NEUTROPHILS TO LYMPHOCYTE RATIO AS A BIOMARKER OF CORONARY ARTERY DISEASE

SHAMS TABREZ*, MOHAMMED NABIL ALAMA**, NASIMUDEEN R. JABIR*, CHELAPRAM K. FIROZ*
*King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia - **Cardiology Unit, Department of Medicine, King Abdulaziz University Hospital, Jeddah 21589, Saudi Arabia

ABSTRACT

Introduction*: Cardiovascular disease is the leading contributor of global death. Because of steadily expanding ascend in death rate, the earnest need is to discover novel prognostic biomarkers. In this attempt, inflammation has been a prominent target because of its importance in the development and behavior of atherosclerotic plaques. The current article came up from the recent suggestions by the scientific community on the role of neutrophils to lymphocyte ratio (NLR) as an inflammatory predictive biomarker of cardiovascular events at an early stage.*

Materials and methods*: Blood samples were collected from 100 chronic coronary artery disease (CAD) patients who have visited the outpatient department (OPD) at King Abdulaziz University Hospital, Jeddah, Saudi Arabia and 100 healthy control individuals. Complete blood examination was done on Cell-Dyn Ruby, USA to calculate NLR. In addition, lipid profile, platelet counts, red cell distribution width (RDW), C-reactive proteins (CRP), fasting glucose, insulin and glycosylated hemoglobin (HbA1c) were also measured by various procedures on different instruments.*

Results*: Total cholesterol and low-density lipoproteins (LDL) showed a significant rise of around 13%. However, non-high-density lipoprotein cholesterol (Non-HDL) level showed a similar decline ($P<0.05$) compared with control. A remarkable rise in fasting glucose, Hb1Ac, and insulin by 58%, 53%, and 44% was recorded in CAD group. Moreover, CRP level also showed a significant rise of 39% in CAD group compared with control individuals. The white blood cell (WBC) analysis revealed a significant enhancement in total WBC, neutrophils and lymphocytes counts. The calculated NLR was found to be significantly ($P<0.001$) elevated by 110% in CAD patients compared with control individuals.*

Conclusions*: In view of significant enhancement of NLR level in our samples, we recommend its use as a predictive biomarker of CAD because it is a simple and inexpensive method and could be performed in a routine blood sample.*

Keywords*: WBC; Neutrophils to lymphocyte ratio; Inflammation; CAD; Cardiac markers*

DOI: 10.19193/0393-6384_2016_5_143

Received *April 30, 2016;* ***Accepted*** *July 02, 2016*

Introduction

Despite, the recent advances in medical research, coronary artery disease (CAD) remain the major cause of death worldwide[1]. Even-though, all the pathogenic components associated with atherogenesis have not been completely elucidated, inflammation has been suggested as a significant triggering factor of myocardial infarction[2].

Identification of a particular blood cell type holds several advantages in predicting CAD over total white blood cell (WBC) count as it is least affected by physiological conditions. Among the various inflammatory markers, neutrophil to lymphocyte ratio (NLR) has gained increased attention recently as a potential biomarker for CAD risk assessment[3]. NLR is an integrated reflection of two dissimilar yet balancing immune pathways and is

comparatively better prognostic marker than neutrophils or lymphocytes alone. Several studies have also suggested NLR as a potential inflammatory marker for cancer and other systemic diseases[4-6].

There are innumerable scientists working in the CAD thrust area, atherogenesis and their secondary complications all across the globe. The scientific work currently carried out in USA, UK, Australia, China, Japan, South Korea and India have quite a relevancy, because the majority of developed and/or developing countries have very high occurrence of CAD in their population. Therefore, the need of the hour is to stop the menace of the chronic inflammation associated with CAD at the early stage. Kingdom of Saudi Arabia (KSA), also does not have different scenario compared with above- mentioned countries. The number of CAD patients in KSA is increasing alarmingly, and it is becoming essential to find out early, an inexpensive and easily measurable biomarker of CAD to stop its progression at an early stage. We believe that the current study will provide new avenues to a cardiologist to check the menace of CAD at its earlier stages. As far as our knowledge goes, we are for the first time suggesting the use of NLR as a potential biomarker of CAD, especially in the Saudi population.

Materials and methods

The study included 200 subjects: 100 confirmed chronic CAD patients who have visited outpatient OPD at KAUH, Jeddah, and 100 healthy control individuals. Written and properly informed consent was obtained from all the individuals before the commencement of the study. It was carried out between October 2014 and March 2015 at KAUH, Jeddah. This study was approved by the ethical committee of the institution. The selection of study subjects was made by confirming CAD based on the presence of wall-motion abnormality on left ventriculography and attendant stenosis of at least 50% in any of the major coronary arteries or in the left main trunk, as documented by coronary angiography. Individuals who have a history of significant or serious uncontrolled disease and unwilling or unable to comply with the protocol were excluded from the study. Body mass index (BMI) was calculated as weight/length^2 (kg/m^2).

The peripheral blood samples were collected in EDTA and EDTA free tubes from selected cardiovascular patients and healthy control individuals. Complete blood cell examinations were done using Cell-Dyn Ruby (Illinois, USA) immediately after the sample collection. In addition, serum separation was done by centrifugation at 2000g for 5 min. The collected serum samples were stored at -80 °C until further analysis. Serum HbA1c and insulin were analyzed on Dimension VistaTM System (Camberley, UK) and Architect ci4100 (Abbott, USA) respectively based on different principal procedures. The protocol provided by the manufacturer's (Siemens Healthcare Diagnostics, Camberley, UK) were strictly adhered. Serum CRP, fasting glucose and lipid profile were estimated on a dedicated Selectra ProM clinical chemistry analyzer system (ELITech, Sees, France). Non-HDL cholesterol determinations were done by subtracting LDL value from total cholesterol. These parameters were estimated according to the conventional laboratory protocols using enzymatic spectroscopic methods. All the instruments used in the study were calibrated daily and quality controls were duly maintained. Information's obtained from complete blood cell counts were used to calculate neutrophil to lymphocyte ratio.

Statistical analyses

A statistical analysis was performed to find out the correlation between CAD patients and healthy control individuals. All statistical variables expressed as mean ± SD and were analyzed by student t-test using GraphPad prism 5 software. P value <0.05 was considered statistically significant.

Results

The study population consisted of 100 confirmed CAD patients [67 men and 33 women] with an average age of 51 years and 100 healthy control subjects [63 men and 37 women] with an average age of 48 years. The study population was categorized into two groups according to presence or absence of significant CAD. Both groups were fairly similar in terms of age, gender and BMI (based on sample availability). The baseline demographic characteristics of both groups are presented in table 1.

Various CAD risk factors such as lipid profile, fasting glucose, HbA1c, and insulin were estimated in serum samples. Among the lipid parameters, total cholesterol and LDL showed an increase in the level from 3.77 to 4.24 (P<0.01) and 2.37 to 2.72 (P<0.05) respectively. However, calculated non-HDL level showed a decline in its level from 3.12 to 2.73 (P<0.05). Triglycerides (TGL) and HDL level did not show any significant change among two studied

groups. Different diabetic markers also showed the remarkable rise in fasting glucose (5.38 to 8.51), Hb1Ac (5.26 to 8.05) and insulin (15.52 to 22.44) in CAD group. The well-known inflammatory marker (CRP), also showed a significant rise of approximately 40% in CAD group compared with control individuals.

Demographic characterization	Control group (n = 100)	CAD group (n = 100)
Age	48±5	51±10
Sex (Man %)	63	67
Weight	79±8	81±15
Body mass index	31±3	29±5

Table 1: Comparisons of demographic characterization of study groups.

Biological parameters	Control group (n = 100)	CAD group (n = 100)	P value
Fasting Glucose	5.38±0.49	8.51±3.82	P < 0.001
Hb1Ac	5.26±0.41	8.05±2.00	P < 0.001
Insulin	15.52±1.67	22.44±17.44	P < 0.001
Total Cholesterol	3.77±0.57	4.24±1.03	P < 0.001
TGL	1.60±0.31	1.70±0.80	Non Significant
HDL-C	1.04±0.16	1.11±0.25	Non Significant
LDL-C	2.37±0.57	2.72±0.85	P < 0.001
Non-HDL	3.12±0.99	2.73±0.57	P < 0.001
CRP	1.55±0.33	2.15±0.93	P < 0.001
PLT	285.41± 46.99	290.83±39.32	Non Significant
RDW-CV	13.61± 0.48	14.03±1.57	Non Significant
WBC	6.93±1.88	7.75 ± 1.32	P < 0.001
Neutrophils	3.02±1.18	4.24± 0.52	P < 0.001
Lymphocytes	2.86±0.69	1.99 ± 0.41	P < 0.001
Monocytes	0.58±0.15	0.58 ± 0.18	Non Significant
Eosinophils	0.43±0.35	0.36 ± 0.19	Non Significant
Basophils	0.05±0.03	0.04 ± 0.02	Non Significant
NLR	1.05±0.29	2.21 ± 0.52	P < 0.001

Table 2: Comparisons of clinical and biochemical characteristics of control individuals and CAD patients.
CAD: Coronary artery disease, Hb1Ac: Glycosylated hemoglobin, TGL: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, Non-HDL: Non-high-density lipoprotein cholesterol, CRP: C- reactive proteins, PLT: Platelet counts, RDW-CV: Red cell distribution width- coefficient of variation, WBC: White blood cell, NLR: Neutrophils to lymphocyte ratio

The WBC analysis revealed a significant increase in its total number, as well as in neutrophils and lymphocytes count compared with healthy control individuals, whereas other cells such as monocytes, eosinophils and basophils did not show any significant change. The calculated NLR was found to be significantly elevated by 150% (P<0.001) in CAD patients compared with control individuals. The results of the above mentioned biological parameters are summarized in table 2.

Discussion

CAD remains a major challenge in the field of cardiovascular research without specific treatment and preventive measure till date.

Since several years, dyslipidemia has been considered as an important risk factor for CAD[7-9]. Moreover, reduction in LDL levels has been well established treatment strategy to reduce CAD events, especially in patients with high risk[10, 11]. Our study also reported an increase in the level of dyslipidemia agents such as total cholesterol and LDL (Table 2). Recently, novel calculated parameter (Non-HDL) has been suggested as a more accurate risk predictor of CAD. The inclusion of very low-density lipoprotein (VLDL) remnants in the calculation of non-HDL cholesterol, makes it more valuable because it represents most of the apoB and apoA1 contents. We have also recorded a significant decline in Non-HDL level in CAD patients compared with control individuals.

It has been suggested in the scientific literature that CAD risk increased up to 4 fold in diabetic subjects[13]. HbA1c has been suggested for its strong relationship with CAD in both diabetic and non-diabetic patients, providing information about the occurrence and severity of the disease[14, 15].

In addition, hyper-insulinemia (a marker of low insulin sensitivity) has been related to CAD in previous prospective and cross-sectional studies[16, 17]. Our study also agrees with the previously confirmed association of diabetic markers, such as level of fasting glucose, insulin, and HbA1c, and CAD[14, 17].

Active involvement of various inflammatory pathways and associated inflammatory mediators have a major role in the initiation and progression of atherosclerotic lesions[18]. Several studies suggested enhanced expression of CRP in cardiovascular patients[19-21]. The present study also noted the significant rise in CRP level in CAD patients, which further confirms the inflammatory involvement in CAD cases[20].

Platelets count has been reported as a measure of inflammatory status in chronic inflammatory conditions[22, 23]. The effect of increased platelet numbers that are still within physiologic ranges remains unclear. Some studies reported an association of higher platelet counts and rapid platelet-aggregation response with coronary death[24, 25]. However, one study suggested that platelet counts and aggregation that are within physiologic range are not related to CAD events[26]. Our study did not show any significant change in platelets count in CAD patients compared with control individuals.

RDW is a hematological parameter routinely obtained as part of the complete blood count which measures red blood cell size heterogeneity. The exact correlation between RDW and coronary complications are not completely elucidated as yet. Moreover, some studies suggested, RDW as a predictor of adverse outcomes in patients with heart failure and in patients with prior myocardial infarction[27-30]. However, our study did not show any significant association of RDW between CAD and control group.

WBC have been proposed as an important factor in the destabilization of coronary artery plaques and its elevated count is considered as one of the risk factors of CAD[31]. Pathogens mediated elevation in WBCs count has also been reported as a key mechanism which could lead to disruption of plaques[32]. Several previous studies highlighted the inflammatory role of WBCs and suggested their clinical practice as an independent predictor of coronary risk[25, 31, 33, 34]. An Atherosclerosis Risk in Communities (ARIC) study on 13,555 CAD patients confirmed the association of elevated WBC count with increased incidence of coronary heart disease, ischemic stroke, and mortality[35].

Moreover, the altered equilibrium of WBC subtypes such as neutrophils and lymphocytes are also associated with the presence of coronary heart disease, peripheral arterial disease and stroke[25]. Our study supports the previous finding and showed significant enhancement in total WBC counts and its subtypes such as neutrophils and lymphocytes in CAD patients compared with healthy control individuals (Table 2). Inflammatory involvement in our study groups is also confirmed by the status of other inflammatory markers which could lead to higher level of WBCs and its subtypes. It is believed in the scientific community that identification of a particular blood cell type could better predict the differential identification of CAD over total WBC count[25].

The search for specific inflammatory markers of CAD led to the development of various novel biomarkers with higher specificity and sensitivity[36]. Among them, NLR has gained increased attention as a potential biomarker for the risk assessment of CAD recently[3]. Several studies reported the association of enhanced NLR with CAD that could be used as a marker for risk prediction, diagnostic assessment and disease severity[37-39]. Arbel et al.[31] suggested, NLR relation to the severity of CAD and clinical outcome in patients undergoing angiography. Sahin et al.[32] suggested NLR as an indicator of the presence of CAD in diabetic patient. In addition, higher NLR has also been associated with increased cardiac mortality in clinically stable patients with CAD compared with total WBCs count[37]. The present study is in accordance with the previous results and further supports the use of NLR as a valuable biomarker of CAD risk and assessment.

Conclusion

Keeping in mind the early acting immunological nature of neutrophils and lymphocytes and their presence in the blood circulation, we recommend the use of NLR as a biomarker of CAD because of their simple, easily measurable and inexpensive method. Along with previous international recommendations, our study supports the use of NLR as a cost- effective biomarker to predict the future cardiovascular risk.

References

1) Firoz CK, Jabir NR, Khan MS, Mahmoud M, Shakil S, Damanhouri GA, et al. *An overview on the correlation of neurological disorders with cardiovascular disease*. Saudi J Biol Sci 2015; 22(1): *19-23*.

2) Jabir NR, Firoz CK, Baeesa SS, Ashraf GM, Akhtar S, Kamal W, et al. *Synopsis on the linkage of Alzheimer's and Parkinson's disease with chronic diseases*. CNS Neurosci Ther 2015; 21(1): *1-7*.

3) Bhat T, Teli S, Rijal J, Bhat H, Raza M, Khoueiry G, et al. *Neutrophil to lymphocyte ratio and cardiovascular diseases: a review*. Expert Rev Cardiovasc Ther 2013; 11(1): *55-9*.

4) Baysal E, Çetin M, Yaylak B, Altntaş B, Altndağ R, Adyaman Ş, et al. *Roles of the red cell distribution width and neutrophil/lymphocyte ratio in predicting thrombolysis failure in patients with an ST-segment elevation myocardial infarction*. Blood Coagul Fibrinolysis 2015; 26(3): *274-8*.

5) Feng J-F, Huang Y, Chen Q-X. *The combination of platelet count and neutrophil lymphocyte ratio is a predictive factor in patients with esophageal squamous cell carcinoma*. Transl Oncol 2014; 7(5): *632-7*.

6) Xie X, Luo KJ, Hu Y, Wang JY, Chen J. *Prognostic value of preoperative platelet-lymphocyte and neutrophil-lymphocyte ratio in patients undergoing surgery for esophageal squamous cell cancer*. Dis Esophagus 2016; 29(1): *79-85*.
7) Lloyd-Jones DM, Wilson PWF, Larson MG, Beiser A, Leip EP, D'Agostino RB, et al. Framingham risk score and prediction of lifetime risk for coronary heart disease. Am J Cardiol 2004; 94(1): *20-4*.
8) Kabakci G, Koylan N, Ilerigelen B, Kozan O, Buyukozturk K. *Impact of dyslipidemia on cardiovascular risk stratification of hypertensive patients and association of lipid profile with other cardiovascular risk factors: results from the ICEBERG study*. Integr Blood Press Control 2008; 1: *5-13*.
9) D'Adamo E, Guardamagna O, Chiarelli F, Bartuli A, Liccardo D, Ferrari F, et al. Atherogenic dyslipidemia and cardiovascular risk factors in obese children. Int J Endocrinol 2015; 2015.
10) Sever PS, Poulter NR, Dahlöf B, Wedel H, Collins R, Beevers G, et al. *Reduction in cardiovascular events with atorvastatin in 2,532 patients with type 2 diabetes: Anglo-Scandinavian Cardiac Outcomes Trial--ipid-lowering arm (ASCOT-LLA)*. Diabetes Care 2005; 28(5): *1151-7*.
11) Cholesterol Treatment Trialists C, Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, et al. *The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials*. Lancet 2012; 380(9841): *581-90*.
12) Banu S, Jabir NR, Manjunath NC, Firoz CK, Kamal MA, Khan MS, et al. *Comparative study of non-high density lipoproteins cholesterol level and lipid profile in pre-diabetic and diabetic patients*. CNS Neurol Disord Drug Targets 2014; 13(3): *402-7*.
13) Mohan V, Venkatraman JV, Pradeepa R. *Epidemiology of cardiovascular disease in type 2 diabetes: the Indian scenario*. J Diabetes Sci Technol 2010; 4(1): *158-70*.
14) Ashraf H, Boroumand MA, Amirzadegan A, Talesh SA, Davoodi G. *Hemoglobin A1C in non-diabetic patients: an independent predictor of coronary artery disease and its severity*. Diabetes Res Clin Pract 2013; 102(3): *225-32*.
15) Mehraj I, Wani IY, Aslam K, Masoodi S, Wani YY. *Association of hba1c with prevalence and severity of coronary artery disease in diabetic and Nondiabetic patients in Kashmir*. AJST 2014; 5(3): *230-2*.
16) Rewers M, Zaccaro D, *D'Agostino R, Haffner S, Saad MF, Selby JV, et al. Insulin sensitivity, insulinemia, and coronary artery disease: the Insulin Resistance Atherosclerosis Study*. Diabetes Care 2004; 27(3): *781-7*.
17) Bertoluci MC, Quadros AS, Sarmento-Leite R, Schaan BD. *Insulin resistance and triglyceride/HDLc index are associated with coronary artery disease*. Diabetol Metab Syndr 2010; *2*.
18) Ridker PM, Lüscher TF. Anti-inflammatory therapies for cardiovascular disease. Eur Heart J 2014; 35(27): 1782-91.
19) Bisoendial RJ, Boekholdt SM, Vergeer M, Stroes ESG, Kastelein JJP. *C-reactive protein is a mediator of cardiovascular disease*. Eur Heart J 2010; 31(17): *2087-91*.
20) Kaptoge S, Seshasai SRK, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al. *Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis*. Eur Heart J 2014; 35(9): *578-89*.
21) Ridker PM. *Clinical application of C-reactive protein for cardiovascular disease detection and prevention*. Circulation 2003; 107(3): 363-9.
22) Milovanovic M, Nilsson E, Järemo P. *Relationships between platelets and inflammatory markers in rheumatoid arthritis*. Clin Chim Acta 2004; 343(1-2): *237-40*.
23) Matowicka-Karna J, Kamocki Z, Polińska B, Osada J, Kemona H. *Platelets and inflammatory markers in patients with gastric cancer.* Clin Dev Immunol 2013; *2013*.
24) Thaulow E, Erikssen J, Sandvik L, Stormorken H, Cohn PF. *Blood platelet count and function are related to total and cardiovascular death in apparently healthy men*. Circulation 1991; 84(2): *613-7*.
25) Madjid M, Fatemi O. *Components of the complete blood count as risk predictors for coronary heart disease: in-depth review and update*. Tex Heart Inst J 2013; 40(1): *17-29*.
26) Meade TW, Cooper JA, Miller GJ. *Platelet counts and aggregation measures in the incidence of ischaemic heart disease (IHD)*. Thromb Haemost 1997; 78(2): *926-9*.
27) Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M, et al. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. Circulation 2008; 117(2): 163-8.
28) Ani C, Ovbiagele B. *Elevated red blood cell distribution width predicts mortality in persons with known stroke*. J Neurol Sci 2009; 277(1-2): *103-8*.
29) Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. *Red blood cell distribution width and mortality risk in a community-based prospective cohort*. Arch Intern Med 2009; 169(6): *588-94*.
30) Cavusoglu E, Chopra V, Gupta A, Battala VR, Poludasu S, Eng C, et al. *Relation between red blood cell distribution width (RDW) and all-cause mortality at two years in an unselected population referred for coronary angiography*. Int J Cardiol 2010; 141(2): *141-6*.
31) Takeda Y, Suzuki S, Fukutomi T, Kondo H, Sugiura M, Suzumura H, et al. *Elevated white blood cell count as a risk factor of coronary artery disease: inconsistency between forms of the disease*. Jpn Heart J 2003; 44(2): *201-11*.
32) Patel P, Mendall MA, Carrington D, Strachan DP, Leatham E, Molineaux N, et al. *Association of Helicobacter pylori and Chlamydia pneumoniae infections with coronary heart disease and cardiovascular risk factors*. BMJ (Clinical research ed) 1995; 311(7007): *711-4*.
33) Madjid M, Awan I, Willerson JT, Casscells SW. *Leukocyte count and coronary heart disease: implications for risk assessment*. J Am Coll Cardiol 2004; 44(10): *1945-56*.
34) Kounis NG, Soufras GD, Tsigkas G, Hahalis G. *White blood cell counts, leukocyte ratios, and eosinophils as inflammatory markers in patients with coronary artery*

disease. Clin Appl Thromb Hemost 2015; 21(2): *139-43*.

35) Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. *White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study*. Am J Epidemiol 2001; 154(8): *758-64*.

36) Yayan J. *Emerging families of biomarkers for coronary artery disease: inflammatory mediators*. Vasc Health Risk Manag 2013; 9: *435-56*.

37) Celik T, Bugan B. *White blood cell count and stable coronary artery disease: the role of neutrophil to lymphocyte ratio*. Cardiol J 2011; 18(6): *720-1*.

38) Arbel Y, Finkelstein A, Halkin A, Birati EY, Revivo M, Zuzut M, et al. *Neutrophil/lymphocyte ratio is related to the severity of coronary artery disease and clinical outcome in patients undergoing angiography*. Atherosclerosis 2012; 225(2): *456-60*.

39) Sahin S, Sarikaya S, Akyol L, Altunkas F, Karaman K. *Evaluation of Neutrophil to Lymphocyte ratio as an Indicator of Presence of Coronary Artery Disease in Diabetic Patients*. Nat J Med Res 2013; 3(4): 300-3.

Acknowledgements:
This work was supported by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (141 - 912 - D1435). The authors, therefore, gratefully acknowledge the DSR technical and financial support.

Corresponding author
Dr. SHAMS TABREZ
King Fahd Medical Research Centre
King Abdulaziz University
P.O. Box 80216, Jeddah 21589
Kingdom of Saudi Arabia