DIABETES TECHNOLOGY & THERAPEUTICS
Volume 16, Number 5, 2014

DOI: 10.1089/dia.2013.0264

DTT
Diabetes Technology & Therapeutics

ORIGINAL ARTICLE

Association of Neutrophil-Lymphocyte Ratio with Glucose Intolerance: An Indicator of Systemic Inflammation in Patients with Type 2 Diabetes

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Abstract

Background: The neutrophil-lymphocyte ratio (NLR) has been demonstrated to be a better risk factor than total white blood cell count in the prediction of adverse outcomes in various medical conditions. This study analyzed the association of NLR with different grades of glucose tolerance and insulin resistance in Asian Indians.

Subjects and Methods: Study subjects were recruited from Phase 3 of the Chennai Urban Rural Epidemiology Study (CURES). For this cross-sectional analysis, subjects with normal glucose tolerance (NGT) ($n=237$), impaired glucose tolerance (IGT) ($n=63$), and type 2 diabetes mellitus (DM) ($n=286$) were selected. The hemogram was done in all subjects using a five-part hematology analyzer (model SF-3000; Sysmex, Kobe, Japan). The NLR was calculated as the ratio between counts for neutrophils and total lymphocytes. Fasting insulin was measured by enzyme-linked immunosorbent assay, and insulin resistance was calculated using the homeostasis model assessment (HOMA-IR).

Results: Subjects with DM showed a significantly higher NLR (2.2 ± 1.12) compared with IGT subjects (1.82 ± 0.63), who in turn had a higher ratio than NGT subjects (1.5 ± 0.41) ($P<0.01$). Pearson correlation analysis showed a significant positive correlation of NLR with glycated hemoglobin ($r=0.411$), fasting plasma glucose ($r=0.378$), and HOMA-IR ($r=0.233$) ($P<0.001$). Regression analysis showed a linear increase in NLR with increasing severity of glucose intolerance even after adjusting for age, waist circumference, blood pressure, triglycerides, and smoking.

Conclusions: This is the first report on the correlation of NLR with different grades of glucose intolerance and insulin resistance. NLR can be used as an adjuvant prognostic marker for macro- and microvascular complications in patients with glucose intolerance.

Introduction

Both prediabetes and overt type 2 diabetes mellitus (DM) are characterized by insulin resistance and are associated with obesity and cardiovascular disease.[1] Several studies have shown the relationship between systemic inflammation and insulin resistance, where an altered immune system plays a decisive role in the pathogenesis of DM.[2] These immunological alterations result in elevated circulating levels of acute-phase proteins and pro-inflammatory cytokines that play a major role in the development of chronic inflammation-induced organ dysfunction in DM.[3,4]

The immune response to various physiological challenges is characterized by increased neutrophil and decreased lymphocyte counts, and it is often recognized as an inflammatory marker to assess the severity of disease pathogenesis.[5,6] It has been suggested that elevated white blood cell (WBC) count may be associated with various components of metabolic syndrome and increased cardiovascular risk in patients with impaired glucose tolerance (IGT).[7,8] Recently, the neutrophil-lymphocyte

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ratio (NLR) has been demonstrated to be a greater risk factor than total WBC count in the prediction of adverse outcomes in various medical conditions like cancer and cardiovascular diseases.[9–12] However, the relationship between NLR and different degree of glucose intolerance has not been evaluated so far. Therefore, in the current study we have analyzed the association of NLR with different grades of glucose intolerance and insulin resistance in Asian Indians.

Research Design and Methods

Study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), a large epidemiological study conducted on a representative population of Chennai, the fourth largest city in India. The detailed study design composed of several phases (Phases 1–5), each dealing with specific epidemiologic questions, has been published elsewhere.[13] Informed consent was obtained from all participating subjects, and institutional ethical committee approval was obtained. In brief, the city of Chennai was divided into 155 corporation wards representing socioeconomic diverse groups. In Phase 1 of CURES, 46 of the 155 wards in Chennai were screened via systematic sampling techniques, providing a total sample size of 26,001 individuals ≥ 20 years of age. Subsequently, in Phase 3, every 10^{th} subject recruited in Phase 1 ($n = 2{,}600$), was invited for detailed testing; 90.4% (2,350 of 2,600) of subjects participated. For the present study, all subjects from Phase 3 were chosen randomly from among those who satisfied the following inclusion criteria: absence of infectious or inflammatory diseases and not taking statins or aspirin. Based on the inclusion criteria, in total, 586 samples were selected for this study, including 237 subjects with normal glucose tolerance (NGT), 63 with IGT, and 286 with type 2 DM. Institutional ethical committee approval was obtained for the study, and informed consent was obtained from all study subjects.

Definitions

Diabetes and prediabetes were diagnosed based on the World Health Organization consulting criteria[14] (i.e., fasting plasma glucose [FPG] of ≥ 7.0 mmol/L [126 mg/dL] and/or a 2-h post-glucose value of ≥ 11.1 mmol/L [200 mg/dL] or subjects receiving treatment for diabetes [insulin or oral hypoglycemic agents]). IGT was identified if the 2-h post-glucose value was ≥ 7.8 mmol/L (140 mg/dL) and < 11.1 mmol/L (200 mg/ dL). NGT was diagnosed if the 2-h post-glucose value was < 7.8 mmol/L (140 mg/dL) and FPG was < 6.1 mmol/L (100 mg/dL).[15] Insulin resistance was calculated using the homeostasis assessment (HOMA-IR) model: fasting insulin (mIU/mL) $\times$ fasting glucose (mmol/L)/22.5. The cutoff value for insulin resistance was 1.93, which is the 75^{th} percentile of the total population in the Chennai Urban Population Study.[16]

Anthropometric measurements

Weight, height, and blood pressure were measured using standardized techniques.[13] Height was measured with a tape to the nearest centimeter. Weight was measured with a traditional spring balance that was kept on a firm horizontal surface. The body mass index (BMI) was calculated using the following formula: weight (kg)/height^2 (m^2). Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus; Diamond®, Pune, India). Two readings were taken 5 min apart, and the mean of the two was taken as the blood pressure.

Biochemical parameters

FPG (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method), and high-density lipoprotein cholesterol (direct method; polyethylene glycol–pretreated enzymes) were measured using a Hitachi-912 autoanalyzer (Hitachi, Mannheim, Germany). The intra- and interassay coefficients of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein cholesterol was calculated using the formula of Friedewald et al.[17] Glycosylated hemoglobin (HbA1C) was estimated by high-pressure liquid chromatography using the Variant™ machine (Bio-Rad, Hercules, CA). The intra- and interassay coefficients of variation of HbA1C were <10%.

Measurement of leukocyte count and NLR

Leukocyte count was assessed using a five-part hematology analyzer (model SF3000; Sysmex, Kobe, Japan) based on flow cytometry. The intra- and interassay coefficients of variation of the leukocyte count were <10%. NLR was calculated as the ratio between (percentage of) neutrophils and total lymphocyte counts in the study subjects.

Statistical analysis

Data for all biochemical parameters are presented as mean $\pm$ SD values. Student's t test or one-way analysis of variance (with Tukey's Honestly Significant Differences) was used as appropriate to compare groups for continuous variables, and the χ^2 test or Fisher's exact test was used as appropriate to compare proportions. All analysis was done using the Windows-based SPSS Statistical Package (version 10.0; SPSS, Inc., Chicago, IL), and P values <0.05 were regarded as statistically significant.

Results

Clinical and biochemical characteristics of the study subjects are shown in Table 1. Age ($P < 0.01$), waist circumference ($P < 0.01$), BMI ($P < 0.001$), systolic and diastolic blood pressure ($P < 0.001$), HOMA-IR ($P < 0.001$), FPG ($P < 0.001$), HbA1C ($P < 0.001$), and serum cholesterol ($P < 0.001$) were higher among subjects with diabetes compared with NGT individuals. Neutrophil and lymphocyte counts were significantly higher in subjects with DM compared with subjects with IGT or NGT ($P < 0.01$). However, there was no significant difference between the groups in terms of monocytes, basophils, and eosinophils (Table 1).

NLR was significantly higher in subjects with glucose intolerance. DM subjects had a significantly higher (2.2 ± 1.12; $P < 0.001$) NLR compared with IGT subjects (1.82 ± 0.63; $P < 0.01$), who in turn showed a significantly higher ratio compared with NGT subjects ($1.5 \pm 0.41 < P < 0.01$) (Fig. 1). A possible gender difference among groups was assessed, and we found no difference in NLR. We have also analyzed the influence of medications on NLR in diabetes subjects by

Table 1. Clinical and Biochemical Characteristics of Study Subjects

Parameter	*NGT* (n = *237*)	*IGT* (n = *63*)	*DM* (n = *286*)
Age (years)	39 ± 7	43 ± 8[a]	47 ± 8[bc]
Waist circumference (cm)	85.9 ± 11.3	89.8 ± 8.2	90.5 ± 9.8[bc]
BMI (kg/m^2)	24.1 ± 4.2	25 ± 3.1	25.6 ± 4.3[b]
Glycated hemoglobin (%)	5.5 ± 0.3	6.1 ± 0.6[a]	8.5 ± 2.2[bd]
Fasting plasma glucose (mg/dL)	82.6 ± 6.36	94.8 ± 12.11	159.3 ± 70.3[bd]
HOMA-IR	1.6 ± 1	3.1 ± 1.5[a]	4.6 ± 2[bc]
Blood pressure (mm Hg)			
Systolic	120.5 ± 15.6	128.3 ± 17.44[a]	129.9 ± 19.3[b]
Diastolic	76.2 ± 9.3	78.6 ± 12.6	78.4 ± 10.5[a]
Serum cholesterol (mg/dL)	180.4 ± 36	193.6 ± 42.5[a]	199.3 ± 41.8[b]
Serum triglycerides (mg/dL)	143.8 ± 91.7	149 ± 90.5	159 ± 95
LDL-C (mg/dL)	117.6 ± 33.3	119.7 ± 39.2	120.5 ± 38.3
HDL-C (mg/dL)	41.8 ± 9.82	40.6 ± 7.9	41.2 ± 9.2
Neutrophils (%)	53.8 ± 7.9	55.9 ± 6.4	59.7 ± 7.3[bc]
Lymphocytes (%)	34.2 ± 6.9	32.6 ± 6.1	29.67 ± 6.2[bc]
NLR	1.5 ± 0.4	1.82 ± 0.6[a]	2.2 ± 1.1[bd]
Monocytes	5.8 ± 2.1	5.3 ± 1.7	5.4 ± 3.3
Eosinophils	5.6 ± 4.2	5.7 ± 3.6	5.0 ± 5

[a]$P < 0.01$, [b]$P < 0.001$ compared with normal glucose tolerance (NGT).
[c]$P < 0.01$, [d]$P < 0.001$ compared with impaired glucose tolerance (IGT).
BMI, body mass index; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, insulin resistance assessed by homeostasis model assessment; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-lymphocyte ratio.

subdividing them into two groups (i.e., those on oral drugs alone and those with oral drugs plus insulin). The groups did not show any statistically significant difference in NLR values.

Figure 2 presents the scatter plot of Pearson correlation analysis. NLR showed significant positive correlation with HbA1C ($r = 0.411$; $P < 0.001$), FPG ($r = 0.378$; $P < 0.001$), and HOMA IR ($r = 0.233$; $P < 0.001$). NLR also showed a significant correlation with age, BMI, and serum cholesterol ($P < 0.01$).

A linear regression analysis was performed, using NLR as the dependent variable and glucose tolerance status as the independent variable, to determine the association of NLR with diabetes (Table 2). For each analysis, two groups were chosen, and the group with more severe glucose intolerance, coded as 1, was tested against the next less severe glucose intolerance group, which was coded as 0. The group coded as 0 was used as reference for the analysis. In Model 1, where NGT was coded as 0 and IGT was coded as 1, IGT showed a significant association with NLR even after adjusting for age, waist circumference, HOMA-IR, and blood pressure (systolic and diastolic) ($P = 0.005$). Similar analysis was performed in Model 2 with IGT and DM using IGT as the reference group. Even after adjusting for age, waist circumference, HOMA-IR, and blood pressure, subjects with diabetes showed a significant association with NLR ($P = 0.012$).

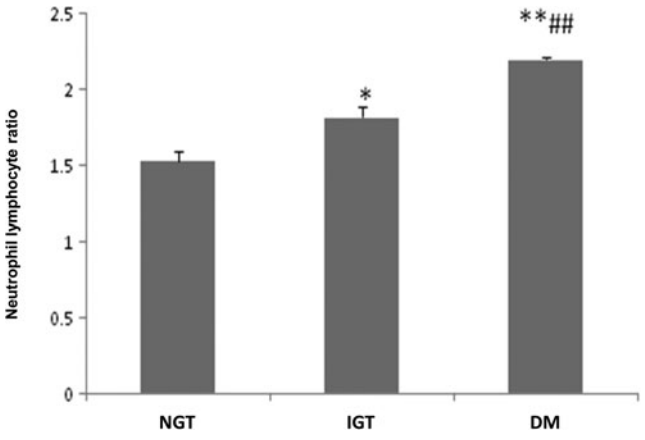

FIG. 1. Neutrophil-lymphocyte ratio in subjects with different grades of glucose intolerance. Data are mean ± SE values. *$P < 0.01$, **$P < 0.001$ compared with normal glucose tolerance (NGT); ##$P < 0.001$ compared with impaired glucose tolerance (IGT). DM, diabetes mellitus.

Discussion

The current study shows that NLR increases with increasing severity of glucose intolerance and is found to be positively associated with insulin resistance as measured by HOMA-IR. Although the pathophysiologic mechanisms of type 2 DM development are multifactorial, many epidemiological studies have highlighted the association of chronic low-grade inflammation with DM. Previously the link between high leukocyte count and impaired insulin sensitivity has been shown in patients with type 2 DM.[18] An elevated leukocyte count has been shown to be associated with risk for ischemic heart disease.[19] Insulin resistance is considered to be one of the underlying causes of glucose intolerance, which in turn clusters together with other metabolic abnormalities resulting in increased risk for coronary artery disease.[20,21] We have reported earlier that leukocyte count is elevated in subjects with diabetes, prediabetes, and metabolic syndrome.[22,23] Unlike the total WBC count, NLR is a dynamic parameter and appear to possess a superior predictive value over total leukocyte count.[24] Moreover, NLR demonstrates the balance of two complementary although paradoxical components of the immune system where neutrophils represent the active nonspecific inflammatory mediator

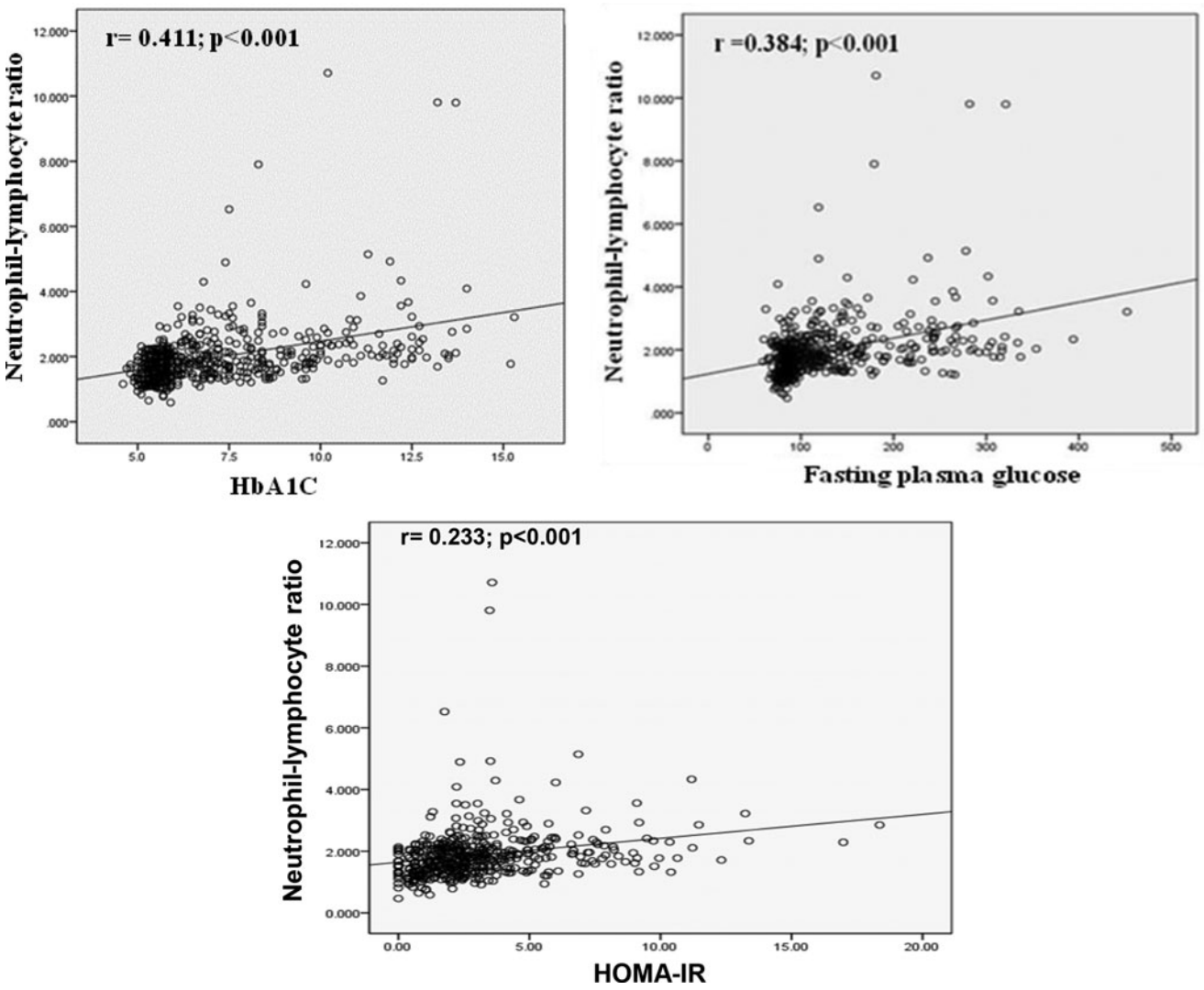

FIG. 2. Pearson analysis showing the positive correlation of neutrophil-lymphocyte ratio with glycosylated hemoglobin (HbA1C), fasting plasma glucose, and insulin resistance assessed by homeostasis model assessment (HOMA-IR).

initiating the first line of defense, whereas lymphocytes represent the regulatory or protective component of inflammation.[25] It is interesting that NLR has been shown positively associated not only with the presence but also with severity of metabolic syndrome.[26] Previously Imtiaz et al.[10] have also suggested that systemic inflammation measured by NLR has a significant association with prevalent chronic conditions such as hypertension and diabetes. Ours is the first study demonstrating the association of NLR with severity of glucose intolerance and insulin resistance. In the current study we found elevated levels of NLR owing to the presence of subclinical inflammation in IGT subjects who are considered to be at an increased risk for cardiovascular diseases. We have shown previously that the inflammatory process may start as early in the IGT stage itself, where the levels of inflammatory markers increase with increasing grades of glucose intolerance.[27]

Although NLR was initially identified as a prognostic marker in many types of cancer that might help in patient stratification and individual risk assessment,[28–30] several recent studies point out that NLR could serve as a prognostic marker for vascular diseases. Tsai et al.[31] demonstrated that NLR is strongly associated with the risk of ischemic cardiovascular disease. Other studies have demonstrated the link between increased NLR and poor survival after coronary artery bypass grafting,[32] and it has been shown to be an independent predictor of major adverse cardiac events in patients with ST-segment elevation myocardial infarction.[33] It is interesting that several recent studies support the view that NLR could even offer a prognostic value for predicting microvascular complications of diabetes. Although NLR has been shown to provide significant information regarding inflammation in chronic kidney disease patients,[34] in a 3-year follow-up study NLR served as a predictor of worsening renal function in diabetes patients.[35] Very recently, Ulu et al.[36] have also shown NLR as a quick and reliable predictive marker not only for diabetic retinopathy but also for its severity.

Leukocytes from diabetes patients generate more reactive oxygen species,[37] which in turn increase vascular endothelial

Table 2. Linear Regression Analysis Using the Neutrophil-Lymphocyte Ratio as the Dependent Variable and Varying Degrees of Glucose Tolerance Status as the Independent Variable

Parameter	β	P *value*
Model 1		
Unadjusted (NGT = 0, IGT = 1)	0.292	< 0.001
Adjusted for		
Age and waist	0.246	< 0.001
Age, waist, and HOMA-IR	0.237	0.002
Age, waist, HOMA-IR, and systolic and diastolic blood pressure	0.221	0.005
Model 2		
Unadjusted (IGT = 0, DM = 1)	0.353	0.009
Adjusted for		
Age and waist	0.316	0.019
Age, waist, and HOMA-IR	0.342	0.012
Age, waist, HOMA-IR, and systolic and diastolic blood pressure	0.340	0.012

DM, diabetes mellitus; HOMA-IR, insulin resistance assessed by homeostasis model assessment; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

permeability and promote leukocyte adhesion, leading to alterations in endothelial function.[38] Deficiency in endothelial-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction.[39] The increased NLR in diabetes subjects may be attributed to differential influence of hyperglycemia on neutrophils and lymphocytes. Increased apoptosis in lymphocytes has been reported earlier both in rats with diabetes and in patients with diabetes,[40] and increased oxidative DNA damage in peripheral blood lymphocytes has been demonstrated in patients with type 2 diabetes.[41] In contrast, hyperglycemia has been shown to reduce the apoptosis in neutrophils, leading to impaired neutrophil clearance and prolonged inflammation in mice with diabetes.[42] One mechanism by which increased levels of neutrophils could mediate insulin resistance could be through exaggerated inflammation. The increase in NLR appear to underlie the elevated levels of pro-inflammation, as evident from the persistent neutrophil activation and enhanced release of neutrophil proteases in patients with type 2 diabetes.[43,44] Increased neutrophil recruitment into adipose tissue (probably via secreted elastase) has been shown in mice fed with a high-fat diet.[45,46] It appears that neutrophils can be added to the extensive repertoire of immune cells that participate in pro-inflammation processes that underlie insulin resistance.

As we have excluded subjects with any active infection or inflammation, it is unlikely that these results are due to any infection among subjects with glucose intolerance. One of the limitations of this study is that we used a cross-sectional design, which cannot investigate any causal associations between NLR and glucose intolerance. However, as this is the first report on the correlation of NLR with different grades of glucose intolerance and insulin resistance, we propose that NLR can be used as an additional or adjuvant prognostic risk marker for both diabetes and its vascular complications. Future research with prospective design and multiple measurements of NLR could provide more robust evidence on role of NLR as an indicator of subclinical inflammation and prognostic risk factor for both diabetes and its vascular complications.

Acknowledgments

The authors wish to thank the Chennai Willingdon Corporate Foundation for its support for the CURES field studies. This is the 132nd study from CURES (CURES-132).

Author Disclosure Statement

No competing financial interests exist.

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