc.) and macrovascular complications which are responsible for much of the burden associated with diabetes.[1,2,3]

Diabetic nephropathy (DN) is manifested as increased albumin urea excretion starting from microalbuminuria to macroalbuminuria, and is now a leading cause of end stage renal disease.[4,5]

The literature suggests that diabetes is associated with chronic systemic inflammation and oxidative stress which results in alteration in immunological system and leads to development of chronic inflammation-induced organ dysfunction.[6,7]

White blood cell count and its subtypes are sensitive and classic markers indicative of inflammation. The increase in the neutrophil count is seen in thrombus formation and ischemic diseases.[8]

It was reported from the various studies that neutrophil lymphocyte ratio (NLR) is used as an inflammatory marker and to predict the prognosis of many cardiac and non-cardiac diseases.[9]

Thus, the present study was performed to evaluate the relationship between diabetic nephropathy and inflammation using NLR and estimate whether NLR can be used as a predictive and reliable marker.

Aims and objectives

- To study the prevalence of albuminuria in type 2 diabetes mellitus.

- To study the neutrophil to lymphocyte ratio in type 2 diabetes patients.

- To compare various glycemic and biochemical parameters in diabetic patients with and without albuminuria.

Material and methods

The present cross-sectional study was conducted at tertiary care hospital, Yashoda Hospitals, Malakpet, Hyderabad, from May 2019 to April 2020after obtaining the ethical permission from the institution over a period of 18 months.

A total of 120 diabetic patients attending OPD/IPD were involved in the study after obtaining the informed consent and divided into 2 groups, group one with urine albumin present (diabetic nephropathy group) and group as normal (urine albumin absent). These groups were compared for various glycaemic and biochemical parameters.

Inclusion criteria

- All patients of type 2 diabetes mellitus patients attending the OPD / IPD.

Exclusion criteria

- 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, skin infection, AIDS; patients with systemic disorder such as CVD, chronic liver disease, blood disorders, malignancy; patients having diseases affecting urinary protein excretion as nephritic syndrome, urolithiasis, renal insufficiency and renal artery stenosis.

A detailed history was collected, clinical examination was done.Demographic data (age, gender, history of illness, etc.) were recorded.

Venous blood samples were drawn from the subjects after 8 hours or overnight fasting. Samples was maintained at room temperature and tested within 1 hour of collection for complete blood count including hemoglobin, total and differential leucocyte count and total platelet counts. Neutrophil to Lymphocyte ratio was calculated as a simple ratio between the absolute neutrophil and the abso-lute lymphocyte counts both obtained from the same automated blood sample.

Other biochemical tests include glycosylated hemoglobin (HbA1c), fasting plasma glucose (glucose oxidase-

peroxidase method) with post prandial glucose, renal function tests (blood urea, serum creatinine), liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total and direct bilirubin, serum albumin), urine albumin, and lipid profile (serum triglycerides, serum HDL cholesterol, serum LDL cholesterol, serum total cholesterol) were done.Detection of albumin in urine was done using the dipstick method which is a semiquantitative screening tool for proteinuria.

Data was recorded and analysed with the help of SPSS 21.0 version. Continuous variables were presented as Mean +- SD whereas categorical variables were presented in frequency and percentage. Student t-test and chi square test was used appropriately. Pearson's correlation coefficient was used to establish a correlation between NLR, eGFR and different other parameters.P<0.05 was considered statistically significant.

Observations and Results

In the present study the majority of the study subjects were in the age group of 61-70 years and there was male predominance and male female ratio was 1.7:1. It was reported that, out of total 120 cases, 38 cases (31.67%) had albuminuria, ranging from 1+ to 3+, and was categorised as diabetic nephropathy (DN) group. Around 82 cases (68.33%) had nil or trace urine albumin and were in normal group.

In DN group, a majority of 15% (n=18) had their age in between 61-70 years, followed by 7.5% of cases had their age >70 years (n=9) and 5.8% of cases had their age between 51-60 years (n=7), while only 3.3% cases had their age in between 41-50 years (n=4). Mean age in DN group was 58.35 ± 11.8 years while in urine albumin negative cases it was 62.37 ± 9.4 years; while the distribution was found to be correlating positively

(Pearson correlation coefficient = 7.25), there was no significant difference between the two groups (p = 0.123).

The mean weight of the study participants was 70.40±7.3kg, mean height was 158.36 ± 3.1 cm, mean BMI was 28.09 ± 3 kg per m2 and the age group of 51-60 years were overweight, whereas frank obesity was found to be highest amongst the study population of 61-70 years (40.9%) age group.

Table 1: BMI VS Urine albumin

BMI	Urine Albumin			
	Absent		Present	
	No.	%	No.	%
Normal	9	7.5	6	5
Overweight	57	47.5	17	14.17
Obese	16	13.33	15	12.5
Total	82	68.33	38	31.67
X^2	7.0712			
P	0.029			

Table 1, depicted that Out of total 38 urine albumin positive cases (DN group), a majority of 14.17% (n=17) were overweight, followed by 12.5% (n=15) of cases were obese and only 5% (n=6) of cases had normal body mass index and there was significant correlation and significant difference.
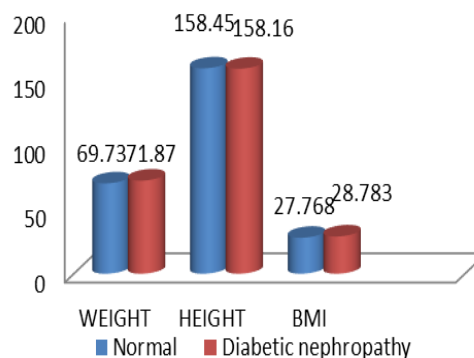

Figure 1: Anthropometric measurement vs urine albumin

It was reported that the overall mean BMI of the diabetic nephropathy group (28.78 ± 3.5) was higher than that of normal population (27.77 ± 2.7 kg per m^2) as shown in figure 1.
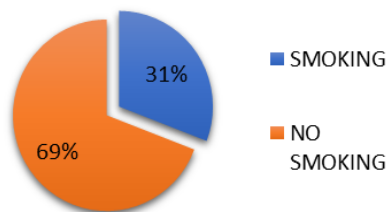

Figure 2: Smoking vs urine albumin

Figure 2, showed that out of total 38 urine albumin positive cases, 24 cases (20%) had a smoking habit and 11.6% (n=14) did not. Out of total 82 urine albumin negative cases, 23 of them (19.17%) were smoker and 49.12% (n=59) were non-smoker. No statistically significant difference was found (p=0.332).

Table 2: Health status

Parameters	%
Hypertension	13
Use of antidiabetic medicines	
- OHA	85
- Insulin	15

The current study reported that 13% patients were hypertensive, 85% patients were on OHA and 15% patients were on insulin as depicted in table 2.
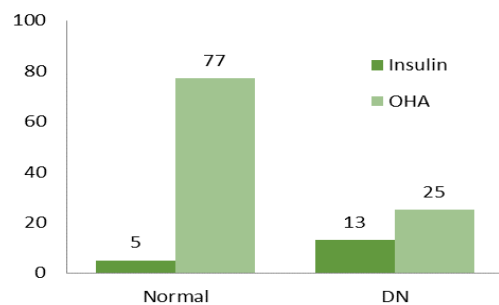

Figure 3: Smoking vs urine albumin

Figure 3, showed that in DN group, about 25 cases (20.8%) and 13 cases (10.83%) were on oral hypoglycaemic drugs and insulin respectively. In normal group, 77 cases (64.17%) and 5 cases (4.17%) were on oral hypoglycaemic drugs and insulin respectively. There was no statistical significant correlation (p=0.000).
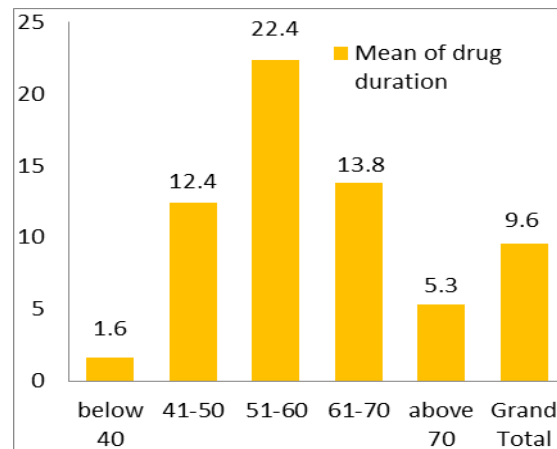

Figure 4: Age vs duration of medication for DM

It was observed that the overall mean of the duration of medications for diabetes in each age group of the study population was 9.6 ± 4.7 years.
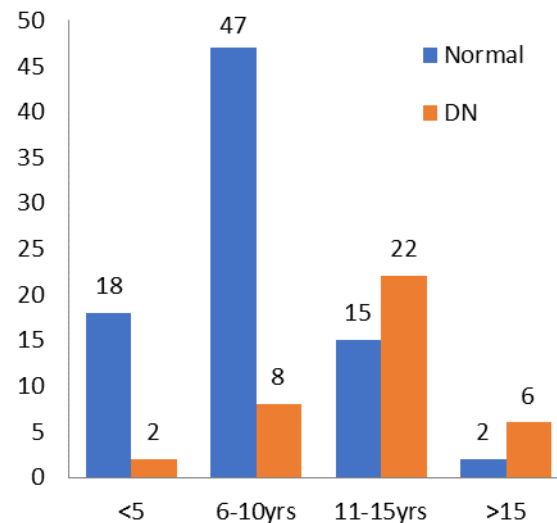

Figure 5: Albuminuria vs duration of DM

In our study, among DN group, a majority of 18.33% (n=22) had their diabetes duration of 11-15 years, followed by 6.67% (n=8) of cases in between 6-10 years and 5% (n=6) of cases >15 years while only 1.67% (n=2) cases had a duration of <5 years. And in normal group,

about 39.17% (n=47) had their diabetes duration of 6-10 years, followed by 15% (n=18) of cases had a duration of <5years and 12.5% (n=15) in between 11-15 years while only 1.67% (n=2) cases had a duration of >15 years. There was positive correlation (correlation coefficient was 31.93) and the difference was significant statistically (p = 0.000).

Table 3: FBS and PPBS vs Urine albumin

FBS (mg/dl)				
	Absent		Present	
	No.	%	No.	%
<126	3	2.5	0	0
>126	79	65.83	38	31.67
PPBS				
<200	31	25.83	6	5
>200	52	42.5	32	31.67

It was reported that mean FBS was> 126 mg/dl, while this difference was found statistically insignificant (p>0.05) and PPBS was> 200 mg/dl which was non-significant (P=0.015).
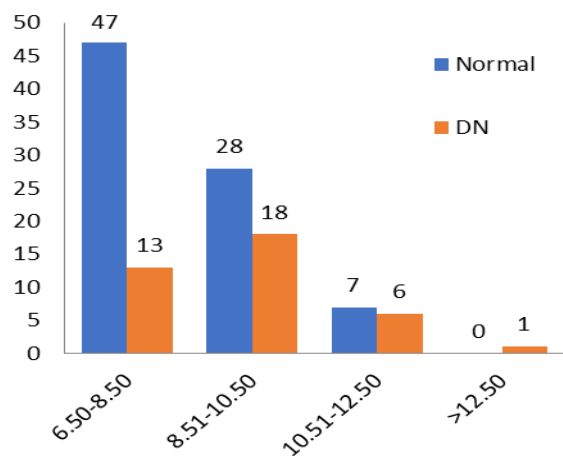

Figure 6: HBA1c vs Urine albumin

A statistically significant difference was reported (P=0.06). Out of 38 urine albumin positive cases, about 10.8% had HbA1c <8.5 suggesting a good proportion had good hypoglycaemic control. However, a majority had poor control (15% >8.5, followed by more than 5% >10.51 HbA1c). In normal group, a majority of cases had

good glucometabolic control (39.17% had HbA1c level <6.50) while 23% and 5.8% of cases had poor control as per HbA1c level 8.51-10.50 and >10.51 respectively as shown in figure 6.
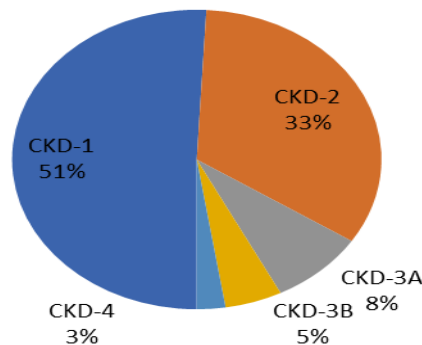

Figure 7: Stages of CKD as per eGFR

The figure 7 showed that the majority (50.83%) of the patients were in CKD -1 stage and a positive correlation and significant difference was found between stages of CKD and urine albumin (P=0.000).

Table 4: Mean NLR

Age Range	N (%)	Mean	SD
below 40years	9 (7.50)	1.74104	0.19
41-50years	13 (10.83)	1.003787	0.36
51-60years	30 (25.00)	0.394449	0.57
61-70years	44 (36.67)	0.360571	0.58
70 & above	24 (20.00)	0.700757	0.39
Total	120 (100.00)	0.120564	0.5

Table 4: showed that the overall mean NLR (Neutrophil-to-Lymphocyte ratio) of the study population was 0.121±0.5. The table also stated the mean NLR in the range of each age group.
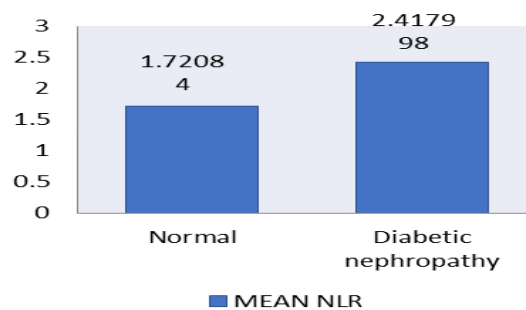

Figure 8: Mean NLR vs urine albumin

The overall mean NLR of the diabetic nephropathy group (2.42±0.5) was higher than that of normal population (1.72±0.3).

Table 5: Correlation between CBC &eGFR

Investigations	eGFR	
	X^2	P
Hb	0.300**	0.000
TLC	-0.048	0.301
Neutrophil	-0.469**	0.000
Lymphocyte	0.532**	0.000
NLR	-0.580**	0.000
Platelets	0.006	0.474

** Correlation is significant at the 0.01 level (1-tailed)

* Correlation is significant at the 0.05 level (1-tailed)

Table 6: Correlation between Sugar level with NLR & eGFR

Sugar level	Parameters			
	NLR		eGFR	
	X^2	P	X^2	P
Fasting	0.340**	0.000	-0.272**	0.001
Post prandial	0.050	0.295	-0.030	0.371

** Correlation is significant at the 0.01 level (1-tailed)

* Correlation is significant at the 0.05 level (1-tailed)

Table 7: Correlation between LFT with NLR &eGFR

Liver Function Test	Parameters			
	NLR		eGFR	
	X^2	P	X^2	P
Serum bilirubin (Total)	0.204*	0.013	-0.173*	0.029
Serum bilirubin (Direct)	0.128	0.081	-.299**	0.000
SGOT	0.343**	0.000	-0.149	0.052
SGPT	0.058	0.264	-0.067	0.234
ALP	0.242**	0.004	-.212**	0.010
Serum ALBUMIN	-.519**	0.000	0.381**	0.000

Table 8: Correlation between RFT with NLR &eGFR

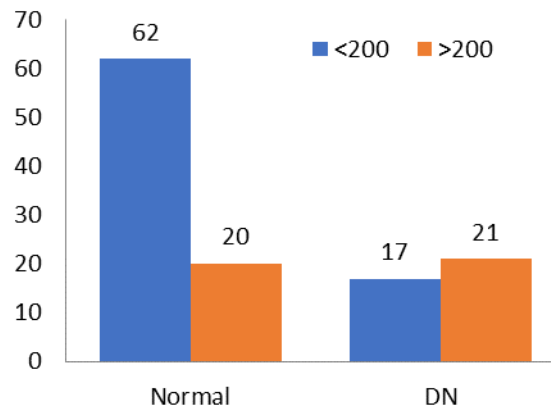| RFT	Parameters			
	NLR		eGFR	
	X^2	p	X^2	p
Blood Urea	0.665**	0.000	-0.632**	0.000
Serum Creatinine	0.661**	0.000	-0.775**	0.000

Figure 9: Total cholesterol level & urine albumin

It was reported that the most of the patients had total cholesterol<200mg/dl and out of which albuminuria was present and absent in 14.17% and 51.67% cases respectively. Similarly, in DN group, about 17.5% (n=21) and in normal group, about 16.6% (n=20) had their total cholesterol level > 200 mg/dl. This difference was found statistically significant (p=0.001).

In our study there was no significant statistical difference between urine albumin with triglycerides (p=0.017), gender (male p=0.181 and female p=0.015), and there was statistically significant difference between LDL cholesterol with urine albumin (p=0.000).

Discussion

In our study the mean age in urine albumin positive cases was 58.35 ± 11.8 years while in urine albumin negative cases was 62.37 ± 9.4 years and this difference was found to be statistically insignificant (p = 0.123) and male to female ratio was 1.7:1. The results are correlated with the study conducted by Srinivas et al. included 40 patients with mean age 53.47 ± 6.14 years; and male preponderance was shown in the distribution.[10]

The overall mean weight and height of the study population was 70.40 ± 7.3 kg and 158.36 ± 3.1 cm

respectively.The majority of the population were overweight, i.e., BMI was in between 25-29.9 kg per m^2 with overall mean of 28.09 ± 3.0 kg per m^2. In similar study conducted by Khandare et al, similarly, showed no difference in distribution of the anthropometric indices (weight, height and BMI) when compared in both the groups.[5]

It was found that 20% patients were smoker, most of the patients were in stage 1 CKD. The findings are in accordance with the studies conducted by Liao et al., Jiang et al. andKhandare et al.[5,11,12]

In our present study, when we compared complete blood count with eGFR**,** haemoglobin, and lymphocyte count had a significantpositive correlation; and with neutrophil count and NLR had a significant negative correlation between the eGFR value and count and NLR (p=0.000). in similar study performed by Khandare et al. showed the mean NLR among diabetic patients with albuminuria (2.83 ± 0.85) was significantly higher than among those without albuminuria (1.94 ± 0.65) with p<0.001, but individually, the total WBC count did not differ in the two groups.[5]

In the diabetic profile, 85% of our study population were on oral hypoglycaemic agents and 15% were on insulin, with the overall mean of the duration of medications being 9.6 ± 4.7 years.About 10.8% in DN group had a good hypoglycaemic control (HbA1c <8.5). However, a more than 20% had poor control (>8.5 HbA1c). In normal group, a majority of cases had good glucometabolic control (39.17% had HbA1c level <6.50) while more than 28% of cases had poor control (>8.51 HbA1c level). The results are in accordance with the study conducted by Khandare et aldid not observe any significant difference with respect to FBS (P = 0.0769), PPBS (P = 0.5674), and HbA1c (P = 0.06) when compared in both groups, although they stated HBA1c to be a risk factor for DN. In another study performed by Chowta et al. observed mean duration of diabetes in albuminuric patients was 10.7±5.0 years while in normoalbuminuric patients was 3.2 ± 2.0 years, which was statistically highly significant. (P< 0.0001).[5,13]

Our study showed SGPT had a positive correlation with NLR and negatively with eGFR, but statistically it was not significant.In Khandare et al., only SGPT was found to be significantly raised in DN group as compared to normal patients group (p = 0.001).[5]

Among the renal function parameters, a significant positive correlation between NLR and blood urea level and serum creatinine level was seen (both p=0.000) with a significant negative correlation between eGFR and blood urea level and serum creatinine level (p=0.000). In contrast, Khandare et al[5] showed that blood urea and serum creatinine had no difference in patient having diabetic nephropathy and without nephropathy groups.

In the lipid profile, 17.5% cases in DN group and 16.6% in normal group had high cholesterol level > 200 mg/dl and the difference was found statistically significant (p=0.001); 21.6% cases in DN group and 30.8% in normal group had high triglyceride; 2.3% female and 5.3% of male patients with DN had high HDL cholesterol; 23.33% cases of DN and 25.83% normal cases had LDL cholesterol level >100 mg/dl. In contrast Khandare et aldid not display any difference in the components of lipid profile (total cholesterol, TG, HDL, LDL) when compared in both groups. Kolharet al[14]found a significant association of DN with elevated total cholesterol, LDL and triglycerides.

Conclusion

The present study concluded that microalbuminuria was found to be one of the earliest markers for diabetic nephropathy. 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 a simple and inexpensive marker that can be easily calculated from a simple peripheral blood count, especially in a setup with limited laboratory facilities.

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