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## Title of the article: Linking of Neutrophil-Lymphocyte Ratio (NLR) to HbA1c in Diabetes Mellitus: A Cross-Sectional Study

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### Abstract:

Diabetes mellitus (DM) is a universal public health problem and is associated with metabolic, cellular, and blood disorders. Haematological deviations have been reported in diabetes and play a vital role in diabetes-associated complications. Hemogram is one of the most common investigations, available in all hospitals where the NLR can be easily derived from the existing set of parameters measured in the blood sample. The NLR has been associated as a systemic inflammatory response marker in numerous diseases. Therefore, this study was carried out to derive correlation between NLR and DM and to analyze the association between the NLR and HbA1c values in patients of DM. In our study a total of 641 diagnosed cases of DM data were analysed and they were categorized as per the HbA1c value into Group A (n=414), Group B (n=102), Group C (n=125). Using a CBC, NLR was calculated and analyzed with IBM SPSS statistical grad pack 29.0 for Mean  $\pm$  SD, Chi-square, and Pearson correlation coefficient. There is a significant association amongst NLR and HbA1c values in diabetic patients ( $p < 0.001$ ). The correlation analysis suggests a relationship amongst NLR and HbA1c levels, indicating that higher NLR values may be associated with higher HbA1c levels.

Keywords: Diabetes mellitus, HbA1c, Neutrophil-Lymphocyte Ratio, Complete blood count

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## Introduction:

Of late Diabetes mellitus (DM) has become a sombre threat globally not only because of the disease per se but because of loss of control in the maintenance of normal indices leading to complications affecting invariably the micro and macro vessels thus reducing the life expectancy. (Zheng Y *et al.*, 2018, Fujita T *et al.*, 2013) Hence, substantial consideration has to be focused on initial diagnosis and active management with follow-up in patients with DM. The trigger for the involvement of the vessels is alleged to be vascular damage ensuing from platelet stimulation which accounts for complications. The utility of inflammatory markers has always been a limitation in day-to-day clinical practice, the reason being a lack of knowledge, standardized techniques, the financial burden on the patient, and technical hitches in measuring. (Fujita T *et al.*, 2013)

Hemogram is one of the most common investigations which is available in all hospitals ranging from advanced care providers to rural health setups. This parameter is wished for at the commencement of the management of virtually any ailment for which the patient has arrived at the hospital. In hemogram, the neutrophil-lymphocyte ratio (NLR) is an effortlessly calculable parameter that can be readily derived from the existing set of parameters measured in the blood sample (Khandare SA *et al.*, 2017, Verdoia M *et al.*, 2015).

NLR has been associated as a systemic inflammatory response marker in numerous diseases and can be thought of as being utilized in a globally relevant disorder i.e., DM because of its convenience and less burden on the pockets of the patient (Huang W *et al.*, 2015).

## Objectives:

1. Assess NLR and HbA1c in patients of DM
2. Derive correlation between NLR and HbA1c.

## Methodology:

**Study Design-** A record-based cross-sectional study was conducted. CBC and HbA1c data were gathered from the medical record section, of the patients who had come to the hospital and were known cases of type 2 diabetes mellitus

**Study Duration-** Two months

**Sample size-** During this period, a convenient sample method was adopted.

**Inclusion criteria-** Data of diabetes mellitus patients of age 18 years and above of either gender who had been investigated for CBC and HbA1c were included.

**Exclusion criteria-** Type 2 DM with associated underlying conditions predisposing to altered hemogram and other biochemical parameters.

As per the HbA1c levels, the patients were grouped as group A - HbA1c  $\leq$  7%, group B - HbA1c 7.1-9.0 %, and group C - HbA1c  $\geq$  9 %. The data of these patients were also collected for CBC. (Hussain M *et al.*, 2017)

Using CBC, the NLR was calculated by entering the values in excel sheet. The normal reference range stated by Sayed AA *et al* 2021 the neutrophil count between 4.28 and 4.64 X  $10^3/\mu\text{L}$ , and a lymphocyte count between 1.95 and 2.1 X  $10^3/\mu\text{L}$ , a normal NLR count was between 2.18 and 2.21. The data gathered for the above-said parameters was analyzed with IBM SPSS statistical grad pack 29.0 for Mean  $\pm$  SD, Chi-square, and Pearson correlation coefficient.

## Results:

In our study, a total of 641 diagnosed cases of DM data were analyzed and they were categorized as per the HbA1c value into Group A (n=414), Group B (n=102), Group C (n=125) as shown in figure 1.

The values of NLR and HbA1c for various groups are depicted in Table 1.

Group C with worst control of HbA1c showed a high leukocyte count ( $p = 0.001$ ), high neutrophil count ( $p = .003$ ) and lower lymphocyte count ( $p = 0.44$ ) when compared to group A. This difference was not statistically significant when group A was compared to group B on the same parameters as shown in table 2.

Correlation between NLR and HbA1c is shown in Table 3.

- There is a significant association between the NLR and HbA1c values in diabetic patients ( $p < 0.001$ ).

- The correlation analysis suggests a relationship between NLR and HbA1c levels, indicating that higher NLR values may be associated with higher HbA1c levels.

## Discussion:

Diabetes is a worldwide public health issue linked to disturbances that may be cellular and/or metabolic. These changes reported in diabetes play a key role in complications that occur in such patients.

CBC is a feasible and inexpensive, blood test that is available in any laboratory. Interpretation of the data of NLR which is a routine test for the assessment of any inflammatory response in

the body for diabetes could be useful in terms of decreasing the monetary burden and monitoring the disease progression. Inflammatory pathways are the primary pathogenic mediators for diabetes mellitus and related complications (Boucher AA *et al.*, 2012). NLR is said to be more consistent than a CBC in detection of subclinical inflammation.

The first line of host immune response are neutrophils which act against pathogens. Numerous mechanisms suggested include cytokines, protein cascades, phagocytosis, reactive oxygen species and many more (Mortaz E *et al.*, 2018). Of all these neutrophils have a role in adaptive immunity and thus are the chief effector cells in any inflammatory response that occurs in our body. Thus, neutrophils as controllers of innate immunity, the functions of the immune system are controlled through the dendritic cells (DCs), B cells, NK cells, CD4, CD8 and T cells, as well as mesenchymal stem cells (Li Y *et al.*, 2019).

As far as our information, our study is the first in central India to reveal a significant association between NLR with HbA1c ( $p < 0.001$ ) independent of random levels of blood glucose in general population with a history of diabetes mellitus. The correlation analysis suggests a connection amongst NLR and HbA1c levels, indicating that higher NLR may be associated with higher HbA1c levels.

Our study is in correlation with the study of Selfil *et al.*, 2014 which discovered that increased NLR may be linked with raised HbA1c in type 2 diabetic patients whereas two different studies done in the Japanese population state varied relation between WBC count and diabetes mellitus and not with NLR (Hong GB *et al.*, 2020, Kashima S *et al.*, 2019).

Studies of Shiny *et al.*, 2014 and Lou *et al.*, 2015 stated that augmented NLR are relatable to glucose intolerance and thus to insulin resistance which is a typical finding in type 2 diabetic patients. Kizilgul *et al.*, 2018 found that different parameters of CBC like WBC, neutrophil, and lymphocyte were elevated in diabetics who had inappropriate glycaemic control inspite of insulin therapy, though NLR did not show any correlation with diabetes.

Apart from diabetes, it is known that there is a positive correlation of NLR with other diseases in which innate and adaptive cellular immunity is disturbed (Zahorec R *et al.*, 2021).

Overall, the study indicates significant associations between NLR and HbA1c values in diabetic patients. These findings support the probable use of NLR as a simple marker for the associated risk of diabetes mellitus and its complications and may indicate an inflammatory process.

## Conclusion:

In our study, we concluded that increased NLR in diabetics seems to be an independent predictor of inflammation.

Though CBC has variable serum patterns which are dependent on the laboratory analyzer evaluation of NLR levels may be useful in the assessment of patients who are at increased risk for complications in DM.

The positives of the study were that all the patients were from the same hospital setup and the blood analyzed was on a single analyzer which was responsible for calibration and giving values of the assessed parameters since it is a known fact that the assessed parameters give variable inputs in different setups. Another drawback is the retrospective design of the study which has been undertaken. Lastly, the small sample size and varied distribution of patients as per HbA1c levels need to be reinvestigated in a larger population. A prospective study that uses the same analyzers with larger sample sizes would be more informative.

To conclude, NLR needs to be evaluated in patients of type 2 diabetes mellitus but what needs to be measured is whether it can be used as a disease-progression tool during the follow-up of diabetic patients.

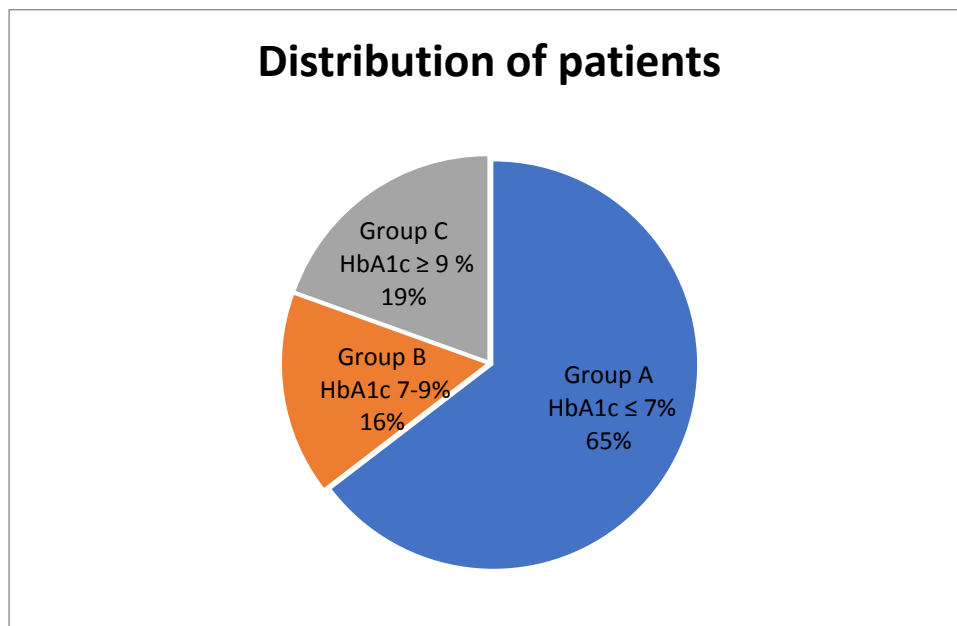
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**Figure 1: Distribution of patients as per the HbA1c levels**

**Table 1: Values of NLR as per the various HbA1c levels**

Group	N (Number of subjects)	NLR (Mean±SD)	HbA1c %
Group A	414	3.085±1.09	5.61±0.89
Group B	102	3.733±5.2	8.082±0.56
Group C	125	3.313±1.07	11.93±2.2

**Table 2: Statistical analysis of components of CBC and HbA1c in all patients**

	Neutrophils	Lymphocyte	Platelets	TLC	HbA1c	NLR
<b>N</b>	641	641	641	641	641	641
<b>Mean</b>	71.380	22.119	277604.41	11259.37	7.2351	5.74229246
<b>Std. Deviation</b>	27.9807	11.5817	232665.604	14112.130	2.76282	8.92368367

**Table 3: Correlation between NLR and HbA1c**

Group	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	68900.736 <sup>a</sup>	62620	<.001
Likelihood Ratio	4529.152	62620	1.000
Linear-by-Linear Association	.011	1	.918
N of Valid Cases	641		