Relationship between neutrophil-lymphocyte ratio and insulin resistance in newly diagnosed type 2 diabetes mellitus patients

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Abstract

Background: Insulin resistance (IR) plays a vital role in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). The mechanism of IR may be associated with inflammation, whereas the neutrophil-lymphocyte ratio (NLR) is a new indicator of subclinical inflammation. Scholars have rarely investigated the relationship between IR and NLR. This study aims to evaluate the relationship between IR and NLR, and determine whether or not NLR is a reliable marker for IR.

Methods: The sample consists of a total of 413 patients with T2DM, 310 of whom have a HOMA-IR value of > 2.0. The control group consists of 130 age and BMI matched healthy subjects.

Results: The NLR values of the diabetic patients were significantly higher than those of the healthy control (P < 0.001), and the NLR values of the patients with a HOMA-IR value of > 2.0 are notably greater than those of the patients with a HOMA-IR value of ≤ 2.0 (P < 0.001). Pearson correlation analysis showed a significant positive correlation of NLR with HOMA-IR (r = 0.285) (P < 0.001). Logistic regression analysis showed that the risk predictors of IR include NLR, TG and HbA1c. NLR (P < 0.001, EXP(B) = 7.231, 95% CI = 4.277–12.223) levels correlated positively with IR. The IR odds ratio increased by a factor of 7.231 (95% CI, 4.277–12.223) for every one unit increase in NLR.

Conclusions: Increased NLR was significantly associated with IR, and high NLR values may be a reliable predictive marker of IR.

Keywords: Neutrophil-lymphocyte ratio, Insulin resistance, Inflammation, Diabetes mellitus

Background

Type 2 Diabetes Mellitus (T2DM) is characterized by insulin resistance and is associated with obesity and cardiovascular diseases [1]. Several studies that explored the relationship between systemic inflammation and cardiovascular diseases [2] indicated that chronic inflammation promotes the acceleration of diabetic microangiopathy in addition to the development of macroangiopathy in diabetic patients [3,4]. Insulin resistance (IR) is a reduction in reaction or sensitivity to insulin and is considered to be the common cause of impaired glucose tolerance, diabetes, obesity, dyslipidemia, and hypertensive diseases. IR syndrome is associated with multiple metabolic disorders and was renamed metabolic syndrome by Zimmet et al. in 1997 [5]. The exact molecular action leading to IR is not yet understood, but several studies have confirmed the relationship between systemic inflammation and insulin resistance, in which an altered immune system plays a decisive role in the pathogenesis of DM [6]. The immune response to various physiological challenges is characterized by increased neutrophil and decreased lymphocyte counts, and NLR is often recognized as an inflammatory marker to assess the severity of the disease [7,8].

The count of white blood cell (WBC) is a basic but cheap, readily available, and sensitive indicator of the inflammatory status [9]. WBCs are positively associated with inflammation, particularly in cardiovascular diseases [10]. An increase in the number of neutrophils is
associated with thrombus formation and ischemic injury [11-13]. WBC subtypes may reflect different aspects of infection or inflammatory processes. In recent years, the presence of neutrophilia and relative lymphopenia was shown to be an independent predictor of mortality in patients with acute heart failure [13,14]. Moreover, NLR was introduced as a novel marker to determine inflammation in cardiac and noncardiac disorders [15,16].

However, there have been few studies evaluating the prognostic value of NLR in IR, especially patients who were newly diagnosed diabetes. In this study that lies in the selection of newly diagnosed diabetic subjects. We aim to evaluate the relationship between IR and inflammation by using NLR, and determine whether NLR can be used as a predictive and reliable marker.

Methods
Study population and design
Conducted from July 2013 to January 2014 in the Department of Endocrinology of Zhujiang Hospital, Southern Medical University and Mingjing Diabetes Hospital, Guangdong, China, our study included 413 patients newly diagnosed with T2DM but without hypertension, any acute inflammation, infection, acute or chronic renal failure, chronic liver, heart diseases, or any microvascular complications of diabetes. The control group was comprised of 130 aged-matched healthy subjects. All participants were surveyed on the followings: age, sex, hyperlipidemia, smoking, family history, chronic disease, dietary compliance, used drugs, and other risk factors. The study protocols were conducted in accordance with the tenets of the Declaration of Helsinki and approved by the Medical Ethics Committee of Zhujiang Hospital of Southern Medical University and Mingjing Diabetes Hospital. Written informed consent was obtained from all patients.

Measurement of NLR and IR
NLR was calculated as the simple ratio between the absolute neutrophil and lymphocyte count, which were both obtained from the same automated blood sample. NLR was computed for each subject. IR was also computed for each patient. The homeostasis model of IR (HOMA-IR) was used as a measure of IR [17]. HOMA-IR was calculated using the following formula: fasting plasma glucose (mmol/L) multiplied by fasting serum insulin (mIU/L) divided by 22.5. A HOMA-IR value of > 2.0 was indicative of IR.

Definitions
Diabetes was diagnosed based on the World Health Organization consulting criteria [18] (i.e., fasting plasma glucose [FPG] of ≥7.0 mmol/L [126 mg/dL] and/or a 2-hpost-glucose value of ≥11.1 mmol/L [200 mg/dL]).

Statistical analysis
For continuous variables with normal distributions, data were expressed as mean ± standard deviation. Categorical variables were expressed as percentages. The Kolmogorov–Smirnov test was used to evaluate the distribution of variables. Student’s t-test (independent-sample t-test) was used for continuous variables with normal distribution, whereas the Mann–Whitney U test was used for continuous variables without normal distribution. The χ² test was used for categorical variables. Pearson’s correlation analyses were used to assess the relationships. Logistic regression analysis was used to assess the associations between IR and the other parameters evaluated. A value of P < 0.05 was accepted as level of significance (two-tailed). The SPSS statistical software (SPSS for Windows, version 19.0; SPSS, Inc, Chicago, IL) was used for statistical calculations.

Results
The groups were similar in terms of age, gender, body mass index, and smoking habits (P > 0.05). All baseline clinical characteristics of the groups are listed in Table 1. The NLR values of the patients were significantly higher than those of healthy subjects. The patient group also showed significantly higher triglyceride (TG) values and HbA1c values than the control group. No significant differences in Cr, TC, HDL, and LDL levels were detected between the patient and control groups.

The demographic and laboratory data of the groups are outlined in Table 1. The diabetic patients were divided into two groups according to their HOMA-IR score after the demographic and laboratory data evaluation. Group 1: HOMA-IR < 2.0; group 2: HOMA-IR > 2.0 [19,20]. Group 2 was found in 310 of the 413 DM patients (75.1%). These groups had similar ages, and BMIs. NLR strongly correlated with neutrophil and lymphocyte values. Mean neutrophil values significantly increased and mean lymphocyte values decreased in group 2; hence, the NLR value was significantly higher in group 2 than in group 1 (Table 1, Figure 1). NLR showed significant positive correlation with HOMA IR (r = 0.285; P < 0.001) (Figure 2).

A logistic regression analysis was also carried out using the enter method to evaluate the risk factors for IR. The measurement of NLR, TG and HbA1c were dependent parameters, whereas age, gender, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), HDL-C, LDL-C, and microalbuminuria were independent parameters. As shown in Table 2, the results showed that IR was independently related to NLR, TG and HbA1c.
Discussion
The present study had shown that the NLR values of the diabetic patients were significantly higher than those of the healthy control (P < 0.001), and the NLR values of the patients with a HOMA-IR value of > 2.0 are notably greater than those of the patients with a HOMA-IR value of ≤ 2.0 (P < 0.001).

Many epidemiological studies have determined that DM is associated with chronic inflammation [21], which may contribute to the acceleration of diabetic microangiopathy.
and the development of macroangiopathy [3,4]; IR is a characterized of T2DM, whereas the exact molecular action leading to IR is not yet understood, several studies have associated IR with inflammation [1,6] experimental studies have demonstrated a link between chronic inflammation and insulin resistance through mechanisms involving obesity [22] and atherosclerosis [23]. NLR has been recently defined as a novel potential inflammation marker for IR. High NLR values were independently re-

deemed by T cells and that IR results in a decrease in

Table 2 Logistic regression analysis of factors independently associated with IR

| Variable      | P value | EXP(B)  | 95% CI       |
|---------------|---------|---------|--------------|
| NLR           | <0.001  | 7.231   | 4.277–12.223 |
| Gender        | 0.303   | 0.709   | 0.368–1.365  |
| Age (years)   | 0.856   | 1.005   | 0.948–1.066  |
| BMI (kg/m²)   | 0.257   | 1.045   | 0.969–1.126  |
| SBP (mm Hg)   | 0.165   | 1.012   | 0.995–1.030  |
| DBP (mm Hg)   | 0.858   | 1.003   | 0.970–1.038  |
| TC (mmol/L)   | 0.691   | 1.078   | 0.744–1.562  |
| TG (mmol/L)   | 0.030   | 1.338   | 1.028–1.741  |
| HDL (mmol/L)  | 0.626   | 1.254   | 0.504–3.121  |
| LDL (mmol/L)  | 0.667   | 1.111   | 0.689–1.792  |
| HbA1c (%)     | 0.027   | 1.164   | 1.018–1.331  |
| Microalbuminuria | 0.571  | 0.997   | 0.988–1.007  |

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated. Hemoglobin. P < 0.05 was accepted as the level of significance.

However, some common physical conditions, such as dehydration and Prostate Specific Antigen of the blood specimen can affect the accuracy of the data. Furthermore, physical exercise and release of catecholamine (CA) can cause a drop in neutrophil granulocyte and lymphocyte. NLR represents a combination of two markers where neutrophils represent the active non-specific inflammatory mediator initiating the first line of defense, whereas lymphocytes represent the regulatory or protective component of inflammation [30]. NLR is superior to other leukocyte parameters (e.g., neutrophil, lymphocyte, and total leukocyte counts) because of its better stability compared with the other parameters that can be altered by various physiological, pathological, and physical factors [17,31]. Thus, as a simple clinical indicator of IR, NLR is more sensitive compared with the neutrophilic granulocyte count and CRP levels, which are widely used as markers of IR [32,33].

A logistic regression analysis of the following risk factors was conducted: NLR, TG and HbA1c. In our study, in conjunction with the rising of the level of HbA1c, the degree of IR increased significantly. HbA1c showed an association with early-phase insulin secretion assessed by insulinogenic index [34]. Heianza et al. [35] reported that elevated HbA1c levels of above 41 mmol/mol (>5.9%) were associated with a substantial reduction in insulin secretion and insulin sensitivity as well as an association with β-cell dysfunction in Japanese individuals without a history of treatment of diabetes. Increased accumulation of TG has been observed in human muscle tissue of obese and type 2 diabetic subjects, and associated with IR [36,37], which is in agreement with the present study. IR reduces the inhibition effect of lipolysis in adipose tissue, resulting in the increase of the free fatty acid (FFA) level in plasma. Plasma FFA levels usually increase in obesity [38]. Infusion of free fatty acids (FFA) has been shown to induce IR in skeletal muscles [39] in several studies. However, in the present study, NLR serves an important function in predicting the risk of IR. IR in diabetic patients is related to chronic inflammation, and NLR may be helpful in assessing the prognoses of these patients.

**Conclusion**

We recommend that the NLR values of diabetic patients be calculated as NLR is a cheap, predictive, and prognostic marker for IR. High NLR values were independently related to IR.

**Competing interests**

The authors declare that they have no competing interests.
Authors’ contributions
ML, with the most contribution to the study, was responsible for the study design, data collection, and manuscript writing. PL, RT, YP, SY, and WH helped with the acquisition and interpretation of data and with manuscript revisions. PL also analyzed the data. LH guaranteed this work, provided academic guidance, and took responsibility for the accuracy of the data analysis. All authors read and approved the final manuscript.

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References
1. DalalEstra M, Mussap M, Gallina P, Bruseghin M, Cernigoi A, Saller A, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol. 2005;16:78–82.
2. Lee GK, Lee LC, Chong E, Lee CH, Teo SG, Chia BL, et al. The long-term predictive value of the neutrophil-to-lymphocyte ratio in Type 2 diabetic patients presenting with acute myocardial infarction. QJM. 2012;105:1075–82.
3. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J. 1999;138:A19–20.
4. Fujita T, Hemmi S, Kajiwara M, Yabuki M, Fuke Y, Satomura A, et al. Complement-mediated chronic inflammation is associated with diabetic microvascular complication. Diabetes Metab Res Rev. 2013;29:220–6.
5. Zimmet PZ, McCarty DJ, de Courten MP. The global epidemiology of non-insulin-dependent diabetes mellitus and the metabolic syndrome. J Diabetes Complications. 1997;1:60–8.
6. Shoelson S, Lee J, Goldfine A. Inflammation and insulin resistance. J Clin Invest. 2006;116:1793–801.
7. Mansoori D, Jamaati H, Arami S, Zadars M, Abbasian L, Reza A, et al. Comparison of lymphocyte number and their subsets in patients with diabetes mellitus type II, tuberculosis and concomitant TB and diabetes. Tansaffos. 2002;1:45–50.
8. Von Vettinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. J Immunol. 2001;188:5183–8.
9. Zazula AD, Procoma-Neto D, Gomes AM, Kruklis H, Barbieri GF, Forte FY, et al. Assessment of neutrophils/lymphocytes ratio in patients suspected of acute coronary artery disease. Arq Bras Cardiol. 2008;90:31–6.
10. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which white blood cell subtypes predict increased cardiovascular risk? Of The American College Of Cardiology. 2005;45:1638–43.
11. Hauner M, Amighi J, Exner M, Melkus W, Saberi S, Schlager O, et al. Association of neutrophils and future cardiovascular events in patients with peripheral artery disease. Journaal Of Vascular Surgery. 2005;41:610–7.
12. Stewart GJ. Neutrophils and deep venous thrombosis. Haemostasis. 1993;1:27–40.
13. Arruda-Olson AM, Reeder GS, Bell MR, Weston SA, Roger VL. Neutrophilia predicts death and heart failure after myocardial infarction: A community-based study. Circ Cardiovasc Qual Outcomes. 2009;2:656–62.
14. Rudiger A, Burckhardt OA, Harpes P, Muller SA, Pollatt 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;24:451–455.
15. Tamhane UL, Aneja S, Montgomery D, Rogers DK, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. Am J Cardiol. 2008;102:653–7.
16. Nühler J, Nühler E, Bodl V, Sanchis J, Mihana G, Mainar L, et al. Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. Am J Cardiol. 2008;101:747–52.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
18. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15:539–53.
19. Sultiyoningrum DC, Gasicv D, Lear SA, Ho J, Mente A, Devlin AM, Total and high molecular weight adiponectin and ethnic-specific differences in adiposity and insulin resistance: across-sectional study. Cardiovasc Diabetol. 2013;12:170.
20. Anurad E, Tracy RP, Pearson TA, Kim K, Berglund L. Synergistic role of inflammation and insulin resistance as coronary artery disease risk factors in African Americans and Caucasians. Atherosclerosis. 2009;205:290–2.
21. Ptasov A, Tampourlou M, Parajogatoke DB, Skoumas Y, Chrysohou C, Nomikos T, et al. Association between Low-grade systemic inflammation and type 2 diabetes mellitus among Men and women from the ATTICA study. Rev Diabet Stud. 2007;4:98–104.
22. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou C, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112:1821–30.
23. Rajvani A, Rubbun GM, Wheatcroft SB. Cell-specific insulin resistance: implications for atherosclerosis. Diabetes Metab Res Rev. 2012;28:662–34.
24. Turkmen K, Guney I, Yelikaya FH, Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail. 2012;34:155–9.
25. Gorasia DG, Dudek NL, Veith PD, Shankar R, Safavi-Hemami H, Williamson NA, Reynolds EC, Hubbard MJ, Pucelli AW. Pancreatic Beta Cells Are Highly Susceptible to Oxidative and ER Stresses During the Development of Diabetes J Proteome Res 2014 Dec 8.
26. Tabak AG, Kivimäki M, Brunner EJ, Lowe GD, Jokela M, Akbaraly TN, et al. Changes In C-reactive protein levels before type 2 diabetes and cardiovascular death: the Whitehall II study. Eur J Endocrinol. 2010;163:89–95.
27. Buyukkaya E, Karakas MF, Karakas E, Akçay AB, Tanboga IH, Kurt M, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clin Appl Thromb Hemost. 2014;20:159–63.
28. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med. 2009;15:94–100.
29. Lorenzo C, Hanley AJ, Haffner SM. Differential white cell count and incident type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes. 2014;57:83–92.
30. Bhutta H, Agha R, Wong J, Tang TY, Wilson YG, Walsh SR. Neutrophil-lymphocyte ratio predicts medium-term survival following elective major vascular surgery: a casecontrol study. Vasc Endovascular Surg. 2011;45:227–31.
31. Gibson PH, Croal BL, Cuthbertson BH, Small GR, Ifezulike AI, Gibson G, et al. Preoperative neutrophil lymphocyte ratio and outcome from coronary artery bypass grafting. Am Heart J. 2007;154:995–1002.
32. Zahorec R. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. Bratil Lek Listy. 2001;102:5–14.
33. Imitz F, Shafique K, Mirza SS, Ayyoozb Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med. 2012;5:2.
34. Kim TN, Park MS, Lee SK, Yang SJ, Lee KW, Nam M, et al. Elevated A1C is associated with impaired early-phase insulin secretion rather than insulin resistance in Koreans at high risk for developing diabetes. Endocrine. 2012;42:584–91.
35. Hesana Y, Aseae Y, Fujihara K, Tsuji H, Saito K, Hsieh SD, et al. High normal HbA1c(1c)s were associated with impaired insulin secretion without escalating insulin resistance in Japanese individuals: the toranomon hospital health management center study 8 (TOPICS 8). Diabet Med. 2012;29:1285–90.
36. Kelley DE, Slasky BS, Janosky J. Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus. Am J Clin Nutr. 1991;54:509–15.
37. Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism. 2000;49:467–72.

38. Gorden ES. Non-esterified fatty acids in blood of obese and lean subjects. Am J Clin Nutr 1960, 740–747.

39. Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest. 1994;93:2438–46.