Int J Clin Exp Pathol 2016;9(10):10833-10838
www.ijcep.com /ISSN:1936-2625/IJCEP0037821

Original Article

Relationship between hyperuricemia and neutrophil-to-lymphocyte ratio in type 2 diabetes mellitus

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Received August 13, 2016; Accepted August 24, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Aims/Introduction: To research the relationship between Serum uric acid (SUA) levels and the Neutrophil-to-lymphocyte Ratio (NLR) of Type 2 Diabetes Mellitus patients. Materials and methods: 290 newly diagnosed Type 2 Diabetes Mellitus patients are selected in accordance with the WHO diagnostic criteria: Male subjects with SUA levels ≥416 μmol/L (60 mg/L), and female subjects with SUA levels ≥357 μmol/L (70 mg/L), a total of 234 subjects, are sorted into the high SUA (HUA) group, and the rest, a total of 56 subjects, into the normal SUA (NUA) group. 130 subjects were selected as a control group. One-Way analysis of variance (etc) was applied to the data of the three groups; Pearson correlation analysis was used to calculate the correlation of SUA levels, NLR and IR; risk factors influencing SUA levels were analyzed with Logistic regression analysis; ROC curve analysis was used to determine the diagnostic value of NLR to HUA, and the optimal threshold value of NLR. Result: (1) The NLR and IR of the HUA group was significantly higher than those of the NUA group (2.54±0.64 vs. 2.06±0.62, P<0.001; 3.70±1.83 vs. 2.71±1.54, P<0.001); (2) In the HUA group, UA was positively correlated with NLR and IR (respectively r=0.512, P<0.001; r=0.357, P<0.001). (3) NLR (P=0.011, EXP(B)=5.237, 95% CI=1.465-18.719) was a risk factor of Hyperuricemia. Conclusion: Our results suggest that NLR may be an independent risk factor of Hyperuricemia.

Keywords: Neutrophil-to-lymphocyte ratio, hyperuricemia, diabetes, insulin resistance

Introduction

In recent years, the incidence of hyperuricemia has gradually increased with improvement in living conditions and changes in the dietary structure. The WHO has confirmed that hyperuricemia is an independent risk factor of cerebrovascular diseases. Moreover, hyperuricemia in the presence of type 2 diabetes mellitus is recognized as a high risk factor of lethal and non-lethal cerebrovascular events [1]. Although the role of hyperuricemia in the occurrence of Type 2 Diabetes Mellitus is not yet determined, numerous researches suggest that the pathogenic mechanism of hyperuricemia is closely related to inflammation and insulin resistance (IR) [2]. Hyperuricemia increases the generation of oxygen free radicals (OFR), and takes a role in the chronic inflammatory reaction of type 2 diabetes mellitus. NLR is a novel indicator of inflammation, research suggest that it is closely correlated to IR [3], and shows good prospects in the assessment grading of cardiovascular diseases and as a outcome indicator of tumors [4, 5]. As of now, there is no research in progress regarding the relationship between patient SUA and NLR, IR. This paper is on the clinical observation of 290 patients in our hospital and the relationship between NLR, SUA and IR.

Materials and methods

Ethics statement

The protocol (2014-NFMK-003) for the research project has been approved by medical ethical committee of Zhujiang Hospital within which the work was undertaken and that it conforms to the provisions of the Declaration of Helsinki.

Materials

All participants signed an informed consent before the procedure. From Jan. 2013 to Dec.

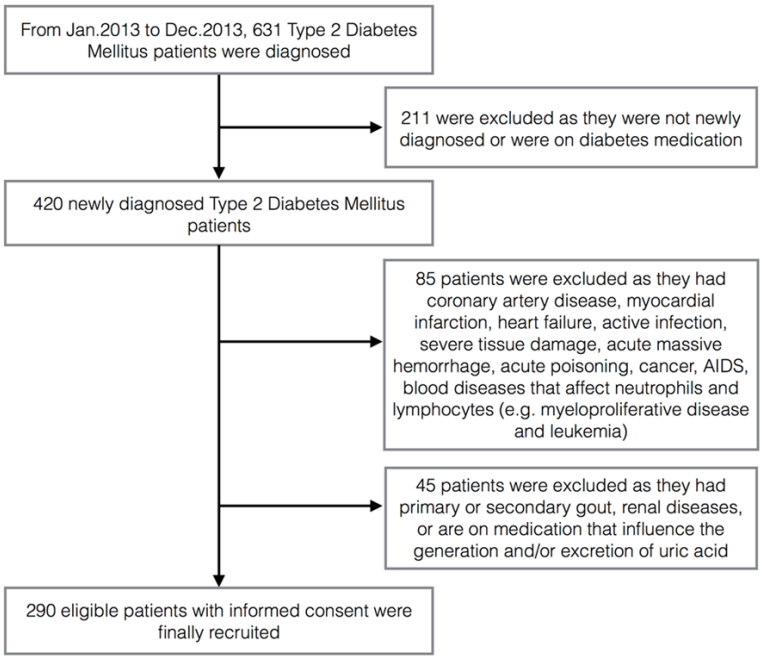

Figure 1. Flow diagram for studies included.

Table 1. Baseline clinical characteristics of groups

Variable	Diabetes patients w/o HUA (n=56)	Diabetes patients w/HUA (n=234)	Healthy subjects (n=130)	*P* value
Age (years)	62.82±8.19	63.40±8.95	63.71±7.34	0.803
Male, n (%)	26 (46.4)	115 (49.1)	60 (46.2)	0.839
BMI (kg/m^2)	24.35±3.99	24.25±3.58	23.70±3.52	0.320
Cr (μmol/L)	82.29±16.45	77.90±16.65	76.73±17.23	0.114
TG (mmol/L)	2.03±1.19	2.49±1.37	1.37±1.19	<0.001
TC (mmol/L)	5.40±0.92	5.53±1.33	5.58±1.41	0.683
HDL (mmol/L)	1.3 ±0.19	1.38±0.25	1.44±0.83	0.413
LDL (mmol/L)	3.10±0.74	3.07±0.89	3.06±0.89	0.970
Fins (mmol/L)	7.70±4.31	9.35±4.44	6.18±3.49	<0.001
FPG (mmol/L)	8.58±4.28	9.87±3.07	5.44±2.62	<0.001
HbA1c (%)	8.12±2.29	9.11±2.06	6.22±1.87	<0.001
IR	2.71±1.54	3.70±1.83	1.42±0.89	<0.001
NLR	2.06±0.62	2.54±0.64	1.45±0.51	<0.001
Neutrophil	3.63±1.00	4.21±4.09	2.87±1.01	0.001
Lymphocyte	1.84±0.49	1.70±1.33	2.01±0.42	0.026
WBC	5.48±1.25	5.90±5.35	4.87±1.29	0.070
Uric Acid (μmol/L)	303.2±76.6	321.6±89.4	507.2±153.1	<0.001

BMI, body mass index; Cr, creatinine; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; Fins, fasting insulin; FPG, fasting plasma glucose; HbA1c, Hemoglobin A1c; IR, insulin resistance; NLR, neutrophil to lymphocyte ratio; WBC, white blood cell.

2013, 290 newly diagnosed type 2 diabetes mellitus patients are selected from the Department of Endocrinology of Zhujiang Hospital, Southern Medical University, and retrospectively evaluated. Male subjects with SUA levels ≥416 μmol/L (60 mg/L), and female subjects with SUA levels ≥357 μmol/L (70 mg/L) are sorted into the high SUA (HUA) group (234 subjects, mean age 62.82±8.19 y/o), and the rest into the normal SUA (NUA) group (56 subjects, mean age 62.82±8.19 y/o). 130 subjects with a mean age of 63.71±7.34 y/o were selected as a control group. Rejection criteria: Patients with primary or secondary gout, renal diseases, or are on medication that influence the generation and/or excretion of UA were rejected.

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 sample. Insulin resistance was calculated using the homeostasis assessment (HOMA-IR) model: fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5. Insulin was estimated by enzyme-linked immunosorbent assay (ELISA), using UniCel DxI 800 (BECKMAN COULTER), selected Access Ultrasensitive Insulin kits.

Measurements of other variables

All study subjects underwent uniform questionnaire and physical examination to measure height, weight, body mass index (BMI); fasting plasma glucose (FPG), fasting insulin (Fins), creatinine (Cr), triglyceride (TG), total cholesterol (TC),

Table 2. The association between UA and NLR, IR in HUA group

	UA	P
NLR	r=0.512	<0.001
IR	r=0.357	<0.001

Table 3. The association between UA and NLR, IR in all subjects

	UA	P
NLR	r=0.480	<0.001
IR	r=0.332	<0.001

Table 4. Logistic regression analysis of factors independently associated with IR

Variable	*P* value	EXP(B)	95% CI
NLR	0.011	5.237	1.465-18.719
Gender	0.891	0.964	0.573-1.621
Age (years)	0.021	1.039	1.006-1.073
BMI (kg/m^2)	0.562	0.979	0.912-1.051
SBP (mmHg)	0.682	0.996	0.979-1.014
DBP (mmHg)	0.156	1.024	0.991-1.059
TC (mmol/L)	0.713	1.074	0.733-1.574
TG (mmol/L)	0.033	1.279	1.020-1.605
HDL (mmol/L)	0.908	0.964	0.521-1.785
LDL (mmol/L)	0.589	0.867	0.517-1.454
HbA1c (%)	0.021	1.189	1.026-1.377
IR	<0.001	1.671	1.446-1.931

SBP, systolic blood pressure; DBP, diastolic blood pressure.

high-density lipoprotein cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and complete blood count (CBC) were determined by standard methods.

Definitions

Diabetes was diagnosed based on the World Health Organization consulting criteria [6] (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]).

Method of statistics

Statistical analysis was completed using SPSS20.0. Quantitative data are expressed as $\bar{x}$ ±s. Comparisons between groups are done using One-way ANOVA. Relevance between factors is analyzed using Pearson's test. The association of risk factors regarding UA levels is assessed using Multi-factor logistic regression. The diagnostic value of NLR to HUA and the optimal threshold value of NLR are determined using the ROC curve. P<0.05 is regarded as statistical significance.

Results

The flow chart of the study selection is shown in **Figure 1**.

Age, gender and BMI difference of the HUA group, NUA group, and control are not statistically significant (P<0.05). Cr, TC, HDL-C, LDL-C and total white blood cells (TWBC) show no statistically significant difference between the groups. As shown in **Table 1**.

Looking from group to group, IR, NLR and neutrophil count of the healthy control, the NUA group and the HUA group are in ascending order (P<0.001). As shown in **Table 1**.

Pearson's correlation analysis of the UA levels, IR and NLR of group HUA show that UA levels are positively correlated with IR (r=0.357, P<0.001) and NLR (r=0.512, P<0.001). As shown in **Table 2**.

Pearson's correlation analysis of the UA levels, IR and NLR of all subjects show that UA levels are positively correlated with IR (r=0.332, P<0.001) and NLR (r=0.480, P<0.001). As shown in **Table 3**.

Age (P=0.021, EXP(B)=1.039, 95% CI=1.006-1.073), NLR (P=0.011, EXP(B)=5.237, 95% CI=1.465-18.719), TG (P=0.033, EXP(B)=1.279, 95% CI=1.020-1.605) and HbA1c (P=0.021, EXP(B)=1.189, 95% CI=1.026-1.377) are all risk factors of HUA. As shown in **Table 4**.

Receiver operator characteristic curve analysis was used to analyze the relation of neutrophil-to-lymphocyte Ratio to serum uric acid levels in all subjects of Type 2 Diabetes Mellitus. For neutrophil-to-lymphocyte Ratio, the AUC value was 0.856 (95% CI, 0.655-0.777; P<0.001) and the reflection point (cut-off value) was 1.85 with a sensitivity of 91.8% and a specificity of 66.7%. As shown in **Figure 2**.

Discussion

NLR is a simple, efficient, and reliable marker of inflammation, due to its high stability and

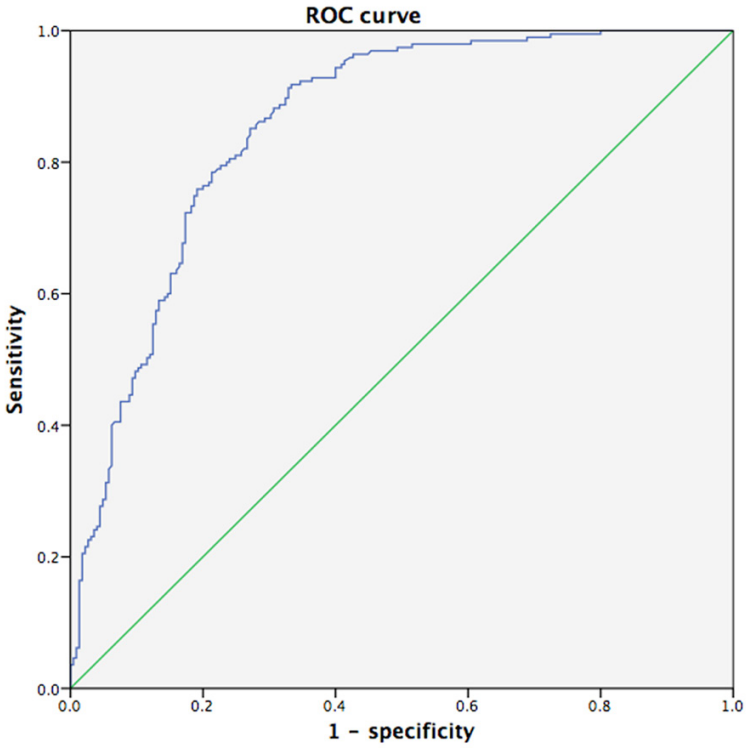

Figure 2. Receiver operator characteristic curve analysis for Neutrophil-to-lymphocyte Ratio's relation to serum uric acid levels in all subjects of Type 2 Diabetes Mellitus.

sensitivity. Azab et al [7] believes that a high neutrophil count is a marker of ongoing destructive nonspecific inflammatory process and a low lymphocyte count is a marker of inadequate regulatory and quiescent immunity pathway. It thus attracts interest in a wide variety of fields such as cardiovascular disease (CVD) assessment and tumor prognosis [4-8]. NLR is at an advantage compared to other markers, such as C reactive protein (CRP), which are often influenced by biological conditions such as dehydration, or other factors such as treatment and processing of the blood sample. Release of substances such as catecholamine result in a decrease in blood WBC and lymphocyte, but these factors are of no significant influence to NLR.

Accompanying the rise of living standards, elements resulting in HUA also become common. The low solubility of UA in the blood results directly in damage of the vascular endothelium when it crystalizes and precipitates on the vascular wall. Furthermore, elevated UA levels promote the oxidization of lipids and generation of free radicals, reduces generation of NO, and damages the endothelia [9]. As pancreatic islets are highly vascularized organs, endothelial cell apoptosis, β cell dysfunctioning occur under the effects of HUA, resulting in glycometabolism disorder.

Research confirms that HUA patients have notable IR. A prospective ARICS study [10] commenced follow-ups to 7990 subjects in a 12-year-span, and discovered that risk of IR increases dramatically mirroring the rise in UA levels. During HUA, UA crystals are likely to precipitate, resulting in β cell dysfunctioning, in turn glycometabolism disorder. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity is also disrupted. Intermediate products of glycolysis, ribose-5-phosphate (R-5-P), and phosphoribosylpyrophosphate (PRPP) transfer into the blood, again boosting the generation of UA. IR also promotes lipid production in the liver, causing disorder in purine metabolism, thus raising UA levels [11]. Moreover, the body's compensatory adaption to IR promotes β cell secretion, the resulting high levels of insulin and insulin precursors stimulate renal tubule Na^+-H^+ exchange, promoting H^+ discharge and at once anions like UA, inducing hyperuricemia [12, 13].

Phagocytes such as neutrophils and macrophages can also recognize elevated UA as an endogenous stimulus, activating the NLRP3 family of inflammatory factors, generating IL-1β, consequently promoting WBC proliferation, synthesis and aggregation [14]. The reason that lymphocyte levels in the HUA group are considerably lower than in the NUA group is still uncertain. Some research suggest that it may be due to the body's lowering the number of $CD8^+$ lymphocytes to suppress the body's anti-inflammation environment [15]. Although the mechanism of which is not yet certain, reduction of lymphocytes under HUA and IR conditions is quite common [16, 17].

The dual-factor logistic regression in this study indicates that HUA is associated with NLR, HbA1c, TG, and age factors, NLR of which displaying higher sensitivity compared to other risk factors. Type 2 diabetes mellitus patients with HUA are in a state of chronic low-level inflammation, while NLR could be used to assess the development of the disease by reflecting the level of inflammation of the body. American Diabetes Association explicitly states that HbA1c is a risk factor of hyperuricemia, and should be controlled to a level of below 7% [18]. Vascular diseases brought about by old age damages the pancreas and the kidneys, accelerating the development of hyperuricemia, consecutively resulting in the more severe metabolic syndrome [19, 20]. The reason that triglycerides cause hyperuricemia is still unknown, but some scholars suspect that high blood TG levels promote generation and usage of free fatty acids, disordering energy metabolism, ultimately resulting in increased SUA.

In conclusion, inflammation exists within patients with Type 2 Diabetes Mellitus complicated by hyperuricemia, while inflammation is closely related to IR. Our study shows that NLR and IR of group HUA are significantly higher than that of group NUA, and that UA is positively correlated with NLR and IR among HUA group patients, indicating that NLR may become a marker for determining the state of inflammation among masses and assessing UA metabolism among type 2 diabetes mellitus patients, thus providing guide for clinical treatment. The shortcoming of this study is, among others, the limit in the number of subjects, as well as the limit in regions. Further study is needed.

Acknowledgements

We would like to thank the Zhujiang Hospital of Southern Medical University, Guangdong, China for providing the data. This work was supported by the Medical Scientific Research Fund Project of Haizhu District, Guangzhou, Guangdong Province (B2013253) (2013/01-2015/12).

Disclosure of conflict of interest

None.

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