<u>Original research article</u>

Study of association of neutrophil lymphocytes ratio with development of cardiovascular disease in type 2 diabetic patients

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Abstract

Background: The neutrophil-to-lymphocyte ratio (NLR) has been studied as a prognostic factor in CVD. This Study was conducted to assess the correlation of Neutrophil Lymphocytes ratio with development of cardiovascular disease in type 2 diabetic patients.
Materials and Methods: It was a hospital based, Prospective, Randomized, Case Control study included all Patients of type II diabetes mellitus who attended outpatient of department of general medicine in Mahatma Gandhi Medical College & hospital, Jaipur for a period of 1 year.
Results: There was a statistically significant difference present in number of subjects with hypertension in case and control group (p=0.043). According to Pearson correlation analysis there was a highly statistically significant positive correlation between NLR and severity of CVD (r=0.56, p<0.01). There was highly statistically significant difference present in mean total cholesterol level and HDL, between case and control group (p<0.01). There was a statistically significant difference present in mean LDL, when compared between case and control group (p=0.007). There was a highly statistically significant difference present in mean levels of White blood cell, Neutrophil (%) and Lymphocyte (%), when compared between case and control group (p<0.01).
Conclusion: The data presented show that high NLR might be an independent risk factor for increased cardiovascular disease. NLR is a simple and easy to calculate. This test is inexpensive and done routinely. In a setup with limited laboratory facilities, NLR can be a cheap effective alternative marker as predictor of cardiovascular disease in diabetes patients.

Keywords: Type-2 diabetes mellitus, cardiovascular disease, neutrophil lymphocytes ratio.

Introduction

India is the diabetes capital of the world, with diabetes and prediabetes prevalence of 9% and 11-14%, respectively [1]. Type-2 diabetes mellitus (T2DM) has an aggressive clinical phenotype in Indians [2].
CVD or stroke is a disease caused by the interaction of several risk factors, including diabetes and genetic predisposition. 34 It is the most common cause of death worldwide [3]. CVD is increased 2 to 4 times and cardiovascular mortality or morbidity is higher in patients with type 2 diabetes mellitus than in those without diabetes [4]. In several studies, a 1% increase in hemoglobin A1c (HbA1c) within the normal range resulted in increased 10-year cardiovascular mortality and CVD mortality doubled in the impaired glucose tolerance (IGT) group compared with the normal glucose tolerance (NGT) group.
The neutrophil-to-lymphocyte ratio (NLR) has been studied as a prognostic factor in CVD. Although the correlation between NLR and CVD has not been fully established, systemic factors such as inflammation, vascular endothelial cell dysfunction, and oxidative stress are thought to have an effect [5]. A higher NLR is expected to lead to faster development and progression of atherosclerosis, and a higher NLR is associated with a higher mortality rate or can be used as a prognostic factor in patients with acute coronary syndrome or percutaneous cardiovascular intervention [6, 7, 8].
Because NLR was also found to be independently associated with the coronary calcium score, 61 it was hypothesized that there exists an association between NLR and the severity of CAD. As, data are lacking on the role of NLR as a predictor of cardiovascular disease in Indians with T2DM, the present research was undertaken to evaluate the role of NLR in cardiovascular disease.

Aims and Objected

Study correlation of Neutrophil Lymphocytes ratio with development of cardiovascular disease in type 2 diabetic patients.

Material & Methods

It was a hospital based, Prospective, Randomized, Case Control study included all Patients of type II diabetes mellitus who attended outpatient of department of general medicine in Mahatama Gandhi Medical College & hospital, Jaipur for a period of 1 year.

Inclusion criteria

All patients with type 2 diabetes who met the 2015 American Diabetes Association standards and classification.

Exclusion criteria

- Patients with T1DM.
- Acute Inflammation.
- Active Infection.
- Leukaemia.
- Chronic Liver diseases.
- Renal disorders.
- Patient who refused consent.

Investigation

- CBC
- RBS
- HbA1c
- S. Creatinine
- SGPT
- SGOT
- Fasting Lipid Profile

Statistical analysis: Data were entered on MS office Excel and analysed by SPSS software. The appropriate statistical tests were applied.

Results

Table 1: Gender distribution among the study groups

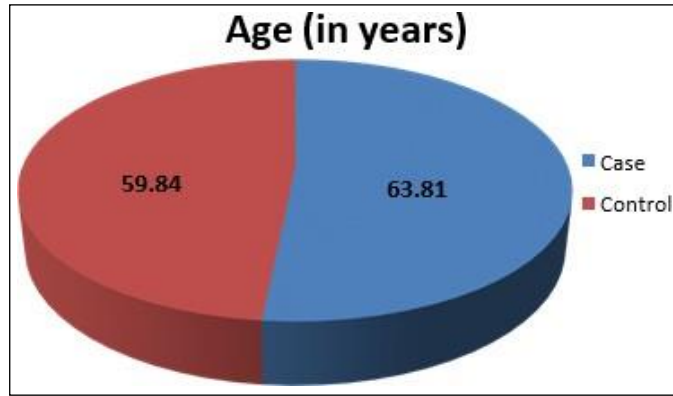| Gender	Case (n=50)		Control (n=50)		p value
	No	%	No	%	
Male	32	64	28	56	0.58
Female	18	36	22	44	
Total	50	100	50	100	

There was no significant difference in gender distribution between case and control group.
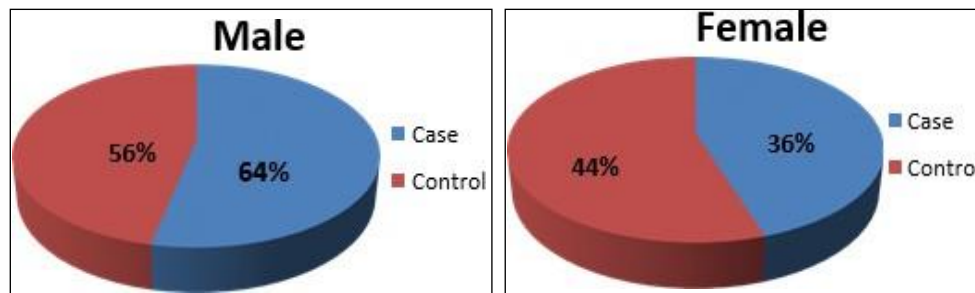
Graph 1: Gender distribution among the study groups

Table 2: Mean age among the study groups

Group	Age (in years)		p value
	Mean	SD	
Case (n=50)	63.81	8.92	0.28
Control (n=50)	59.84	10.09	

Graph 2: Mean age among the study groups

The mean age of subjects in case and control group was 63.81±8.92 and 59.84±10.09 years, respectively. There was no significant difference present in age of subjects between case and control group. (Table 2)
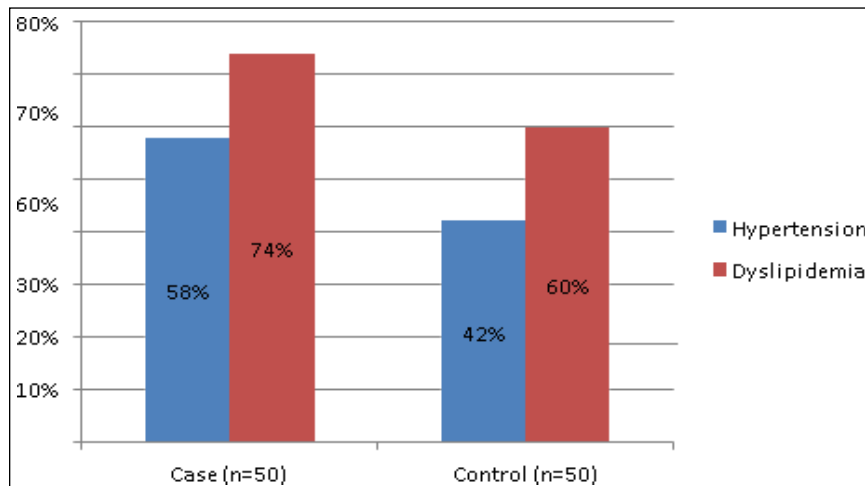
Table 3: Hypertension and Dyslipidemia among the study groups

Variables	Case (n=50)		Control (n=50)		p value
	N	%	N	%	
Hypertension	29	58	21	42	0.043*
Dyslipidemia	36	74	30	60	0.06

*:Statistically significant

There was a statistically significant difference present in number of subjects with hypertension in case and control group (p=0.043).
The number of subjects having Dyslipidemia in case and control group were having difference i.e., 74% and 60%, respectively, but this difference was not significant. (Table 3)

**Graph 3:** Hypertension and Dyslipidemia among the study groups

Table 4: Lipid profile among the study groups

Lipid Parameters	Case (n=50)		Control (n=50)		p value
	Mean	SD	Mean	SD	
Total cholesterol (mg/dL)	166.60	21.04	139.22	16.57	<0.01**
Triglyceride (mg/dL)	142.36	19.11	137.29	14.90	0.32
HDL (mg/dL)	37.24	9.38	44.19	7.20	<0.01**
LDL (mg/dL)	143.67	27.96	105.54	30.05	0.007*

*: statistically significant, **: highly significant

The mean level of Total cholesterol (mg/dL) in case and control group was 166.60±21.04 and 139.22±16.57, respectively. The mean level of HDL (mg/dL) in case and control group was 37.24±9.38 and 44.19±7.20, respectively. The mean level of LDL (mg/dL) in case and control group was 143.67±27.96 and 105.54±30.05, respectively. There was highly statistically significant difference present in mean total cholesterol level and HDL, between case and control group (p<0.01). There was a

statistically significant difference present in mean LDL, when compared between case and control group (p=0.007). (Table 4)
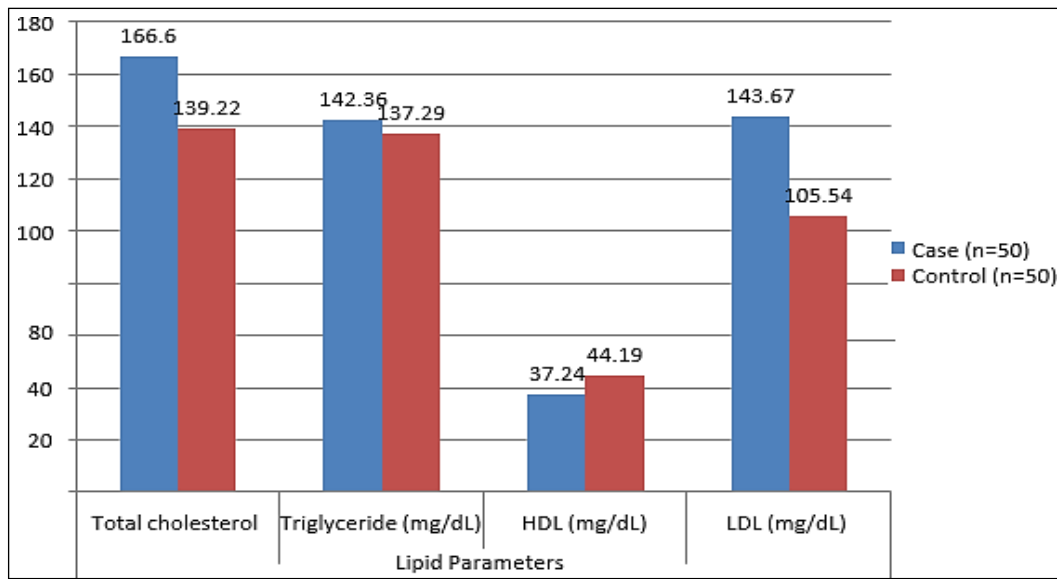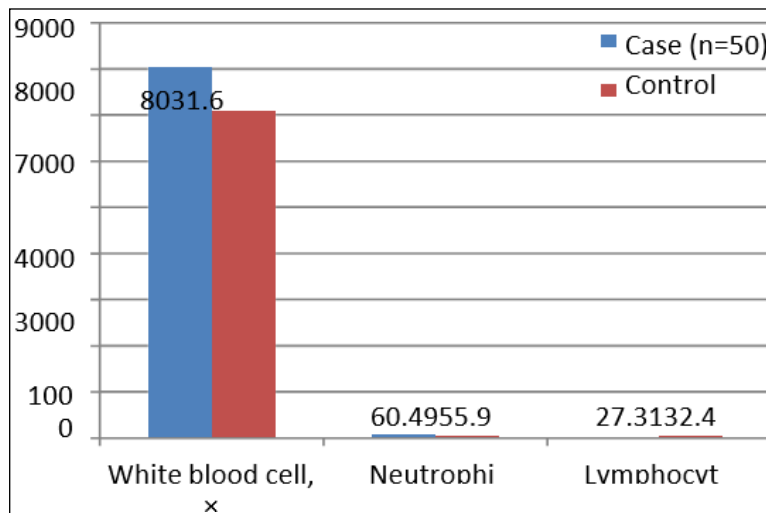

Graph 4: Lipid profile among the study groups

Table 5: WBC profile among the study groups

Variables	Case (n=50)		Control (n=50)		p value
	Mean	SD	Mean	SD	
White blood cell, $\times 10^3/\mu L$	8031.6	2189.5	7091.34	1906.99	<0.01**
Neutrophil (%)	60.49	9.36	55.97	8.19	<0.01**
Lymphocyte (%)	27.31	7.67	32.48	6.83	<0.01**

**: highly statistically significant

Graph 5: WBC profile among the study groups

The mean level of White blood cell, $\times 10^3/\mu L$ in case and control group was 8031.6±2189.5 and 7091.34±1906.99, respectively. The mean level of Neutrophil (%) in case and control group was 60.49±9.36 and 55.97±8.19, respectively. The mean level of Lymphocyte (%) in case and control group was 27.31±7.67 and 32.48±6.83, respectively. There was a highly statistically significant difference present in mean levels of White blood cell, Neutrophil (%) and Lymphocyte (%), when compared between case and control group ($p<0.01$). (Table 5)

Table 6: Comparison of Neutrophil Lymphocytes ratio (NLR) among the study groups

Group	NLR		p value
	Mean	SD	
Case (n=50)	2.21	0.99	<0.01**
Control (n=50)	1.72	1.07	

**: highly statistically significant
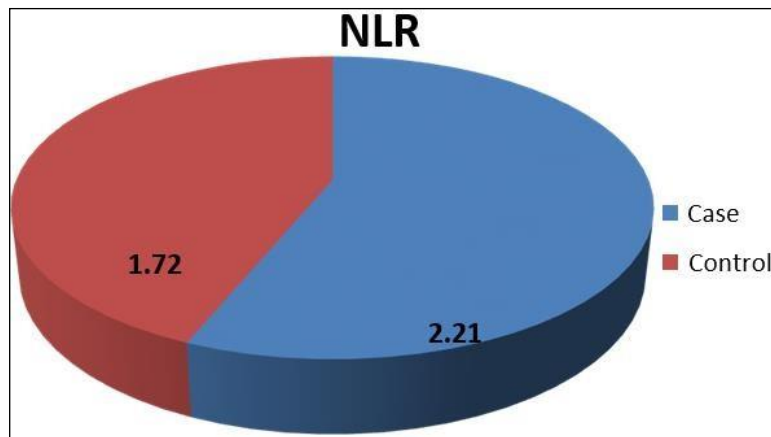
Graph 6: Comparison of Neutrophil Lymphocytes ratio (NLR) among the study groups

The mean Neutrophil Lymphocytes ratio (NLR) in case and control group was 2.21±0.99 and 1.72±1.07, respectively. The difference in mean Neutrophil Lymphocytes ratio (NLR) in case and control group was highly statistically significant ($p<0.01$). (Table 6)

Table 7: Correlation between NLR and severity of CVD

Parameters	Value
r value	0.56
value	<0.01**

**: highly statistically significant

According to Pearson correlation analysis there was a highly statistically significant positive correlation between NLR and severity of CVD (r=0.56, p<0.01). (Table 7)

Discussion

Type 2 diabetes is a major public health problem worldwide, and vascular complications still represent the main cause of morbidity and mortality in type 2 diabetes patients. The main pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls and ischemia throughout the body.

NLR is defined as a novel potential biomarker to determine inflammation in diabetes and its complications [9]. Calculation of NLR is a very simple method compared with other inflammatory cytokines tested [1].

The mean age of subjects in case and control group was 63.81±8.92 and 59.84±10.09 years, respectively. The mean age of subjects in control group was less than age of subjects in case group, suggesting cardiovascular disease is common in patients with older age group, but the difference was not significant. Hypertension was present in 29 (58%) subjects in case group and 21 (42%) subjects in control group. There was a statistically significant difference present in number of subjects with hypertension in case and control group (p=0.043). The number of subjects having Dyslipidemia in case and control group were having difference i.e., 74% and 60%, respectively, but this difference was not significant.

The mean level of White blood cell, $\times\ 10^3$/μL in case and control group was 8031.6±2189.5 and 7091.34±1906.99, respectively. The mean level of Neutrophil (%) in case and control group was 60.49±9.36 and 55.97±8.19, respectively. The mean level of Lymphocyte (%) in case and control group was 27.31±7.67 and 32.48±6.83, respectively, this suggest that there is an inverse relation present in levels of Lymphocyte and presence of cardiovascular disease. There was a highly statistically significant difference present in mean levels of White blood cell, Neutrophil (%) and Lymphocyte (%), when compared between case and control group ($p<0.01$).

The mean Neutrophil Lymphocytes ratio (NLR) in case and control group was 2.21±0.99 and 1.72±1.07, respectively. The difference in mean Neutrophil Lymphocytes ratio (NLR) in case and control group was highly statistically significant ($p<0.01$).

According to Pearson correlation analysis there was a highly statistically significant positive correlation between NLR and severity of CVD (r=0.56, $p<0.01$). These findings were in accordance to findings of Kim BJ *et al.*, (2016) [10].

According to study done by Kim BJ *et al.*, (2016) [145] patients with significant coronary artery disease (CAD) had a positive correlation between NLR and the severity of coronary artery disease.

Conclusion

The data presented show that high NLR might be an independent risk factor for increased cardiovascular disease. NLR is a simple and easy to calculate. This test is inexpensive and done routinely. In a setup with limited laboratory facilities, NLR can be a cheap effective alternative marker as predictor of cardiovascular disease in diabetes patients. Early screening of patients might help distinguish a high-risk group and guide prophylactic initiatives.

References

1. Dutta D, Mukhopadhyay S. Intervening at prediabetes stage is critical to controlling the diabetes epidemic among Asian Indians. Indian J Med Res. 2016;143:401-4.
2. Dutta D, Maisnam I, Shrivastava A, *et al*. Serum Vitamin-D predicts insulin resistance in individuals with prediabetes. Indian J Med Res. 2013;138:853-60.
3. Dutta D, Mondal SA, Kumar M, *et al*. Serum fetuin-A concentration predicts glycaemic outcomes in people with prediabetes: A prospective study from Eastern India. Diabet Med. 2014;31:1594-9.
4. Dutta D, Choudhuri S, Mondal SA, *et al*. Tumor necrosis factor alpha -238G/A (rs 361525) gene polymorphism predicts progression to type-2 diabetes in an Eastern Indian population with prediabetes. Diabetes Res Clin Pract. 2013;99:e37-41.
5. Rudiger A, Burckhardt OA, Harpes P, Müller SA, Follath F. The relative lymphocyte count on hospital admission is a risk factor for long-term mortality in patients with acute heart failure. Am J Emerg Med. 2006;24:451-4.
6. Twig G, Afek A, Shamiss A, *et al*. White blood cells count and incidence of type 2 diabetes in young men. Diabetes Care. 2013;36:276-82.
7. Jiang H, Yan WH, Li CJ, Wang AP, Dou JT, Mu YM. Elevated white blood cell count is associated with higher risk of glucose metabolism disorders in middle-aged and elderly Chinese people. Int. J Environ Res Public Health. 2014;11:5497-509.
8. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002;51:455-61.
9. Horne BD, Anderson JL, John JM, *et al*. Which white blood cell subtypes predict increased cardiovascular risk? J Am Coll Cardiol. 2005;45:1638-43.
10. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: Atherosclerosis risk in communities study. Am J Epidemiol. 2001;154:758-64.