ORIGINAL ARTICLE

EVALUATION OF NEUTROPHIL TO LYMPHOCYTE RATIO AS AN INDICATOR OF PRESENCE OF CORONARY ARTERY DISEASE IN DIABETIC PATIENTS

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ABSTRACT

Introduction: Cardiovascular disease (CVD) is the leading cause of death in type 2 diabetes mellitus (DM). DM is a substantial risk factor in atherosclerotic cardiovascular disease. We aimed to investigate relationship between the presence of established neutrophil to lymphocyte ratio (NLR) and coronary artery disease (CAD) in DM.

Methods: 104 diabetic patients who had coronary lesion with a diameter stenosis of at least 50% and 64 diabetic patients who had normal coronary anatomy matched with age and sex were retrospectively selected and classified CAD (+) and CAD (-) group respectively. Baseline NLR in two groups was compared.

Results: NLR was higher in CAD (+) group compared to group without CAD (-) (2.69±1.74 vs. 1.98±.85, p<0.001). C reactive protein (CRP) was higher in CAD (+) group compared to group without CAD (-) (1.71±99 vs. 1.38±.99 p=0.02). Multivariate analysis indicated CRP and NLR were an independent indicator of presence of CAD in diabetic patients (Odds ratio: 1.405, %CI: 1.035-1.908, p=0.029 and odds ratio: 1.759, %CI: 1.226-2.525, p=0.002 respectively).

Conclusion: NLR was higher in diabetic patients with angiographically proven CAD compared to those without CAD. NLR may be a useful marker to predict the presence of CAD in type 2 diabetic patients.

Keywords: Coronary artery disease, diabetes mellitus, neutrophil to lymphocyte ratio.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in type 2 diabetes mellitus (DM) [1]. Type 2 DM is a significant risk factor in atherosclerotic CVD [2, 3]. DM is considered as a coroner artery disease (CAD) risk equivalent [4]. DM is associated with an increased risk of cardiovascular morbidity and mortality [5, 6]. It has been known that inflammatory processes play a crucial role in CAD [7]. It has been demonstrated that inflammatory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and interleukin-6 are significantly associated with CVD [7, 8].

Neutrophil to lymphocyte ratio (NLR) is the sign of balance between neutrophil and lymphocyte levels in the body and an indicator of systemic inflammation [9]. Recently, NLR has been shown to provide a reliable inflammatory index to be used in the CAD for prognostic stratification [10, 11]. In the present study, we aimed to investigate relationship between the presence of established NLR and CAD proven with angiography in diabetic patients.

METHODS

Patients Selection: The study design is retrospective cohort study. DM patients, diagnosed according to the American Diabetes Association criteria [12], who underwent coronary angiography were retrospectively examined and selected matched with age and sex between March 2011 and March 2013 in Gaziosmanpasa and Bozok Universities. Overall, 104 diabetic patients who had coronary lesion with a diameter stenosis of at least 50% were included CAD (+) group, and 64 diabetic patients who had normal coronary anatomy were included CAD (-) group. Acute coronary syndromes, valvular or dilated heart diseases, acute or chronic infectious diseases, uncontrolled hypertension, cerebrovascular diseases, thrombosis, malignants, thyroid disorders, hematological disorders, and liver or renal insufficiency were excluded from the study. Hypertension was diagnosed on the basis of JNC-VII criteria[13]. Body mass index (BMI) was calculated as weight/length^2 (kg/m^2).

Laboratory measurements: The white blood cell (WBC), neutrophil, and lymphocyte count, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride (TG), hemoglobin A1c (HbA1c), were retrospectively recorded from patient files. Baseline NLR was measured by dividing neutrophil count to lymphocyte count. These whole blood samples were then analyzed within one hour of venipuncture on an automated blood cell counter .WBC counts more than 12.0 (x 10^3 cells/ mm³) and less than 4.0 (x 10^3 cells/ mm³) were exclusion criteria for the study.

Statistical analysis: The statistical analyses were performed using software (SPSS 18.0). Parametric values were given as mean±standard deviation, and non-parametric values were given as a percentage. To compare parametric continuous variables, Student's t-test was used; to compare nonparametric continuous variables, the Mann–Whitney U-test was used. Categorical data were compared by Chi-square distribution. Variables found to be statistically significant in univariate analyses were entered into multivariate logistic regression analysis. Multivariate logistic regression models were created to identify independent predictors of presence of CAD. Two-tailed P-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Baseline characteristic of patients is showed in table 1. Glucose, TG, TC, LDL, HDL, Hemoglobin (Hb), HbA1c levels and BMI, hypertension in two groups

were not difference. NLR was higher in CAD (+) group compared to group without CAD (-) (2.69±1.74 vs. 1.98±0.85, p<0.001). CRP was higher in CAD (+) group compared to group without CAD (-) (1.71±0.99 vs. 1.38±0.99, p=0.02). All parameters were entered to univariate analysis. In univariate analysis, parameters which had statistically important were entered multivariate analysis. Multivariate analysis indicated CRP and NLR were an independent indicator of presence of coronary artery disease in diabetic patients (Odds ratio: 1.405, %CI: 1.035-1.908 p=0.029 and odds ratio: 1.759, % CI: 1.226-2.525, p=0.002 respectively).

Table1: Baseline characteristic of patients

	CAD(-)	CAD(+)	p
Subjects	64	104	
Age	62.64±7.64	64.53±7.57	0.074
Sex(woman)%	53.8	52.9	0.5
Glucose	140.84±49.95	147.88±50.81	0.323
HT%	41.3	46.2	0.288
Creatinin	0.82±0.17	0.83±0.18	0.672
TC	184.81±45.48	180.10±43.05	0.526
TG	189.88±111.44	171.32±83.91	0.190
HDL	40.22±6.50	38.80±5.87	0.108
LDL	118.46±24.51	124.16±15.25	0.05
HbA1c	8.66±0.83	8.84±0.91	0.161
BMI	31.91±6.28	31.23±4.84	0.427
WBC	8.13±2.19	7.93±1.74	0.483
NLR	1.98±0.85	2.69±1.74	0.001
Hb	13.21±1.40	13.33±1.46	0.545
CRP	1.38±0.99	1.71±0.99	0.02

WBC: white blood cell, NLR: neutrophil to lymphocyte ratio, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: triglyceride, HbA1c: hemoglobin A1c, CRP: C reactive protein, HT: hypertension, BMI: body mass index

Table 2: Univariate and multivariate analyses of predictor of presence of coronary artery disease in diabetic patients

	Univariate Analysis			Multivariate Analysis		
	odds ratio	%CI	p	odds ratio	%CI	p
NLR*	1.836	1.299-2.595	0.001	1.759	1.226-2.525	0.002
CRP*	1.397	1.052-1.855	0.021	1.405	1.035-1.908	0.029
LDL*	0.986	0.973-1.000	0.052	1.908	0.972-1.001	0.987
Age	1.034	0.997-1.072	0.076			
Glucose	1.003	0.997-1.008	0.322			
HT	1.216	0.703-2.105	0.485			
BMI	0.979	0.928-1.032	0.425			
TC	0.998	0.990-1.005	0.523			
TG	0.998	0.995-1.001	0.191			
LDL	0.986	0.973-1.000	0.052			
HDL	0.946	0.902-0.992	0.021			
HbA1c	1.256	0.912-1.728	0.162			
WBC	0.951	0.829-1.093	0.481			
Hb	0.061	0.876-1.286	0.543			

WBC: white blood cell, NLR: neutrophil to lymphocyte ratio, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: triglyceride, HbA1c: hemoglobin A1c, CRP: C reactive protein, HT: hypertension, BMI: body mass index
*Variables found to be statistically significant in univariate analyses were entered into multivariate logistic regression analysis

DISCUSSION

In present study, we demonstrated that NLR was higher in angioraphicly proven CAD compared to those

without CAD in diabetic patients. Also we showed that CRP was higher in CAD groups than those without CAD. There was positive correlation between presence

of CAD and CRP and NLR. But, NLR was strongly associated with presence of coronary artery disease compared to CRP.

Patients with diabetes develop vascular complications including macrovascular complications (CAD, peripheral vascular disease, and stroke) and microvascular complications (diabetic nephropathy, diabetic retinopathy, and peripheral neuropathy) [14]. Continuous hyperglycemia may cause endothelial dysfunction and vascular lesions, resulting in diabetic vascular complications [15, 16]. Several studies have suggested that chronic, low grade; subclinical inflammation play a major role in development of insulin resistance which may then proceed to development of clinically overt DM [17, 18]. Also, researches have demonstrated that WBC and CRP are associated with increased macrovascular and microvascular complications in type 2 diabetes mellitus [19, 20]. In present study, we showed that CRP level was higher in coronary artery disease with DM than without coronary artery disease but WBC level was not different between groups.

Blood NLR is a new indicator of the overall inflammatory status of the body and a simple, inexpensive, and useful marker of subclinical inflammation. Increased NLR is associated with major adverse cardiac events in diabetic patients [21, 22]. Atherosclerosis is a chronic inflammatory disease [7, 8]. Several studies have revealed the relationship between systemic inflammation and cardiovascular mortality [23-25]. Elevated levels of systemic inflammatory markers have been found related to incidence of CVD [23-25]. It has been suggested that inflammatory markers such as erythrocyte sedimentation rate, CRP and interleukin-6 are significantly associated with CVD [7, 8].

The main function of NLR in CAD may be explained by secretion of various inflammatory mediators; elastase, myeloperoxidase, and oxygen free radicals, which can cause tissue damage [26-28]. Ricevuti et al. showed increased neutrophil aggregation and oxidase activity in the coronary sinus of patients with CAD [29]. In another study, elevated levels of NLR were also found associated with poor survival of patients undergoing coronary artery bypass graft[30]. Present study showed that CRP and NLR were associated with CAD in diabetic patients. These finding were consistent with previous studies. Also we showed that NLR was stronger marker to predict presence of CAD disease compared to CRP.

Limitation

The main limitation of our study was the small sample size. A small sample size can result in a low statistical power for equivalency testing, leading to false-negative results. Also we did not examined relation between severity of CAD.

CONCLUSION

NLR was higher in diabetic patients with angiographicly proven CAD compared to those without CAD. NLR may be useful marker to predict presence of CAD in type 2 diabetic patients.

REFERENCES

1. N.J. Morrish, S.L. Wang, L.K. Stevens, J.H. Fuller, and H. Keen, "Mortality and Causes of Death in the Who Multinational Study of Vascular Disease in Diabetes," *Diabetologia,* vol. 44 Suppl. 2, pp. 14-21, 2001.

2. S. Kitada, Y. Otsuka, N. Kokubu *et al.,* "Post-Load Hyperglycemia as an Important Predictor of Long-Term Adverse Cardiac Events after Acute Myocardial Infarction: A Scientific Study," *Cardiovasc Diabetol,* vol. 9, pp. 75, 2010.

3. R. Nishimura, T. Nakagami, H. Sone, Y. Ohashi, and N. Tajima, "Relationship between Hemoglobin A1c and Cardiovascular Disease in Mild-to-Moderate Hypercholesterolemic Japanese Individuals: Subanalysis of a Large-Scale Randomized Controlled Trial," *Cardiovasc Diabetol,* vol.10, pp. 58, 2011.

4. L. Whiteley, S. Padmanabhan, D. Hole, and C. Isles, "Should Diabetes Be Considered a Coronary Heart Disease Risk Equivalent?: Results from 25 Years of Follow-up in the Renfrew and Paisley Survey," *Diabetes Care,* vol. 28, no. 7, pp. 1588-1593, 2005.

5. W.B. Kannel, and D.L. McGee, "Diabetes and Cardiovascular Disease. The Framingham Study," *JAMA,* vol. 241, no. 19, pp. 2035-2038, 1979.

6. A.D. Mooradian, "Cardiovascular disease in type 2 diabetes mellitus: current management guidelines," *Archives of Internal Medicine,* vol. 163, no. 1, pp. 33–40, 2003.

7. R. Ross, "Atherosclerosis--an Inflammatory Disease," *N Engl J Med,* vol. 340, no. 2, pp. 115-126, 1999.

8. P. Libby, P.M. Ridker, and A. Maseri, "Inflammation and Atherosclerosis," *Circulation,* vol. 105, no. 9, pp. 1135-1143, 2002.

9. R. Zahorec, "Ratio of Neutrophil to Lymphocyte Counts--Rapid and Simple Parameter of Systemic Inflammation and Stress in Critically ill," *Bratisl Lek Listy,* vol. 102, no. 1, pp. 5-14, 2001.

10. X.H. Shen, Q. Chen, Y. Shi, and H.W. Li, "Association of Neutrophil/Lymphocyte Ratio with Long-Term Mortality after St Elevation Myocardial Infarction Treated with Primary Percutaneous Coronary Intervention," *Chin Med J,* vol. 123, no. 23, pp. 3438-3443, 2010.

11. U.U. Tamhane, S. Aneja, D. Montgomery, E.K. Rogers, K.A. Eagle, and H.S. Gurm, "Association between Admission Neutrophil to Lymphocyte Ratio and Outcomes in Patients with Acute Coronary Syndrome," *Am J Cardiol,* vol. 102, no. 6, pp. 653-657, 2008.

12. "Diagnosis and Classification of Diabetes Mellitus," *Diabetes Care,* vol. 36, Suppl. 1, pp. 67-74, 2013.

13. A.V. Chobanian, G.L. Bakris, H.R. Black *et al.,* "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The Jnc 7 Report," *JAMA,* vol. 289, no. 19, pp. 2560-2572, 2003.

14. M. Murea, L. Ma, and B.I. Freedman, "Genetic and Environmental Factors Associated with Type 2 Diabetes and Diabetic Vascular Complications," *Rev Diabet Stud,* vol. 9, no. 1, pp. 6-22, 2012.

15. S.H. Bae, J. Lee, K.H. Roh, and J. Kim, "Platelet Activation in Patients with Diabetic Retinopathy," *Korean J Ophthalmol,* vol. 17, no. 2, pp. 140-144, 2003.

16. M. Brownlee, "Biochemistry and Molecular Cell Biology of Diabetic Complications," *Nature,* vol. 414, no. 6865, pp. 813-820, 2001.

17. C. Pitsavos, M. Tampourlou, D.B. Panagiotakos *et al.,* "Association between Low-Grade Systemic Inflammation and Type 2 Diabetes Mellitus among Men and Women from the Attica Study," *Rev Diabet Stud,* vol. 4, no. 2, pp. 98-104, 2007.

18. G.D. Friedman, I. Tekawa, R.H. Grimm, T. Manolio, S.G. Shannon, and S. Sidney, "The Leucocyte Count: Correlates and Relationship to Coronary Risk Factors: The Cardia Study," *Int J Epidemiol,* vol. 19, no. 4, pp. 889-893, 1990.

19. P.C. Tong, K.F. Lee, W.Y. So, *et al.,* "White Blood Cell Count Is Associated with Macro- and Microvascular Complications in Chinese Patients with Type 2 Diabetes," *Diabetes Care,* vol. 27, no. 1, pp. 216-222, 2004.

20. S. Moradi, S.R. Kerman, F. Rohani, and F. Salari, "Association between Diabetes Complications and Leukocyte Counts in Iranian Patients," *J Inflamm Res,* vol. 5, pp. 7-11, 2012.

21. B. Azab, V. Chainani, N. Shah, and J.T. McGinn, "Neutrophil-Lymphocyte Ratio as a Predictor of Major Adverse Cardiac Events among Diabetic Population: A 4-Year Follow-up Study," *Angiology,* vol. 64, no. 6, pp. 456-465, 2013.

22. G.K. Lee, L.C. Lee, E. Chong *et al.,* "The Long-Term Predictive Value of the Neutrophil-to-Lymphocyte Ratio in Type 2 Diabetic Patients Presenting with Acute Myocardial Infarction," *QJM,* vol. 105, no. 11, pp. 1075-1082, 2012.

23. A.R. Folsom, K.K. Wu, W.D. Rosamond, A.R. Sharrett, and L.E. Chambless, "Prospective Study of Hemostatic Factors and Incidence of Coronary Heart Disease: The Atherosclerosis Risk in Communities (Aric) Study," *Circulation,* vol. 96, no. 4, pp. 1102-1108, 1997.

24. A.R. Folsom, W.D. Rosamond, E. Shahar et al., "Prospective Study of Markers of Hemostatic Function with Risk of Ischemic Stroke. The Atherosclerosis Risk in Communities (Aric) Study Investigators," *Circulation,* vol. 100, no. 7, pp. 736-742, 1999.

25. A.R. Folsom, N. Aleksic, D. Catellier, H.S. Juneja, and K.K. Wu, "C-Reactive Protein and Incident Coronary Heart Disease in the Atherosclerosis Risk in Communities (Aric) Study," *Am Heart J,* vol. 144, no. 2, pp. 233-238, 2002.

26. S. Baldus, C. Heeschen, T. Meinertz et al.,"Myeloperoxidase Serum Levels Predict Risk in Patients with Acute Coronary Syndromes," *Circulation,* vol. 108, no. 12, pp. 1440-1445, 2003.

27. M. Hoffman, A. Blum, R. Baruch, E. Kaplan, M. Benjamin, " Leukocytes and coronary heart disease, " *Atherosclerosis,* vol. 172, no. 1, pp.1-6, 2004.

28. B.D. Horne, J.L. Anderson, J.M. John, et al., " Which white blood cell subtypes predict increased cardiovascular risk? " J Am Coll Cardiol, vol. 45, no.10, pp.1638-1643, 2005.

29. G. Ricevuti, A. Mazzone, S. De Servi, G. Specchia, and P. Fratino, "New Trends in Coronary Artery Disease: The Role of Granulocyte Activation," *Atherosclerosis,* vol. 78, no. 2-3, pp. 261-265, 1989.

30. P.H. Gibson, B.L. Croal, B.H. Cuthbertson *et al.,* "Preoperative Neutrophil-Lymphocyte Ratio and Outcome from Coronary Artery Bypass Grafting," *Am Heart J,* vol. 154, no. 5, pp. 995-1002, 2007.