



NLR As Novel Marker In Diabetic Microvascular Complication

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Introduction:-

Various epidemiological studies have shown that chronic inflammation plays a central role in the pathogenesis of diabetes and its various micro and macro-vascular complications. Neutrophil-lymphocyte ratio (NLR) is a novel potential marker in determining inflammation. The aim of this study was to evaluate the relationship between NLR and diabetic micro-vascular complications, namely retinopathy, nephropathy, neuropathy in type 2 diabetic patients.

Materials & Methods:-

This was an observational cross-sectional study done on 100 type 2 diabetes mellitus patients. NLR was calculated by dividing neutrophil to lymphocyte in complete blood picture. Diabetic nephropathy detected by urine albuminuria by MICRAL-II test strips by dipstick method, Diabetic Retinopathy by fundus examination, and diabetic peripheral neuropathy by 10-grams Semmes-Weinstein monofilament, applying the test on 9 different sites on the plantar surface of the foot.

Results:-

A total of 100 type 2 diabetes mellitus patient were enrolled in our study. A statistically significant difference found between NLR values in patient with and without DR (2.24 ± 0.16 v/s 1.39 ± 0.036 respectively $p < 0.001$). Similarly, NLR was higher in patient with DPN (mean NLR 2.42 ± 0.46) and patient with DN (mean NLR 2.64 ± 0.46). Also patients associated with all 3 micro-vascular complication had mean NLR 2.63 ± 0.38 , few patient associated with DR and DPN had mean NLR 2.44 ± 0.45 , some patient associated with DR and DN had mean NLR 2.65 ± 0.45 .

Conclusion:- Based on the results of this study, we can conclude that NLR, which is an efficient, simple and stable marker of inflammation, can serve as an important predictor of micro-vascular complications in patients with type 2 diabetes.

Keywords: DN (diabetic nephropathy), DPN (diabetic peripheral neuropathy), DR (diabetic retinopathy), NLR (Neutrophil to Lymphocyte ratio)

Introduction

India is the diabetes capital of the world, with diabetes and pre-diabetes prevalence of 9% and 11-14%, respectively.⁽¹⁾ This increased burden of diabetes in Indians is a awful precursor of an exponential increase in diabetes-related end-organ

damage and associated morbidity in the next few decades. There is an urgent need for cheap and easy to measure predictors of the occurrence of diabetes-related end-organ damage in Indians.

Patients with diabetes mellitus commonly develop various chronic vascular complications, including macro-vascular diseases (heart disease, stroke and peripheral vascular disease) and micro-vascular diseases (retinopathy, neuropathy and nephropathy)^[2]. The inflammatory process plays a crucial role in the pathogenesis of type 2 diabetes and precedes the onset of the disease. Subclinical inflammation contributes to further deepening of metabolic disturbances and finally development of vascular diabetic late complications^[3], which are the major cause of morbidity and mortality in diabetic patients^[4]. Moreover, there is evidence that chronic inflammation may contribute to both development and acceleration of micro-angiopathy and macro-angiopathy in diabetic patients^[5]. Inflammatory cytokines such as TNF- α , IL-1 β , IL-6, hs-CRP were found to play a central role in the vicious circle of inflammation, endothelial dysfunction and atherosclerosis in patients with diabetic micro- and macro-vascular complications^[6].

Neutrophil-lymphocyte ratio (NLR) may be considered a novel marker of chronic inflammation. It represents a ratio of two markers; neutrophils, which represent the active nonspecific mediator initiating the first line of defence and lymphocytes, representing the regulatory or protective component of inflammation^[7]. NLR is found to be superior to other leukocyte parameters (e.g. neutrophil, lymphocyte and total leucocyte count) due to its stability which is less influenced by physiological, pathological and physical factors^[8].

Long-term hyperglycemia, reflected by an enhanced level of HbA1c, also changes metabolism of neutrophils and causes their activation, increases their interactions with other cells and prolongs their circulation time in the blood stream^[9].

NLR that can be easily calculated from a simple peripheral blood count, is simpler and cheaper than measuring other inflammatory cytokines, such as IL-6, IL-1 β and TNF- α ^[10]. In this respect, NLR has emerged as a novel surrogate marker. In the present study, the association of NLR with diabetes micro-vascular complication in Indian patients is studied, whether or not NLR can be used as a surrogate marker of DM in this population.

Materials And Methods:-

This study was conducted in the medical ward at MBS Hospital associated with Govt. medical college Kota Rajasthan between 2019-2020. Local Ethical Board approval and informed consents from all study subjects were obtained before enrollment. Study was carried out on 100 type 2 diabetic patients, 53 of them were male and 47 of them were female. Data were collected through a predesigned structured questionnaire to collect information about age, smoking habits, type of diabetes, duration of the disease, previous screening for diabetic complications, history of renal disease (dialysis or transplantation), history of retinal laser photocoagulation as well as symptoms of diabetic neuropathy. Neurological examination was performed using the 10-grams Semmes-Weinstein monofilament, applying the test on 9 different sites on the plantar surface of the foot and diagnosing sensory neuropathy when less than seven sites were felt by the patient. Vibration Perception Threshold (VPT) was also measured, using a biothesiometer, to define the presence of diabetic neuropathy with a cut-off VPT of more than 25 volts for the diagnosis of loss of protective sensation. Retinopathy examination was done through fundus examination, performed by an Ophthalmologist, with fully dilated pupils and severity of retinopathy, if any, was assessed. Diabetic retinopathy was graded as no signs of retinopathy, non-proliferative retinopathy or proliferative retinopathy. Urinary albumin-to-creatinine ratio (UACR) was done for all study patients, and repeated whenever positive for confirmation. Patients were classified as having 'no albuminuria' when the UACR is less than 30 mg/g, incipient nephropathy with UACR 30-300 mg/g or overt nephropathy with values \geq 300 mg/g. For all study subjects, fasting plasma glucose, glycosylated hemoglobin (HbA1c), erythrocyte sedimentation rate (ESR) as well as estimation of neutrophil-lymphocyte ratio (NLR), by dividing the absolute neutrophil count by the absolute lymphocyte count on a peripheral blood film, were performed. Statistical analysis of the data: Data were analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and

D'Agstino test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between the two studied groups were done using independent t-test while for more than two groups F-test (ANOVA) was used. Correlations between two quantitative variables were assessed using Pearson coefficient. Significance of the obtained results was judged at the 5% level.

Results:-

A total of 100 subjects have been enrolled in this study; Out of 100, Each subject was allocated to one of 2 groups; group I included 71 type 2 diabetic patients with at least one micro-vascular complication of diabetes (retinopathy, nephropathy and/or neuropathy), group II included 29 type 2 diabetic patients without any micro-vascular diabetic complication. (Table no. 1)

As compared to patients without diabetic retinopathy, patient associated with retinopathy had higher mean

NLR (2.24±0.16) [Table no.2]. Statistically significant difference of mean NLR was found in diabetic patients with and without neuropathy (2.42±0.46 v/s 1.88±0.46 respectively, p value <0.001) [Table no.3]. Similarly value of mean NLR had higher in patient with diabetic nephropathy as compared to patients without nephropathy (2.64±0.46 v/s 1.87±0.41 respectively) [Table no. 4]. Also patient associated with all 3 micro-vascular complication had higher mean NLR as compared with patient not associated with all 3 micro-vascular complication (2.63±0.38 v/s 1.93±0.47 respectively) [Table no. 5], similarly few patients associated with retinopathy and peripheral neuropathy had higher mean NLR as compared to not associated with retinopathy and neuropathy [Table no. 6]. Some patients associated with diabetic retinopathy and nephropathy had higher mean NLR as compared to patient without diabetic retinopathy and nephropathy [Table no.7], also (values of mean NLR was statistically significant with p value <0.001).

Tables:- Table no.1 The baseline characteristics and laboratory data of all studied subjects

Variable	Diabetic patient with micro-vascular complication (n=71)	Diabetic patient with no micro-vascular complication (n=29)
Age (years)	60.37±12.49	49.34±9.12
Duration of diabetes (Year)	6.85±3.08	3.72±1.20
Fasting blood glucose (mg/dl)	176.45±32.68	143.59±17.11
Post prandial blood sugar (mg/dl)	216.15±41.45	177.62±20.54
HBA1C %	8.25±1.15	7.08±0.55
hs-CRP (mg/l)	2.48±0.67	1.63±0.17
NLR	2.26±0.48	1.56±0.24
Triglycerides (mg/dl)	191.31±28.76	153.41±15.7
LDL-Cholesterol (mg/dl)	161.42±20.38	114.24±16.71
HDL-Cholesterol (mg/dl)	38.17±6.27	41.45±6.92

Above table suggest that diabetic patients with at-least one micro-vascular complications (group I) were having higher neutrophil-lymphocyte ratio (NLR), fasting blood sugar, PPBS, HBA1C, hs-CRP, Triglyceride, LDL-Cholesterol compared with diabetic patients without complications (group II) ($p < 0.001$). HDL-Cholesterol is higher in diabetic patient without any micro-vascular complication.

Table no.2 Relationship between Neutrophil-lymphocyte ratio (NLR) and Diabetic Retinopathy

Diabetic retinopathy	Number of cases	Mean NLR±SD	P VALUE
Absent	30	1.39±0.036	<0.001
Present	70	2.24±0.16	

- In our study population, 30% people not had diabetic retinopathy with mean NLR 1.39 with SD 0.036 and 70% people had diabetic retinopathy with mean NLR 2.24 with SD 0.16.
- P value <0.001, which was statistically significant.

Table no. 3 Relationship between Neutrophil Lymphocyte ratio and Diabetic Peripheral Neuropathy

Diabetic neuropathy	No. of cases	Mean NLR±SD	P value
Absent	66	1.88±0.46	<0.001
Present	34	2.42±0.46	

- In this above table showed that 66% of cases not had D. Neuropathy were mean NLR±SD 1.88±0.46
- 34% of cases had of D. Neuropathy were Mean NLR±SD 2.42±0.46. P value <0.001, which is significant statistically.

Table no.4 Relationship between Neutrophil-lymphocyte ratio (NLR) and diabetic nephropathy

Urine albuminuria	Number of cases (%)	Mean NLR	Std. Deviation	P value
Present	23 (23.0%)	2.64	0.46	<0.001
Absent	77 (77.0%)	1.87	0.41	

- In this study table, 23% people with diabetic nephropathy had mean NLR 2.64 with standard deviation 0.46
- 77% people without diabetic nephropathy had mean NLR 1.87 with standard deviation 0.41. P value <0.001, it is statistically significant.

Table no. 5 Association of mean NLR with all 3 micro-vascular complication of diabetes

Pt. with all 3 micro-vascular complication	Number of pt.	Mean NLR	SD	P VALUE
Absent	82	1.93	0.47	<0.001
Present	18	2.63	0.38	

Above table showed that 82% cases not associated with all 3 micro-vascular complication of diabetes had mean NLR 1.93 with SD 0.47 while 18% cases associated with all 3 micro-vascular complication of DM2 had mean NLR 2.63 with SD 0.38, difference between them was statistically significant and P value was <0.001

Table no. 6 Association of mean NLR with D. Retinopathy+ D. Peripheral Neuropathy

D. Retinopathy+ D. Neuropathy	Number of pt.	Mean NLR	SD	P VALUE
Absent	67	1.87	0.46	<0.001
Present	33	2.44	0.45	

Above study table showed that 67% cases without D. Retinopathy & D. Neuropathy had mean NLR 1.87 with SD 0.46, While 33% cases with combined D. Retinopathy & D. Neuropathy had mean NLR 2.44 with SD 0.45. Difference between them was statistically significant and P value was <0.001.

Table no. 7 Association of mean NLR with pt. of D. retinopathy + D. nephropathy

Pt. with D. retinopathy + D. nephropathy	Number of pt.	Mean NLR	SD	P VALUE
Absent	77	1.88	0.41	<0.001
Present	23	2.65	0.45	

Above study table showed that 77% cases without D. Retinopathy & D. Nephropathy had mean NLR 1.88 and SD 0.41 While 23% cases with D. retinopathy & D. nephropathy had mean NLR 2.65 and SD 0.45. The difference between them was statistically significant and the P value was <0.001.

Discussion:-

The most important finding of our study was that neutrophil-lymphocyte ratio (NLR) levels were significantly higher in diabetic patients with micro-vascular complications (retinopathy, nephropathy and peripheral neuropathy) than among diabetic patients with no complications (p<0.001). Several epidemiological studies have previously highlighted that chronic low grade inflammation is associated with diabetes mellitus [11].

Currently, there is evidence that diabetic retinopathy and other complications of diabetes may be considered to be a state of generalized inflammation [5, 12].

Clinical studies have shown elevated levels of pro-inflammatory cytokines in the vitreous fluid of patients with proliferative diabetic retinopathy, which are related to the activity and progression of retinal injury. These data highlighted the central and causal

role of chronic low-grade subclinical inflammation in the pathogenesis of Diabetic retinopathy [13]. In our study studied 70% people were found to be affected with Diabetic retinopathy and in the rest 30% cases no Diabetic Retinopathy changes were detected on fundus examination. Mean NLR with SD for people having Diabetic retinopathy was found 2.26±0.48 and people not having Diabetic retinopathy 1.57±0.24, P value <0.001 which is statistically significant, it means that mean NLR among patients with retinopathy was significantly higher than among Diabetic patients without retinopathy or any other micro-vascular Diabetic complication (p<0.001). Thus increased NLR value is associated with more chances of developing Diabetic retinopathy. **Ulu S et al** from the Faculty of Medicine, Afyon Kocatepe University, Turkey, found that NLR values of the Diabetic patients with Diabetic retinopathy were higher than those of patients without retinopathy [14]. Another study, carried out by **Yue S et al** in the Fengyutan

Health Care Center, Shenhe District, Shenyang City, China, has similarly showed that patients with Diabetic retinopathy had higher NLR values than Diabetic patients without evidence of the disease [15]. In contrast to our results, **Ciray H et al**, from the Department of Internal Medicine, Karaman State Hospital, Karaman, Turkey, found that NLR was not significantly different between patients with and without Diabetic retinopathy, arguing that there is no independent association between NLR and the presence of retinopathy in patients with type 2 Diabetes [16].

Diabetic peripheral neuropathy develops as a result of hyper-glycemia induced local metabolic, enzymatic and microvascular changes. It has been demonstrated that endogenous TNF- α production is accelerated with microvascular permeability, hypercoagulability and nerve damage, thus initiating and promoting the development of characteristic lesions of diabetic polyneuropathy. In our study, we found that NLR was significantly higher among patients with Diabetic neuropathy, compared with patients without evidence of peripheral neuropathy. Neutrophil-lymphocyte ratio was consistently higher among diabetic patients with loss of protective lower extremity sensation, detected by abnormal monofilament test ($p < 0.001$). 34% cases were found to be affected with diabetic peripheral neuropathy, having mean NLR with SD 2.41 ± 0.46 , which is statistically significant with P value < 0.001 . Different studies have tested the association of chronic subclinical inflammation and Diabetic peripheral neuropathy. **Tingting Xu et al** in their study showed that pt. having Diabetic peripheral neuropathy had mean NLR with SD 2.58 ± 0.50 , and pt. not having DPN had mean NLR with SD 2.18 ± 0.6 , which is near to our study [17]. **KP ranjith et al** in 2018 showed that mean NLR was significantly high in DPN group [18]. **Herder C et al**, from the Institute for Clinical Diabetology, German Diabetes Center, Heinrich Heine University, Düsseldorf NN5N,2, Germany, found that several immune mediators might be associated with the development of Diabetic polyneuropathy [19].

Diabetic nephropathy is a common Diabetic complication, but its pathogenesis remains unclear. However, a cascade of pathological events (with glomerular damage being an early sign, which gives rise to proteinuria, followed by progressive renal

damage, fibrosis, inflammation, and finally loss of functional nephrons) is known to play an important role in the development and progression of DN [20-22]. Renal inflammation in the setting of DN is known to play a critical role. WBC count and its subtypes are among the readily available and inexpensive classic inflammatory markers [23]. Multiple studies have established that inflammatory markers such as neutrophilia and relative lymphocytopenia are independent markers of many diseases, especially complications of DM, such as DN. [24, 25]. 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 [26]. **Asfar B et al**, in the Division of Nephrology, Konya Numune State Hospital, Konya, Turkey, have shown that neutrophil-lymphocyte ratio (NLR) could be related to the presence of diabetic nephropathy and it has been correlated as an indicator of end stage renal disease [26]. In our study, 23 Diabetic patient with nephropathy having mean NLR 2.64 ± 0.46 and 77 pt. not having nephropathy having mean NLR 1.87 ± 0.41 ($p < 0.001$). NLR was higher among patients with overt nephropathy, compared with that in incipient nephropathy, which is statistically significant. In agreement with our results, **Huang W et al**, from the Department of Endocrinology, Zhujiang Hospital, Southern Medical University, Guangzhou, China, have found that neutrophil-lymphocyte ratio 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) and healthy control subjects (1.80 ± 0.64) [27]. Similarly, **Asfar B et al** studied the relationship between neutrophil lymphocyte ratio and urinary protein and albumin excretion in type 2 Diabetic patients who are recently diagnosed, and reported that increased NLR was independently associated with both 24-hour urinary protein ($p < 0.001$) and urinary albumin excretion ($p < 0.001$) in newly diagnosed Turkish patients with type 2 Diabetes [26]. **Sachin chittawar et al** [28] the results of our study show that there was a significant relation between NLR and DN. Therefore, NLR may be considered as a novel surrogate marker of DN in early stages. Similar study done by **Ahmed ateia Abdelaziz et al** [29] July 2018 showed increased mean

NLR in Diabetic pt. with macro-albuminuria than micro-albuminuria, normo-albuminuria.

The most important finding of our study was that neutrophil-lymphocyte ratio (NLR) levels were significantly higher in diabetic patients with microvascular complications (retinopathy, nephropathy and peripheral neuropathy) than among diabetic patients with no complications and the control group ($p < 0.001$).

18% people in our study population associated with all 3 micro-vascular complication had mean NLR 2.63 ± 0.38 while 82% people without all 3 micro-vascular complication had mean NLR 1.93 ± 0.47 p value < 0.001 , which was statistically significant.

Eman Youssef Moursy et al. ⁽³⁰⁾ in 2015, in their study "Relationship between NLR and Micro-vascular Complications in Egyptian Patients with Type 2 Diabetes" showed that mean NLR with SD in pt. with all complication of diabetes 2.39 ± 1.01 and without complication pt. had mean NLR 1.41 ± 0.30 .

Sachin chittawar et al. ⁽²⁸⁾ in their study analysis based on the number of microvascular complications a patient has revealed that patients with a larger number of microvascular complications had a significantly longer duration of Diabetes, higher WHR, higher blood pressure, NLR (with a comparable total leukocyte count), creatinine, and urine albumin excretion. Mean NLR in pt. without complication was 1.69, with one complication 2.06, with two complication was 2.71 and with all 3 micro-vascular complication of Diabetes was 2.69. Our study results were also in concordance with his study. 23% people with Diabetic retinopathy + Diabetic nephropathy had mean NLR 2.65 ± 0.45 , 77 people without Diabetic retinopathy + Diabetic nephropathy had mean NLR 1.88 ± 0.41 p value < 0.001 , which was statistically significant.

33% people with combined Diabetic retinopathy + Diabetic neuropathy had mean NLR 2.44 ± 0.45 , 67% people without Diabetic retinopathy + Diabetic neuropathy had mean NLR 1.87 ± 0.46 p value < 0.001 , which was statistically significant.

Conclusion:-

We could highlight here the importance of a routine assessment the neutrophil-lymphocyte ratio (NLR), which can be easily calculated from a simple peripheral blood count and, therefore, is much

simpler and cheaper than measuring more sophisticated inflammatory cytokines in patients with diabetes. Based on the results of our study, neutrophillymphocyte ratio (NLR), which is an efficient and stable marker of inflammation, can serve as an important predictor for the presence of micro-vascular Diabetic complications, namely retinopathy, neuropathy and/or nephropathy in patients with type 2 Diabetes.

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