# STUDY OF NEUTROPHILS TO LYMPHOCYTE RATIO(NLR) AS A MARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

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## ABSTRACT

**AIM:** To correlate relationship of NLR Ratio in Type 2 DM patients having raised Albumin in urine/ elevated UACR and no Albuminuria / normal UACR.

**MATERIAL & METHODS**: Analytical Cross Sectional study involving data from 96 patients visitng JSS Hospital OPD and Inpatients of JSS Hospital, Mysuru over a period of 18 months. General physical examination was done which included weight, body mass index, height, heart rate, blood pressure, any cyanosis, clubbing, pallor, yellowish discoloration of sclera. Routine Investigations such as Complete Hemogram, Glycated hemoglobin (HbA1c) ,Hepatic function tests (LFTs), Renal function (RFT), Urine microscopy and routine, Chest Xray & specific investigation such as spot urine Albumin(Protein) to creatinine(UACR) ratio was done.

**RESULTS:** The majority of subjects with and without nephropathy were among the age group of 51 to 60 years & majority of subjects with and without nephropathy were males. There was no correlation between gender and nephropathy (P = 1.0). The mean FBS levels were significantly higher among subjects with diabetic nephropathy compared to their normal counterparts (P= 0.038). The mean HbA1c levels among subjects with and without diabetic nephropathy were almost similar (P = 0.878) and was not significant. It was observed that, the median spot UACR, mean N/L ratio, mean blood urea, mean serum creatinine & GFR(glomerular filtration rate) was higher among subjects with diabetic nephropathy(DN) compared to their normal counterparts and p value was found to be significant.

**CONCLUSION:** The study revealed that elevated NLR was associated with the development of DN(diabetic nephropathy), suggesting that inflammation and the dysfunction of the endothelium could be contributing factors to this condition. The NLR test is an easy-to-use and inexpensive way and can be calculated using differential leukocyte count. The NLR test will allow researchers to gain a deeper understanding of role of inflammation in the development of DN and how it could be used as a prognostic and predictive marker of diabetic nephropathy especially in areas with limited resources.

**KEYWORDS:** Diabetic nephropathy, NLR, albuminuria

## INTRODUCTION

Diabetes mellitus (DM) is one of the most common non communicable disease which includes different forms of metabolic disorders which directly or indirectly leads to hyperglycemia. The most

important complications associated with the long duration of the illness is microvascular and macrovascular complications out of which the predominant complications is End stage renal disease (ESRD) and Diabetic retinopathy etc. Diabetic kidney disease (DKD) or Diabetic Nephropathy is a leading cause for chronic kidney disease and ESRD which requires renal replacement therapy.

The most important factor responsible for diabetic nephropathy is duration of diabetes and chronic hyperglycemia which in turn is associated with production of reactive oxidation species and advanced end products of glycation (AGE).

The presence of metabolic products activate profibrotic and proinflammatory gene expression which promote cellular injury & insulin resistance, which are important factors in the development of pathogenic differences between type 1 as well as type 2 diabetes.[1,2].

The relationship between vascular diseases and systemic inflammation plays a major pathway in not only development but also in faster progression leading to microvascular complications and macrovascular complications in diabetic patients. Since inflammation plays a major role, total white blood cells (TWBC) can help as an indicator which might not act as a specific marker but provide a sensitive as well as crude indicator . The biggest advantage being it can be done at any peripheral centres without any difficulty and is relatively cheaper than any other investigations. Numerous studies have also shown that neutrophils are increased in patients with thrombotic phenomena and other ischemic diseases ,thus neutrophils to lymphocyte ratio have shown to have a prognostic significance in patients with myocardial infarction(MI), as well as heart failure and cerebrovascular accident.[3,4]

The relation of certain inflammatory markers like transforming growth factor beta1, cytokines, interleukins like IL1, IL6, IL8, and tumor necrosis factor-alpha (TNF-α) helps in development of diabetic nephropathy.[5,6] NLR being easy to carry out in a limited setup provides the advantage over these inflammatory markers and can be done in rural area with limited setup as well and can act as a marker to identify diabetic patients with nephropathy.

Thus, this study aimed to correlate relationship of NLR Ratio in Type 2 DM patients having raised Albumin in urine/ elevated UACR and no Albuminuria / normal UACR.

## MATERIAL & METHODOLOGY

An Analytical Cross Sectional study involving data from 96 patients visitng OPD and Inpatients of JSS Hospital, Mysuru over a period of 18 months.

Inclusion criteria:

 1. All diagnosed T2DM patients

2. All patients who are above 18 yrs of age , admitted or visiting JSS Hospital, Mysuru.

Exclusion criteria:

- Patients with Type 1 DM
- Patients with various infections such as urinary infection (UTI), upper or lower respiratory infections, otitis media, any viral infection, chronic liver disease, parasitic infestations, tuberculosis, pyrexia of unknown origin.
- Patient who does not want to participate for study.
- Hematological disorder, malignancy, poisoning, any steroids(topical or systemic), alcohol, autoimmune disorder.

- Renal disorder like nephrotic syndrome, urolithiasis and nephritic syndrome.

A structured proforma was used for the evaluation of patients, detailed case history was taken, and the sample collection was done by the primary investigator.

Patient's data was divided into two arms

1.Diabetics with Nephropathy

2.Diabetics without Nephropathy

Details of habits such as smoking, alcoholism, age, drug history, family history, any prior chronic ilness, duration of diabetes, sex was collected.

General physical examination including weight, body mass index, height, heart rate, blood pressure, cyanosis, clubbing, pallor, yellowish discoloration of sclera was done.

Routine Investigations :-
- ➢ Complete Hemogram
- ➢ Glycated hemoglobin (HbA1c)
- ➢ Hepatic function tests (LFTs)
- ➢ Renal function (RFT)
- ➢ Urine microscopy and routine Chest Xray

Specific Investigation:-
- ➢ Spot urine Albumin(Protein) to creatinine ratio.

DATA ANALYSIS:-

The data collected for this study was analyzed using the software known as SPSS 21 after being entered and arranged in MS excel.. The student's t test was used to determine the significance of the quantitative data, while the Mann Whitney and chi-square tests was used to determine the significance of the qualitative data. p value is considered to be significant if its $<0.05$.

## RESULTS

In our study 96 diabetic patients were included. Among 96 patients, 48 patients had elevated UACR (suggestive of DN) and 48 had normal spot urine albumin to creatinine ratio. All the patients were screened and selected based on no habits of smoking and drinking and similar in terms of age, dietary habits and other parameters. Both the groups(Diabetics with Nephropathy and Diabetic without Nephropathy) were compared for multiple variables such as FBS, PPBS , eGFR and HbA1c, ratio of neutrophils and lymphocyte, serum creatinine, and blood urea.

Age & Sex distribution of the study subjects (TABLE 1& 2)

| Age Groups | Groups | | Total | $X^2$ | P |
|---|---|---|---|---|---|
| | Diabetics without Nephropathy | Diabetics with Nephropathy | | | |
| <50 | 11 | 5 | 16 | 3.482 | 0.323 |
| 51-60 | 17 | 24 | 41 | | |
| 61-70 | 14 | 13 | 27 | | |
| 71 and above | 6 | 6 | 12 | | |
| Total | 48 | 48 | 96 | | |

| Gender | Groups | | Total | $X^2$ | P |
|---|---|---|---|---|---|
| | Diabetics without Nephropathy | Diabetics with Nephropathy | | | |
| Female | 23 | 23 | 46 | 0.000 | 1.000 |
| Male | 25 | 25 | 50 | | |
| Total | 48 | 48 | 96 | | |

From the above table it can be observed that, majority of subjects with and without nephropathy were among the age group of 51 to 60 years. There was no statistically significant association between age and nephropathy (P= 0.323) & majority of subjects with and without nephropathy were males. There was no correlation between gender and nephropathy (P = 1.0).

TABLE 2: Comparison of FBS, PPBS & HBA1c among subjects with and without Diabetic Nephropathy

| FBS | Groups | | | T | P |
|---|---|---|---|---|---|
| | | Diabetics without Nephropathy | Diabetics with Nephropathy | | |
| | Mean | 156.77 | 180.04 | 2.102 | 0.038 |
| | Std. Deviation | 50.155 | 57.671 | | |
| PPBS | Groups | | | T | P |
| | | Diabetics without Nephropathy | Diabetics with Nephropathy | | |
| | Mean | 249.2 | 275.31 | 1.446 | 0.152 |
| | Std. Deviation | 79.107 | 92.476 | | |
| HBA1C | Groups | | | T | P |
| | | Diabetics without Nephropathy | Diabetics with Nephropathy | | |
| | Mean | 8.58 | 8.52 | 0.153 | 0.878 |
| | Std. Deviation | 2.00 | 2.07 | | |

From the above table it can be observed that, mean FBS levels were significantly higher among subjects with diabetic nephropathy compared to their normal counterparts (P= 0.038). The mean PPBS levels were higher among subjects with diabetic nephropathy compared to their normal counterparts but the p value was not found to be significant (P= 0.152). The mean HbA1c levels among subjects with and without diabetic nephropathy were almost similar (P = 0.878).

TABLE 4: Comparison of SPOT URINE ALBUMIN TO CREATININE RATIO(UACR) among subjects with and without Diabetic Nephropathy

| SPOT UACR | Groups | | | Z | P |
|---|---|---|---|---|---|
| | | Diabetics | Diabetics | | |

|  |  | without Nephropathy | with Nephropathy |  |  |
|---|---|---|---|---|---|
|  | Median | 9.8 | 625.0 | 8.367 | 0.001 |
|  | IQR | 5.74-18.55 | 310.8-982.5 |  |  |
| N/L RATIO | Groups |  |  | T | P |
|  |  | Diabetics without Nephropathy | Diabetics with Nephropathy |  |  |
|  | Mean | 1.684 | 2.552 | 8.114 | 0.001 |
|  | Std. Deviation | 0.252 | 0.697 |  |  |
| UREA | Groups |  |  | T | P |
|  |  | Diabetics without Nephropathy | Diabetics with Nephropathy |  |  |
|  | Mean | 20.58 | 32.08 | 8.215 | 0.001 |
|  | Std. Deviation | 6.546 | 7.157 |  |  |
| CREATININE | Groups |  |  | T | P |
|  |  | Diabetics without Nephropathy | Diabetics with Nephropathy |  |  |
|  | Mean | 0.601 | 0.846 | 6.338 | 0.001 |
|  | Std. Deviation | 0.182 | 0.195 |  |  |
| GFR | Groups |  |  | T | P |
|  |  | Diabetics without Nephropathy | Diabetics with Nephropathy |  |  |
|  | Mean | 107.75 | 92.35 | 4.960 | 0.001 |
|  | Std. Deviation | 12.073 | 17.794 |  |  |

From the above table it can be observed that, the median SPOT UACR, mean N/L RATIO, mean blood urea, mean serum creatinine & GFR was higher among subjects with diabetic nephropathy compared to their normal counterparts. The comparison showed that p value was significant and UACR, N/L RATIO & GFR was significantly higher in diabetics with nephropathy (P = 0.001).

## DISCUSSION

The study was conducted among 96 diabetic patients among whom 48 had early stage DN and 48 had normal albumin to creatinine ratio. The NLR among the two-group showed significantly elevated (P = 0.001) among the patients with DN.

Numerous studies have been done over past few decades which shows the role of inflammatory mediators (such as cytokines, adipokines, chemokines etc.) and endothelial malfunction which eventually leads to development of diabetic microvascular and macrovascular complications [7-12]. There are multiple theories and explained pathogenesis leading to DN however exact mechanism is

still unclear. The most acknowledged mechanism responsible for DN, shows early glomerular damage leading to proteinuria following which progressive renal damage, inflammation, fibrosis and later on complete loss of functional nephrons leading to development and progression of DN [12-16]. Renal inflammation plays a significant role in the development of DN [12].

Over the years several studies have shown the role of inflammation and various markers such as elevated neutrophil counts and relatively decreased lymphocytes have served as an independent markers in many diseases including diabetes and its complications such as DN. WBC count and differential count are easily available and relatively inexpensive inflammatory markers [11,12 17,18,19,20]. However, isolated WBC count, neutrophils or lymphocyte counts for the diagnosis or accessing risk factors in diseases leads to bias and shortfall unlike NLR ratio which is more dynamic and is prognostically more significant[18,19,21].

Neutrophils to lymphocyte ratio is a relatively newer marker which determines chronic inflammatory state and helps to correlate importance of two independent variables of immune system (neutrophils and lymphocyte). Neutrophils are one of the most important mediators of inflammation and is also the primary response against infection whereas lymphocytes helps in regulating as well as protecting inflammation [22]. Interestingly, NLR was found to be positively associated not only with the presence of metabolic syndrome, but also with its severity[23].

Study done by Imtiaz et al. [24] suggests that diseases which are of chronic nature such as diabetes and hypertension are significantly associated with increased inflammatory response as evident with elevated NLR.

Study done by Shiny et al. [25] also revealed that NLR is significantly elevated in diabetics with high insulin resistance and severe glucose intolerance and helps in determining prognosis for macrovascular and microvascular complications. Over few decades a lot of studies were conducted to see the association between NLR and diabetic complication which helped the researchers to conclude that NLR may have a role to predict the development of multiple complications mostly small arteries.

A study by Ulu et al.[26] showed that NLR is a quick as well as trustable marker which helps in determining prognosis and severity in diabetic retinopathy. Similarly another study done by Ulu et al.[20] helps in concluding that NLR helps in predicting as well as determining prognosis of sensorineural hearing loss in diabetic patients. Similar study done on elderly patients also shows that NLR was significantly elevated and serves as an independent marker of microvascular complications in DM [27]. NLR has also proven to be a simple and inexpensive laboratory parameter in CKD patients which provides important information about inflammation. [28] NLR also functioned as an indicator of renal function decline over 3 years follow up in a diabetic patient. [29] Study done by Afsar et al. also corresponds towards role of NLR in determining diabetic nephropathy as well as predicting ESRD.[30]. Akbas et al.[31] showed NLR was increased among patients with elevated albumin excretion in urine, suggesting there is a role of dysfunction in endothelium as well as inflammation in diabetic patients who has developed nephropathy. In our study we compared NLR ratio among patients with diabetes who has elevated urine albumin to creatinine ratio (UACR) to patients with normal urine albumin to creatinine ratio and it showed the mean NLR was significantly elevated (2.552) in diabetics with elevated UACR compared to diabetics with normal UACR (1.684)

## CONCLUSION

The study revealed that the level of NLR was associated with the development of DN, suggesting that inflammation and the dysfunction of the endothelium could be contributing factors to this condition. It also showed that the test could be used as a predictor of DN. The NLR test is an easy-to-use and

inexpensive way using differential leukocyte count. It can be used in a setup with limited resources and is an alternative to expensive markers of inflammation such as cytokines, ILs, and C-reactive protein. A prospective design study and multiple measurements of the NLR test will allow researchers to gain a deeper understanding of its role in the development of DN and how it could be used as a prognostic and predictive marker of diabetic nephropathy.

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