Correlation between Neutrophil-Lymphocyte Ratio and Cardiac Autonomic Neuropathy in Diabetes Mellitus Type 2 Patients

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Abstract

Objective: To analyze the correlation between cardiac autonomic neuropathy (CAN) and neutrophil-lymphocyte ratio (NLR) in DM type 2 patients.

Methods: This was a cross-sectional study which was conducted at the Internal Medicine Clinic of Dr. Hasan Sadikin General Hospital, Bandung and Chronic Disease Management (CDM) Club in Garut District, West Java, Indonesia from October to December 2015. Subjects were 57 DM type 2 patients who met the inclusion criteria. Cardiac autonomic neuropathy examination and complete blood count (CBC) were performed to discover the subjects' neutrophil-lymphocyte ratio.

Results: A strong correlation was found between CAN in DM type 2 patients and NLR (r=0.679; p=0.000) based on Rank Spearman correlation test. The NLR to CAN cut off point was 1.34.

Conclusions: NLR examination can be used as an indicator of CAN in DM type 2 patients with NLR cut off point. There was a correlation between CAN and DM type 2 diagnosis duration.

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Introduction

Diabetes mellitus (DM) is a complication of metabolical diseases that is characterized by hyperglycemia due to insulin secretion and disorder or their combination.[1,2] In this disease, hyperglycemia becomes the primary risk factor for micro- or macroangiopathy complications. It also augments the oxidative and nitrosative stress that leads to neuron dysfunctions. Because endothelial dysfunction causes ischemic neuron, the condition may be worsened.

Axons, which contain many mitochondria, are easily exposed. both directly or indirectly, by oxidative and nitrosative stress. Augmented oxidative stress stimulates poly (ADP-ribose) polymerase (PARP) activation which activates the polyol and hexonamine pathways and also produces glycation and protein kinase C. This problems cause oxidative stress exacerbation which induces gene expression changes. Hence, the transcription factors can cause neuron disorders and deaths. Activation of the pathways causes microvascular regulation disturbances and endothelial dysfunctions, leading to decreased neurovascular perfusion, and cell dysfunction and apoptosis, as well.[3–5]

Cardiac autonomic neuropathy (CAN) is a DM complication that is rarely noticed by the patients. It is regarded as one of the major factors of a higher number of morbidity and mortality.[4] A study stated that the prevalence of CAN in DM type 2 was 34.4% while CAN can be discovered after 1 year of DM type 2 diagnosis.[6] In addition, it causes disorders in the nerve fibers that innervate the heart and blood vessels, leading to abnormal heart beat and blood vessel control. In the early stage of CAN, sub-clinical symptoms can be discovered.

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Therefore, the patients' condition may worsen because of this.[4,7]

Diabetes mellitus influences the majority of the autonomic nervous systems. Vagus nerve is autonomically considered as the longest nerve. It also physiologically maintains 75% of parasympathetic and sympathetic activities. In the early stage of CAN, parasympathetic nervous function may decrease and causes sympathetic nervous dominance. The gradual increase of sympathetic nervous dominance occurs until the later stage of CAN in apex to the lower part of of heart, namely symphatetic nervous denervation.[4]

Ewing has already proposed a 5-simple non-invasive test to assess the cardiac autonomic functions based on heart rate and blood pressure to certain physiologic maneuvers.[4] The test includes heart rate response to deep breathing, heart rate response to standing, heart rate response during valsalva maneuver, blood pressure response to standing, and blood pressure response to hand grip.

The first two tests are able to describe parasympathetic activity disturbances while the last two tests are useful to describe changes in the sympathetic function. Among the five tests, none is considered the best. However, heart rate response to deep breathing is the mostl frequently used because it shows high specificity and reproducibility as well as easy to use.[4]

Neutrophil-lymphocyte ratio (NLR) is used as a marker of neutrophil and lymphocyte balance level in body. It is regarded as the recent indicator of systemic inflammation status. It is simple, inexpensive, and useful for sub-clinical inflammation diagnosis.[6] Chronic hyperglycemia in DM will increase the release of reactive oxygen species (ROS) from the neutrophils and decrease lymphocyte levels. Decreased lymphocyte proliferation is caused by lower levels of interleukin-2 (IL-2) receptor expression in DM type 2. In addition, DM type 2 patients with uncontrolled glucose level may experience lower lymphocytes and higher neutrophils.

Several studies observed NLR with blood glucose level regulation (HbA1C). The results showed that NLR will increase if the HbA1C value is 7%. The Higher levels of HbA1C are associated with increased cardiovascular complications found in DM type 2 patients.[8–11] Another study examined the correlation between NLR and insulin resistance in newly diagnosed DM type 2 patients.[12] The study stated that NLR can increse in patients with insulin resistance when compared to healthy patients. This finding determines that higher NLR can be used as an indicator to diagnose insulin resistance.

This study was initiated to analyze the correlation between NLR and CAN in DM type 2 patients using Ewing test.

Methods

This was a cross-sectional study conducted at the Internal Medicine Clinic of Dr. Hasan Sadikin General Hospital, Bandung and Chronic Disease Management (CDM) Club in Garut District, Indonesia in the period of October to December 2015. The sampling method used to calculate the subjects was non-intervention consecutive sampling.

The inclusion criteria in the study were DM type 2 patients aged 18–60 years without cognition disturbance and ability to stand up. The exclusion criteria were chronic diseases, hypertension, pulmonary tuberculosis, and acute exacerbation of chronic obstructive pulmonary disease.

The subjects' CAN and NLR in this study were assessed using simultant Ewing tests. Ewing tests performed were heart rate response to internal respiration, position change from sitting to standing, and blood pressure response to standing. Meanwhile, the subject results were assessed based on Ewing test standard assessment.

The NLR score were described and examined to reveal the correlation between NLR and CAN through Ewing tests. If the correlation between NLR and CAN was found, the NLR cut off point would be examined to detect the possibility of CAN incidence.

The CAN examinations in this study were (1) heart rate response to internal respiration, (2) heart rate response while standing, and (3) blood pressure response to sitting. Actually, CAN examination using Ewing tests includes (1) heart rate response to internal respiration, (2) heart rate response while standing, (3) heart rate response on valsalva maneuver, (4) blood pressure to standing, and (5) blood pressure to grasping.

The Ewing test is considered as the most appropriate test when compared to other tests. The test frequently used is the heart rate response to internal respiration due to its higher specificity, reproducibility, as well as efficiency.[6] In this study, the subjects were assessed by using the tests number 1, 2, and 4.

The NLR examination was conducted by drawing 3 cc blood sample which was then

subjected to the differential count test. The differential blood counts were used to assess neutrophil and lymphocyte ratio.

The data were statistically analyzed with p value <0.05. The normality test was performed using Shapiro Wilks and Rank Spearman correlation tests. The Shapiro Wilks test was used due to the small number of subjects and the data were measured on an interval scale.

The Rank Spearman correlation test was used for an ordinal measurement scale and performed to analyze the correlation between the two variables without making distribution frequency assumption from the variables. The protocols in this study were approved by the Health Research Ethich Commitee, Faculty of Medicine, Universitas Padjadjaran.

Table 1 Subject Characteristics

Subject Characteristics	Median (Min-Max)	n
Sex		
Males		11
Females		46
Age (yrs.)	55 (35–59)	57
Weight (kg)	62 (40–110)	57
Height (cm)	156 (144–178)	57
Diabetes mellitus diagnosis duration (month)	24 (1–144)	57
Neutrophil-lymphocyte ratio	1.58 (0.47–4.44)	57

Results

The normality test result using Shapiro Wilk showed that the subject characteristic value related to body mass index (BMI) was normally distributed. For other variables that are not normally distributed, the Rank Spearman correlation test was performed.

A significant correlation between CAN in DM type 2 patients and NLR was found statistically using the Rank Spearman correlation test with 95% confidence interval, (p=0.0000, r=0.679). A significant correlation was found between CAN and diagnosis duration of DM type 2 (p=0.0188, r=0.3188).

The resulted scatter diagram showed a positive correlation pattern between the two variables, CAN and NRL (Fig. 1). Based on the receiver operator curve (ROC), the best two cut-off points of NRL to CAN was 1.3400 with a sensitivity value 0.872 and 1-specificity 0.167 resulted area under the curve 92.2% (Fig. 2).

Discussion

Fifty seven subjects were included in this study, consisting of 46 females and 11 males. A finding has been made showing that the prevalence of DM in females (11.2%) is higher than males (9.6%).[13] This prevalence is associated with improper diet, high adipose tissue, genetic factors, and inactivity. The average age for this disease is fifty two years old and the median is 55 years with 35 years as the youngest age and 59 years as the oldest. Based on the data from the Ministry of Health, the highest proportion of DM type 2 in Indonesia was found in 55–64 years old group.[11]

A similar study had reviewed many studies in the period of 1975–2013 to discover the prevalence of CAN in DM type 2.[4] The results revealed that CAN in DM type 2 occurred in 27–59 years with median 44 and 55 years. Median weight was represented by 65 kg and median height was represented by 156 cm. The average BMI was 24.9 with standard deviation (SD) 3.64 and median 25.2.

The study included 117 DM type 2 patients with CAN and resulted in a BMI of 28.1+5.6.[15] In this study, the average BMI is on the upper limit of normal BMI. Higher BMI may become a risk factor which stimulates complications caused by DM.

The duration of DM diagnoses reported were between 1–144 months with the average diagnosis duration of 37.4 months and median of 24 months. The duration varies with high blood glucose levels. High levels of blood glucose cause disorders to several organs.

Hyperglycemia persists in a very long time and causes changes in organs that leads to both microvascular and macrovascular complications. The most frequently found complication in DM type 2 is neuropathy which occurred in early and acute conditions.[13] Seven percents of the cardiac autonomic neuropathy can be diagnosed in the early diagnosis of DM type 1 and 2.[4,16]

Based on the correlation between CAN and NRL, CAN (-) means lower NLR value and CAN (+) means higher NLR value. A previous study has examined the correlation between NLR and insulin resistance in newly diagnosis of DM type 2 patients and it is reported that NLR increases in subjects with insulin resistance when compared to healty subjects.[12] In addition, high NLR levels become

a predictive sign of insulin resistance. This condition can occur because white blood cells have a positive association with acute or systemic inflammation process caused by hyperglycemia.[17]

The pathogenesis of cardiac autonomic neuropathy includes several mechanisms and factors which enhance ischemic neurons that may lead to direct cell dysfunctions or deaths. Hyperglycemia can increase oxidative and nitrosative stress that lead to nerve disorders.

Acute and chronic inflammations as well as increased immunology cells can increase NLR in DM patients. Hyperglycemic condition causes endothelial cell disorders leading to neutrophil adhesion. Increased number of endothelial cells leads to higher neutrophil levels due to the release of reactive oxygen species (ROS). Higher neutrophil levels are associated with the process of thrombus shape and ischemic injury caused by hyperglycemia.

Lymphocyte levels in chronic inflammation should be increased. On the contrary, lower lymphocyte levels in DM type 2 patients occur due to decreased proliferation.[3,18] It is stated in a previous study that lymphocyte proliferation insufficiency (LPI) occurs in DM patients.[8]

The study reported that LPI incidences are mostly found in uncontrolled DM type 2 patients compared to in controlled DM type 2 patients. Another study revealed that the lymphocyte levels in uncontrolled DM type 2 patients are lower than in controlled DM type 2 patients.[9] However, lymphocyte levels in DM type 1 patients are lower than in healty people.[19]

Lower lymphocyte levels occur in DM type 2; thus, decreased lymphocyte proliferation is caused by lower levels of IL-2 receptor expression. This leads to decreased lymphocyte levels and increased neutrophil levels.

Lower levels of IL-2 receptor expression are caused by CD25 deficiency. CD25 plays a vital role which enhances IL-2 receptor to produce T cells. Stimulated by antigen, CD25 is important for T cells clonal expansion after antigen has been discovered. Interleukin 2 expression is increased by the antigen stimulation because T cells are the main cells which proliferate in specific immune response. Therefore, IL-2 increase the proliferation and differentiation of lymphocyte.[8]

The correlation between CAN and DM type 2 diagnosis duration is considered statistically significant in this study. A study which has similar result to this study revealed that the average prevalence of CAN in DM type 2 increases 65% along with increased DM duration.[20] However, another study stated different results that no correlation was found between DN duration and CAN incidences in DM type 2.[4]

It is reported in this study that DM duration becomes an independent factor of CAN incidences. Cardiac autonomic neuropathy may be discovered 1 year after DM type 2 diagnosis or 2 year after DM type 1 diagnosis. However, CAN symptoms cannot be noticed in the early stage. A possible CAN symptom mostly found is heart beat variability.[4,6] Uncontrolled hyperglycemia becomes a major risk factor associated with incidences of CAN.

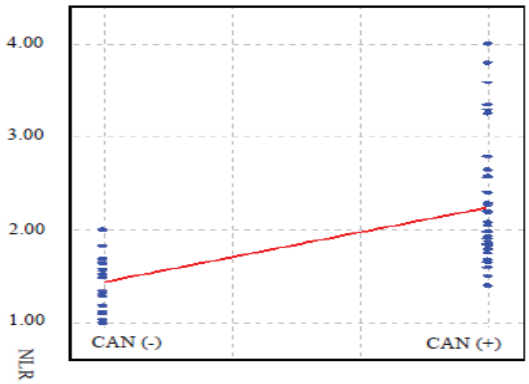

Fig. 1 A Scatter Diagram of Cardiac Autonomic Neuropathy (CAN) and Neutrophil-Lymphocyte Ratio (NLR) Correlation Pattern

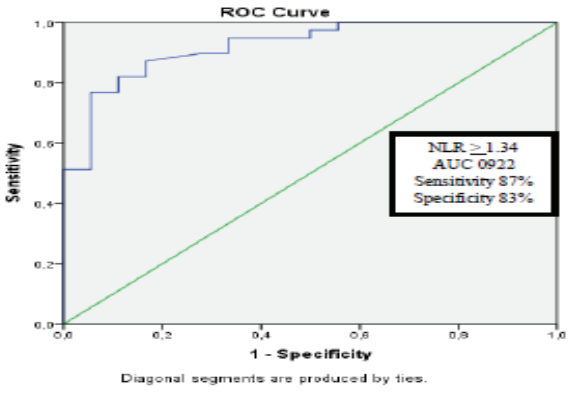

Fig. 2 Receiver-operating Characteristic (ROC) Curve Analysis of Neutrophil-Lymphocyte Ratio (NLR) for Predicting Cardiac Autonomic Neuropathy (CAN) in Diabetic Patients

The Indonesian Society for Endocrinology described that 50% diabetic patients had not been diagnosed suffering from DM and only one-third of DM type 2 patients who was treated properly.[1] Uncontrolled hyperglycemic condition and DM type 2 duration are the major risk factors of CAN progresivity. This condition may be caused by longer duration of hyperglycemic exposure which will increase the oxidative stress and decrease the NO levels. Cell proteins are disrupted, while leukocyte adhesion in endothelium may increase due to PARP activation. The activation will activate polyol and hexonamine pathways and produce glycations and kinase C proteins. Activations of the pathways will cause oxidative stress exacerbation which induces gene expression changes and transcription factors leading to neuron disorders or deaths.

The activation of the pathways causes various microvascular regulation disorders and also endothelial dysfunctions. A decrease in the level of neurovascular perfusion may be seen, which then leads to cell dysfunction and apoptosis.[4] Nitric oxide formation in the endothelium will be prohibited and worsen endothelial dysfunctions. Acute endothelial dysfunctions exacerbate cell protein disorder and leukocyte adhesion in endhotelium. These changes can cause neuron, axon, and Schwann cell metabolism abnormalites, which will then disturb axonal transport. Increased vascular resistance and decreased neuronal blood flow can cause endoneurial hypoxia.

Hypoxia will induce advanced disorders of capillary such as axonal atrophy and CAN conduction that are caused by axonal transport disorders as well as decreased Na,K-ATPase activity. Chronic inflammation will stimulate microangiopathy in DM patients, increasing the incidences of microangiopathy in DM patients.[6]

There are several possible mechanisms related to the correlation between CAN and NRL, which include hyperglycemia in DM type 2 that causes ischemic neuron and affects persistent inflammation process, leading to repeated antigen exposures.[4,12] These underlie the process of chronic inflammation. The results in this study reveal that the relevant inflammations as well as nerve cell disorders in DM type 2 patients are mainly due to hyperglycemia.

Endothelial cell disorders cause neutrophil adhesion while a higher number of endothelial cells increase neutrophil levels. The presence of persistent and uncontrolled hyperglycemia will worsens inflammation since repeated antigen exposures may cause chronic inflammation. Cytokines, derived from the T and macrophage cells, suppresses neutrophil and lymphocyte releases. However, T and macrophage cells are released in chronic inflammation. As persistent hyperglycemia, acute and chronic inflammations may continuously occur in DM patients.[4] Diabetes mellitus type 2 patients with uncontrolled hyperglycemia may experienced lower lymphocyte levels because lymphocyte proliferation is prohibited. This is caused by lower levels of IL-2 receptor expression due to CD25 deficiency.[15] In addition, higher neutrophil levels increase NLR.

There were several limitations in this present study, such as the use of the cross-sectional design which may not be suitable for discovering the cause and effect between CAN and NLR while HbA1C examination was not performed to distinguish controlled and uncontrolled DM type 2 patients. In this study, there was no IL-2 examination to observe lower lymphocyte levels caused by decreased IL-2 levels. The exclusion criteria were pulmonary tuberculosis while non-pulmonary tuberculosis was not excluded because there is no advanced study which discovers the correlation between NLR and non-pulmonary tuberculosis in Asia. In conclusion, a strong correlation is found between CAN and NLR creating a possibility to use NLR value as a predictor of CAN in DM type 2 patients with a cut off point of 1.34.

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