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Relationship of subclinical inflammatory markers with glycemic status and duration of diabetes among rural Indian type 2 diabetic population

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Abstract: *Background:* Neutrophil-to-Lymphocyte Ratio (NLR) and the Platelet-to-Lymphocyte Ratio (PLR)are the inflammatory markers in Type 2 Diabetes Mellitus (T2DM) and there are few documented Indian rural studies. The aim of this study is to evaluate the relationship between Neutrophil Lymphocyte Ratio (NLR), Platelet Lymphocyte Ratio (PLR) with glycemic status (HbA1c) and duration of diabetic detection in rural Indian T2DM. *Methods:* This is a prospective observation study, T2DM between the ages of 30-60 years, attending to Sree Sidhi Vinayaka Diabetic Centre (2013-2019). NLR, PLR was compared with duration of diabetes and glycemic status. T2DM were divided into three groups, Group I with HbA1c <7[excellent control], Group II with HbA1c 7.01-9.0% [poor control] and Group III - HbA1c >9 [worst control]. *Results:* 5163 patients attended the clinic, among them, 1303 fulfilled the inclusion criteria. The mean age of T2DM was 47.02±7.81 yrs, with 637 females and 666 males (Male: Female ratio is ≈1). A significant increase in the NLR & PLR levels in T2DM more than 5 years of duration was noted. There is a significant positive strength of association between duration of T2DM, level of HbA1c, PLR and NLR levels. Mean level of NLR & PLR were significantly lower in the Group I diabetics compared with the Group II and Group III ($p<0.01$), *Conclusions:* There is significant positive strength of association between glycemic status and duration of diabetes with NLR & PLR as inflammatory marker in rural T2DM.

Keywords: Neutrophil-lymphocyte ratio, Platelet-Lymphocyte ratio, HbA1c, Duration of Diabetes Mellitus, Rural Indian Population

Introduction

Global prevalence of Diabetes Mellitus (DM) is expected to be over 217 million and is increasing over the next few years [1]. The increase in type 2 diabetes in South Asian countries is expected to be more than 150% between 2000 and 2035. The burden of diabetes is on the rise in middle- and lower-income countries like India with a prevalence of 9%, due to urbanization, and sedentary life style [2-3]. Type 2 Diabetes mellitus (T2DM) is a silent killer and most of the patients are asymptomatic before they develop complications [3], systemic inflammation, increased insulin resistance and a more violent loss of beta-cell mass [4]. There is a relationship between systemic inflammation, and diabetic microand macrovascular complications [5].

Inflammatory markers such as interleukin (IL)-1, IL6, IL8, tumour necrosis factor, and transforming growth factor-1 are linked to the end organ damage [6]. Estimation of these markers is not possible in routine practice as they are expensive or not easily available [7-8]. In recent years, the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), which are derived

from the measurement of blood count parameters, have been introduced as possible inflammatory markers in cardiac disorders, neoplasms and complications associated with diabetes [9]. High blood glucose levels are toxic to the body, resulting in protein glycation, hyperosmolality and a high intracellular sorbitol content. Glycation is an irreversible, non-enzymatic binding reaction between glucose and protein – e.g. Haemoglobin, the word glycated haemoglobin (HbA1c), emerged.

The key haemoglobin, with subtypes A1 and A2 is haemoglobin A (HbA). Complete HbA1 refers to those molecules that are most negatively charged by the addition of glucose and other carbohydrates. The A1c fraction is present in HbA1c, and glucose is bound to the N-terminal valine beta-chain. HbA1C has become an important method for diagnosing diabetes mellitus (DM), maintaining glycaemic regulation and predicting the risk of vascular complications, in addition to blood glucose [10]. The aim of this study is to evaluate the relationship between Neutrophil Lymphocyte Ratio (NLR), Platelet Lymphocyte Ratio (PLR) with Glycaemic status (HbA1c)and duration of Diabetic detection in rural Indian type 2 Diabetic populations as a simple cost effective early inflammatory maker in T2DM.

Material and Methods

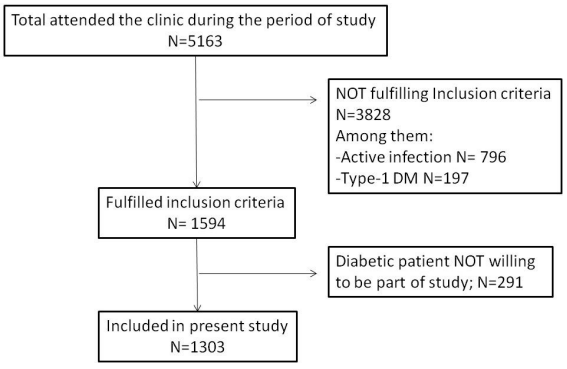
This is a prospective observation study carried out on T2DM between the ages of 30-60 years, attending to outpatient clinic of Sree Sidhi Vinayaka Diabetic Centre, Sangareddy between July 2013 to June 2019. Informed consent was obtained from all the participants, and the study protocol was approved by Institutional Ethics Committee. Diabetes was defined as fasting plasma glucose>126mg/dl or HbA1c >6.5% [11]. Patients with signs of active inflammation, infection, malignancy and pregnancy, history of connective tissue disorders, rheumatic heart disease, crohn's disease, hypertension, CAD, Thyroid disorders, patients on immuno suppressants and patients with increase in ESR were excluded from the study.

The demographic details; Age, gender, height, weight, waist circumference and duration of diabetes were obtained. A body mass index (BMI) was determined by dividing of the weight in kilograms to the square of height in meters [32]. Blood samples were drawn after 12h fasting, for estimation of glucose, HbA1c[Fully automated H.P.L.C., using Biorad Variant II Turbo] [32], White Blood Cell Count (WBC), Neutrophils Count, Lymphocyte Count, Haemoglobin, Haematocrit, and Platelet Count, which were analysed within 2 hours of venepuncture [Fully automated bidirectional analyser (6 Part Differential SYSMEX XN-1000)] [5]. NLR is calculated by neutrophil to lymphocyte count and PLR is calculated by platelet count to lymphocyte count.

They were divided into three groups, Group I with HbA1c <7[excellent control], Group II with HbA1c 7.01-9.0% [poor control] and Group III - HbA1c >9 [worst control]. The Inflammatory markers, NLR and PLR were comparedwith glycaemic status (HbA1c), and duration of diabetes [<5yrs diabetic detection and >5yrs]. The ratio of NLR and PLR is contrasted between the groups and the influence of the duration of diabetes on the ratio of NLR and PLR was analysed.

Statistical analysis: All the data were entered in Microsoft excel sheet and analysed using SPSS version 21 operating on windows 10. The Demographic details of the patients are represented as frequency, percentage, Mean and SD. The mean differences between the multiple variables were analysed using student t-test and ANOVA analysis with bonferroni post hoc analysis for continuous variable. The strength of association between the continuous variable was assessed using the Pearson's correlation. A p-value lower than 0.05 percentile was considered statistically significant.

Fig-1: Study Design



Results

Total of 5163 patients attended the clinic during the period of study. Among them, 1303 fulfilled the inclusion criteria and included for analysis. The mean age of diabetic individuals was 47.02±7.81 yrs, with 637 females and 666 males (Male:Female ratio is ≈1). The other demographic and physical details are presented in Table 1 & 2.

Table-1: Gender distribution of the diabetic population.

	Frequency	Percent
Female	637	48.9
Male	666	51.2
Total	1303	100.0

Table-2: Demographic details of diabetic population.

	Minimum	Maximum	Mean	SD
Age in Yrs	30.00	60.00	47.02	7.81
Weight in kgs	30.00	110.00	62.41	11.99
Height in Mts	1.34	1.91	1.58	0.09
BMI in kg/M2	13.0	42.00	23.91	6.72
Duration of Diabetes Mellitus in Yrs	.00	25.0	3.02	5.12
FBS in mg/dL	37.00	600.00	203.20	90.60
PPBS mg/dL	68.0	812.0	282.17	112.60

Table 3: Mean difference in NLR and PLR in diabetic population based on duration of diabetes mellitus.

	Duration of DM	Mean	Std. Deviation	p-value
NLR	< 5 yrs	2.13	1.22	0.004**
	> 5yrs	2.41	1.34	
PLR	< 5 Years	10.06	6.00	0.01**
	> 5 Years	11.24	5.40	

**p-value <0.01 is statistically highly significant (HS); NLR – Neutrophil-Lymphocyte Ratio; PLR – Platelet-Lymphocyte Ratio; DM – Diabetes Mellitus.

Comparing the NLR according to the duration of diabetes mellitus duration, we documented a significant increase in the NLR levels in diabetics more than 5 years of duration. Likewise the PLR levels were significantly higher in the diabetic duration more than 5 years. (Table 3)

We found significant positive strength of association between duration of diabetes mellitus, level of HbA1c, PLR and NLR levels amongT2DM (Table 4).

Table-4: Strength of association between NLR, PLR, HbA1c and Duration of Diabetes Mellitus.

		NLR	PLR
Duration of DM	r	.089**	.079*
	sig	.004	.010
HbA1c	r	0.067*	0.096**
	sig	0.015	0.001
PLR	r	.745**	
	sig	.000	

*p-value<0.05 is statistically significant; **p-value <0.01 is statistically highly significant (HS).

Table-5: Comparison of mean levels of NLR, PLR in different HbA1c groups of study.

	Group I (HbA1c <7%) Mean±SD (a)	Group II (HbA1c 7-9%) Mean±SD (b)	Group III (HbA1c >9%) Mean±SD (c)	p-value
NLR	1.74±1.33 (b)(c)	2.16±1.17 (a)	2.20±1.31 (a)	0.001**
PLR	7.51±5.78(b)(c)	10.41±5.90 (a)	10.29±5.95 (a)	0.001**

**p-value <0.01 is statistically highly significant (HS); NLR – Neutrophil-Lymphocyte Ratio; PLR – Platelet-Lymphocyte Ratio. Post-Hoc analysis is performed using bonferroni testing post ANOVA testing.

The mean level of NLR was significantly lower in the Group I diabetics compared with the Group II and Group III ($p<0.01$), similarly the PLR level was significantly lower in Group I diabetic compared with Group II and Group III ($p<0.01$). However, there is no significant difference between the mean level of NLR and PLR between the Group II and Group III diabetic population (Table 5).

Discussion

Among the different parameters of the complete blood count and the Neutrophil-Lymphocyte ratio (NLR) were extensively studied as inflammation markers in cardiac and non-cardiac disorders. NLR in acute myocardial infarction, heart failure and stroke has been proposed as a prognostic marker.

Prolonged hyperglycaemia causes the development of multiple inflammatory disorders, such as neuropathy [12], autonomic neuropathy [13] retinopathy, renal failure, hypercoagulability, hypertension, myocardial infarction, stroke, and peripheral vascular disease, and Neutrophil-to-Lymphocyte Ratio (NLR) is a marker of inflammation, and a high NLR negatively affects the frequency and prognosis of CAD [14]. Aclose relationship was found between high NLR and presence and complexity of coronary artery disease, and in CAD, NLR was an independent risk marker for long-term complications in diabetes [15].

High WBC count is a predictor for the development complications of T2DM [16]. NLR and PLR stand out as a marker of chronic inflammation, neutrophils are the nonspecific mediators of inflammation, whereas lymphocytes are protective or regulatory component of inflammation [17-18]. Many studies have demonstrated the association between DM and atherosclerosis, where systemic inflammation plays an important role in the development of atherosclerosis which were revealed by biomarkers, including high-sensitive C-reactive protein and IL-6, TNF-α, fibrinogen, p-selectin, and serum amyloid A [19-20]. In present study, we have found a significant relation of the NLR and PLR with the duration of diabetes mellitus and level of HbA1c.

In present study, we have found a significant lower level of the NLR and PLR in patients with HbA1c level lower than 7% (Group I) compared to HbA1c >7% (Group II& Group III). Which suggest that, HbA1c target of <7% reduces the circulating inflammatory marker and their consequences. The NLR in the poorly controlled DM group was significantly higher than in well-managed DM group, and mean NLR value improved with glycaemic control [21-23]. HbA1c levels >7% are associated with an increased risk of permanent organic damage.

We found a significant positive strength of association between duration of Diabetes Mellitus, level of HbA1c, PLR and NLR levels, similarly other study revealed correlation between NLR and duration of DM, BMI, and HbA1c was strongly positive 24]. Researchers also recorded that the longerdiabetic duration is associated with early development of micro and macrovascular complications [24-32].

Limitation of study: It is a monocentric study; here we did not investigate the relation of different antidiabetic agents as treatment for T2DM with NLR & PLR, as some antidiabetic drugs have also anti-inflammatory activity in addition to glycemic control.

Conclusion

Present studies suggest a significant positive strength of association between the glycaemic status and duration of diabetic detection with inflammatory markers. As duration of diabetes is non modifiable risk factor, it is mandatory to maintain A1c <7%, which will help in long way in controlling the ongoing inflammation and postponement of micro and macro vascular complications. Neutrophil Lymphocyte Ratio (NLR), Platelet Lymphocyte Ratio (PLR) serves as a simple cost effective early inflammatory maker in diabetic rural population.

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Conflicts of interest: There are no conflicts of interest.

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