Research Articles

The Role of Neutrophil Lymphocyte Ratio, Vitamin D, and NGAL as Cardiovascular Disease Marker in Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) patients have higher risk to develop cardiovascular disease (CVD) than non-CKD patients. Neutrophil lymphocyte ratio (NLR) in CKD patients reflects inflammation status and role as complementary prognosticmarker to evaluate cardiovascular risk in stage 3 to 5 CKD. High prevalence of vitamin D deficiency commonly found in CKD patients, leads to endothelial dysfunction and increase inflammation. NGAL is used as renal injury biomarker but nowadays NGAL has been known plays important role in CVD pathophysiology. CVD identification in CKD patients is necessary to obtain CVD risk and to stratify mortality earlier in CKD patients. This study aimed to obtain the differences and continued to obtain cut off of NLR, vitamin D and NGAL in CKD patients undergoing hemodialysis (HD) with and without CVD if the differences was significantly. A cross-sectional study was conducted in dr. Cipto Mangunkusumo National Hospital Jakarta, in December- February 2020. Total subjects were 83, consists of two groups with and without the CVD. There were no significant differences of NLR and vitamin D in CKD patients undergoing hemodialysis (HD) with and without CVD. The significant difference was found only in NGAL with cut off 5.64 ng/ml. NLR was lower, meanwhile vitamin D and NGAL were higher in CKD patients undergoing HD with and without CVD.

Keywords: chronic kidney disease, hemodialysis, neutrophil lymphocyte ratio, Vitamin D, neutrophil gelatinase associated lipocalin.

Peran Rasio Neutrofil Limfosit, Vitamin D, dan NGAL sebagai Penanda Penyakit Kardiovaskular pada Penyakit Ginjal Kronik

Abstrak

Pasien dengan penyakit ginjal kronis (PGK) memiliki risiko penyakit kardiovaskular (PKV) lebih besar dibandingkan dengan yang tidak memiliki PGK. Rasio neutrofil limfosit (RNL) pada pasien PGK mengambarkan status inflamasi dan merupakan marker prognostik komplementer untuk mengevaluasi risiko kardiovaskular pada PGK tahap 3 sampai 5. Prevalensi defisiensi vitamin D tinggi pada penderita PGK dan dapat menyebabkan disfungsi endotel serta meningkatkan inflamasi. NGAL selama ini digunakan sebagai biomarker kerusakan ginjal namun kini NGAL diketahui berperan penting pada patofisiologi PKV. Identifikasi PKV pada pasien PGK diperlukan untuk mengetahui risiko PKV dan menstratifikasi mortalitas lebih awal pada pasien PGK. Penelitian ini bertujuan untuk mendapatkan nilai NLR, vitamin D dan NGAL pada pasien PGK dengan dan tanpa PKV yang mengalami hemodialisis, serta menentukan titik potong. Penelitian cross-sectional ini dilakukan di Rumah Sakit Umum Pusat Nasional dr. Cipto Mangunkusumo pada bulan Desember – Febuari 2020. Jumlah subjek 83 orang dan terdiri atas dua kelompok yaitu kelompok dengan PKV dan tanpa PKV. Tidak terdapat perbedaan bermakna antara RNL dan vitamin D pada pasien PGK yang menjalani hemodialisis dengan PKV dibandingkan dengan tanpa PKV. Perbedaan bermakna terdapat pada kadar NGAL dengan titik potong 5,64 ng/ml. Nilai RNL lebih rendah sedangkan vitamin D dan NGAL lebih tinggi pada pasien PGK dengan PKV dibandingkan tanpa PKV.

Kata kunci: penyakit ginjal kronis, hemodialisis, rasio neutrofil limfosit, vitamin D, neutrophil gelatinase-associated lipocalin.

Introduction

Chronic kidney disease (CKD) has become an epidemic in the world, and still a substantial condition that burdens economically for patients, health workers and the social environment.[1] Based on an epidemiological study, CKD can increase the risk of cardiovascular disease (CVD) by twofold.[2] Acute myocardial infarction, atherosclerotic heart disease, congestive heart failure, stroke, cardiac arrest, cardiac arrhythmias are common CVD in CKD patients. If patients with end stage renal disease (ESRD) are not treated properly, the risks of mortality from CVD increases 20 times greater than the normal population.[3]

The neutrophil lymphocyte ratio (NLR) in CKD patients represents the inflammatory status and NLR is considered a complementary prognostic marker for evaluating cardiovascular risk in CKD stages 3 to 5. An increase in the number of neutrophils accompanied by a decrease in the number of lymphocytes can predict mortality in patients undergoing hemodialysis and peritoneal dialysis.[1]

In general, CKD patients are deficient in vitamin D, this is judged by the low levels of 25-hydroxy and 1.25 dihydroxy Vitamin D. Vitamin D deficiency is associated with higher cardiovascular mortality in both patients with CKD and the general population.[4]

Neutrophil Gelatinase-Associated Lipocalin (NGAL) has been used as a biomarker for renal injury because it is rapidly released in response to tubular damage, however, several studies have shown that NGAL is not only a simple biomarker but is known to play an important role in kidney pathophysiology as well as CVD. NGAL is also involved in various processes such as inflammation and fibrosis.[5]

Mortality in patients with CKD and ESRD remains high, which is associated with a high incidence of CVD such as coronary artery disease, cardiac hypertrophy, and heart failure. Identification of CVD in patients with CKD or ESRD is significant importance for diagnosis and treatment in the early stages of CVD. Although more important measures are needed to identify and use cardiovascular biomarkers to describe the risk of CVD and to predict mortality earlier.[6]

The aim of this study was to obtain the differences of NLR, Vitamin D, and NGAL and to determine cut off and obtain description of NLR, Vitamin D and NGAL in CKD undergoing HD patients with and without CVD.

Methods

The subjects were chronic kidney patients who had hemodialysis. The subjects were obtained from patients of the Hemodialysis Unit of the Kidney Hypertension Division, Department of Internal Medicine, National Central General Hospital dr. Cipto Mangunkusumo (CMHNH). The inclusion criteria were: CKD patients who routinely undergo hemodialysis at least twice per week, aged ≥ 18 years, and willing to participate in this study and sign an informed consent.

The study design is cross-sectional. Data processing and analysis were performed using the Statistical Package for Social Sciences (SPSS) program version 20 and Microsoft Excel 2007. The characteristics of the research subjects were presented descriptively. Data distribution was checked by the Kolmogorov-Smirnov test. Numerical data with normal distribution were presented in the form of mean and standard deviation and tested by independent parametric t-test, while numerical data with abnormal distribution were presented in median and range forms and tested by the non-parametric Mann Whitney rank test. The results of the analysis will then be displayed in tabular and narrative form.

Determination of the cutoff point of NLR, Vitamin D, and NGAL as a differentiator between the CVD group and those without CVD was carried out by measuring the Area Under Curve (AUC) based on the Receiver Operating Characteristic (ROC) curve. Analysis of the relationship between NLR, Vitamin D, and NGAL with CVD and without CVD was calculated by using the Prevalence Ratio (PR) value along with the calculation of sensitivity, specificity, positive predictive value, and negative predictive value.

This study is a further analysis of research entitled "Stool Microbiota Pattern and Amino Acids, Electrolytes, and Blood Hormones as Development of Supportive Therapy in Patients with Chronic Kidney Failure with Hemodialysis". with the ethical review number 0051/UN.2F1/ETIK/2019 and then an ethical amendment was carried out with the number 0057/ UN2.F1 /ETIK /2019.

Results

The total subjects were 83 people (Table 1), who were divided into two groups. Most of them are within the range of 18-79 years; more subjects without CVD (52 people) than those with CVD (31 people).

Table 1. The Characteristics of Research Subject

| Characteristics | Cardiovascular Disease | | Total |
	Yes (n = 31)	No (n = 52)	(n = 83)
Gender (%)			
Male	15 (48.4)	26 (50)	41 (49.4)
Female	16 (51.6)	26 (50)	42 (50.6)
Age (years), median (min-max)	56 (20-71)	47.5 (18-79)	52 (18-79)
Duration of HD (years), median (min-max)	4 (0.5-30)	5 (0.5-21)	48 (0.5-30)
Neutrophils (µl), median (min-max)	4880 (1550-12130)	4545.145 (2720-8020)	4648 (1550- 12130)
Lymphocytes (µl), median (min-max)	1660 (300-3120)	1470 (590-3598.35)	1490 (590-3598)
NLR, median (min-max)	2.96 (0.67- 7.46)	3.186 (0.96-9.19)	3.143 (0.67-9.19)
Vitamin D (ng/ml), median (min-max)	16.2 (5.0-108.6)	15.3 (6.4-105.2)	16.10 (5.0-108.6)
NGAL (ng/ml), median (min-max)	6.53 (3,29-10.32)	5.12 (2.28-9.78)	5.57 (2.276-10.32)
Prevalence of diabetes mellitus	9 (29%)	9 (17.3%)	18 (21.7%)
Prevalence of hypertension	18 (58.1%)	26 (50%)	53,8%
Glomerulonephritis prevalence	1 (3.2%)	2 (3.8%)	3 (36%)
Prevalence of other diseases	3 (9.7%)	15 (28.8%)	18 (21.7%)

Table 2 shows the difference in NLR between CKD patients with heart disease is a median value of 2.95 with a range of 0.67-7.46 and a median of CKD patients without heart disease is 3.19 with a p value of 0.39, so it was not significant.

Vitamin D between CKD patients with heart disease was a median value 16.20 ng/mL with a range of 5.0 - 108.6 and a median CKD patients without heart disease was 15.30 ng/mL with a range of 6.40-105.20 ng/mL with a p value of 0.37, thus it was not significant.

Table 2. Differences of NLR, Vitamin D and NGAL in the Group with CVD and without CVD

| Variable | Cardiovascular Disease | | p |
	Yes	No	
NLR, Median (min-max)	2.95 (0.67-7.46)	3.186 (0.96-9.19)	0.39
Vitamin D (ng/ml), median (min-max)	16.20 (5.0-108.6)	15.30 (6.40-105.20)	0.366
NGAL, (ng/ml) median (min-max)	6.53 (3.29-10.32)	5.13 (2.28-9.78)	0.005

NGAL in CKD patients with heart disease is a median value 6.53 ng/mL with a range of 3.29 - 10.32 and in CKD patients without heart disease is 5.13 ng/mL with a range of 2.28 - 9.78 ng/mL with a p value of 0.05, so statistically significant.

Discussion

In this study, it was found that female subjects were more than male subjects. This is in contrast to a similar study conducted by Taher et al,[7] which found more male subjects. The age range of the study subjects was 18-79 years, a study conducted by Shaarawy et al,[8] in hemodialysis patients also showed a similar age range 19-80 years.

The NLR in CKD patients with heart disease was not significant difference. This is not in accordance with the previous hypothesis, where NLR in CKD patients with CVD is expected to have a higher value than CKD patients who do not have CVD. In a 2012 Hyogo College study where decreased kidney function and increased leukocytes were associated with increased NLR in cardiovascular patients. The increase in NLR was associated in CKD patients with chronic heart failure. Jagadish,[9] also showed an association between increased NLR according to the severity of coronary artery disease, so that it can be used as an independent predictor of coronary heart disease.[10]

There was no significant difference in NLR levels, some of the causes were low BMI, especially in the elderly, where the age distribution in this study was quite wide. The causes of low BMI are persistent inflammation that can cause protein energy wasting, leading to malnutrition in CKD. In addition, uremia also has the potential for intestinal dysbiosis in CKD and increases the translocation of intestinal bacteria and their components to the circulation thereby activating systemic inflammation. Hemodialysis therapy also plays a role in increasing inflammation which is influenced by several factors such as the impurity of the dialysis fluid, the microbiological quality of the dialysate, and the bioincompatible factors in the extracorporeal dialysis circuit.[11] Another cause is HDL cholesterol levels because it has a protective role, and the presence of other comorbidities, namely diabetes mellitus, therapy with statins and ACE-inhibitors also has an effect because it has anti-inflammatory potential.[12]

Obtained differences in Vitamin D between CKD patients with heart disease was not significant. Low 25 (OH) D levels in patients with CKD and ESRD have been associated with a high risk mortality. Epidemiological studies, in general population report moderate to severe deficiency associated increased risk of cardiovascular events, peripheral vascular disease, hypertension and congestive failure. Vitamin D deficiency may affect the cardiovascular system via parathyroid hormone in independent or dependent manner.[9,13-16] In this study, there was no significant differences, this was due to several things Vitamin D levels were found to have an abnormal distribution and the range was quite wide, so it can be seen that vitamin D levels varied greatly in each patient, and it was not known whether the patient took vitamin supplements. Other influencing factors are sun exposure, uremia and hyperparathyroidism inhibition of 25-hydroxylation of cholecalciferol in the liver.[17]

There was a significant difference in NGAL between CKD patients with heart disease. The study by Furuya[18] stated that there was an association between NGAL serum levels and the prevalence of CVD in HD patients. NGAL is a potential cardiac biomarker related to inflammatory response and risk factors for atherosclerosis. Increased levels of NGAL are an indicator of the severity of the atherosclerosis process. Low of NGAL levels can be found in HD patients and can be marker of malnutrition.[19] Immaki et al[20] also found that low of NGAL levels were associated with the patients current malnutrition status and also represented a progressive worsening of HD patients.

Conclusion

A study was conducted on 83 CKD patients who underwent HD with and without CVD. The significant difference only found in NGAL levels in CKD patients with and without CVD. Thus, the NGAL value can be used as a marker for diagnosis and risk stratification in CKD patients with heart disease.

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