WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

Neutrophil lymphocyte ratio (nlr) was significantly associated with diabetic nephropathy at sanglah general hospital, denpasar, bali, indonesia: a case-control study

Ivana Beatrice Paulus[1], Kadek Adi Satrya Palguna[1], Jourdan Wirasugianto[1], I Gede Putu Supadmanaba[2], I Made Siswadi Semadi[3], Ketut Suastika[3]

[1] Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

[2] Department of Biochemistry, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

[3] Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Udayana University, Sanglah Hospital, Denpasar, Bali, Indonesia

Abstract

Background: Diabetic nephropathy (DN) is caused by chronic complications due to microvascular changes in patients with T2DM associated with the inflammatory process. Neutrophils to Lymphocyte Ratio (NLR) is a ratio that can describe the acute and chronic inflammatory systemic and allegedly the inflammatory process in the kidney. This study aims to determine the association between NLR with diabetic nephropathy in patients with type 2 diabetes. Method: This study is an analytical study with a case-control design. Samples were taken from the medical record installation room at Sanglah General Hospital Denpasar. Data was taken by using a simple random sampling method for 1 year that is 2017 according to inclusion and exclusion criteria. Statistical analysis including univariate analysis, normality test, Mann Whitney test, chi-square test, and AUC was done by using SPSS 23. Results: 143 sample data were used in this study with an average age of 62 years old (S.D 11.5). Median NLR for DN as case group were found 3.19 ranges within 1.19-29.2 while median NLR for non-DN as control group were found 3.2 (0.82-15.5) with p=0.000. NLR proportion ≥2.5 in the non-DN group was found 30 (42.2%) and DN group were found 49 (68%) with p=0.002. Risk analysis between NLR and Diabetic nephropathy obtained OR 2.91 and 95% CI: 1.45 - 5.76. Other variables such as age, sex, and diabetic status towards NLR levels were found not statistically significant. The results of AUC were 69.6% with 95%CI within the range (61.1% -78.2%). Conclusion: There is a significant association between NLR and diabetic nephropathy which potentially reflects the inflammation of the diabetic nephropathy process.

Introduction

Diabetes Mellitus (DM) is a metabolic disease that is currently a global burden disease and one of the main health problem in the world [1]. The number of patients with Type 2 Diabetes Mellitus (T2DM) reached around 90% of all DM patients globally [2]. T2DM is referred as non-insulin dependent, due to the occurrence of post-receptor signal interference. T2DM process begins with insulin resistance in peripheral cells which induces compensation for pancreatic β cells. When the progression of DM continues to occur, at one point the pancreatic cell is exhausted and unable to compensate for the situation. Which is lead into the final stage and cause inadequate insulin secretion just like T1DM. The progressivity of T2DM can be followed by acute or chronic complications [3].

Diabetic nephropathy (DN) is one of the chronic complications of T2DM. After 20 to 25 years being diagnosed with T2DM, patients are at risk of developing complications such as DN[4]. Diabetic nephropathy is microvascular changes in patient with

T2DM, caused by the thickening of the capillary membrane and mesangial cells of the glomerulus which is lead into glomerulosclerosis and renal insufficiency [4]. This is also can lead into cardiovascular disease, which increases the occurrence of mortality and morbidity [5,6]. The mechanism of DN with the activation of Renin Angiotensin Aldosterone System (RAAS), Advanced Glycation End Products (AGEs) and Reactive Oxygen Species (ROS) related to the inflammatory process and sclerosis in mesangial cells and tubulointerstitial renal [1,4,7,9].

The inflammatory process that occurs in the kidneys is characterized by increased expression of inflammatory cytokines such as NF-κB, TNF-α, Interleukin 1, 6, and 18 (IL-1, IL-6 and IL-18) which increases in patient with T2DM [8].

Neutrophil to Lympocyte Ratio (NLR) is a ratio between the calculation of neutrophils and lymphocytes which can describe systemic inflammation [10,11] The stability of NLR in describing acute and chronic inflammation systemically is

better than the other leukocyte parameters such as; neutrophils, lymphocytes and total leukocyte counts that can change by physiological, pathological and physical factors [12]. The use of NLR in describing systemic inflammation has advantages over cytokine tests as it is more applicable and economically feasible, but still can describe the inflammatory process [12]. This study aims to determine the association between NLR with diabetic nephropathy in patients with type 2 diabetes.

Method and Materials

This study is an analytical study with case-control design. Place and time of this study is held at Sanglah General Hospital in February until April 2018. Samples were taken from the medical record installation room of Sanglah Hospital Denpasar. Sampling method using simple random sampling according to inclusion and exclusion criteria. Inclusion criteria are patients who diagnosed with T2DM by clinical manifestation and laboratory findings at Sanglah Hospital for one year in 2017 with diabetic nephropathy as case group and non-diabetic nephropathy as control group, while exclusion criteria are patients with either active infection, cancer, tumor or autoimmune diseases. 143 sample were collected; 71 sample as control group and case group with 72 sample. Secondary data was taken by using medical record. Diagnosed T2DM patients were based on ADA/IDF criteria. Information about duration of DM, age, sex, and other chronic illness was collected. While DN was diagnosed using DM patients with urinary albumin excretion ratios (UAE) reaching 20 lg/min to 200 lg/min or 30 mg/24 h to 300 mg/24 h more than twice in 6 months have early-stage of DN and collected according to ICD-10-CM. Routine blood tests investigation such as complete blood picture, kidney function tests, urine routine and microscopy, lipid profile, fasting blood glucose (FPG)/ postprandial blood glucose (2 PPG), chest x-ray, and glycated haemoglobin (HbA1c) was collected. Fundus examination was done to assess diabetic retinopathy; nerve conduction velocity of all limbs was done to assess diabetic neuropathy; ABI and angiography to assess PAD. This research has been obtained and approved with ethical negligence letter by the commission for research ethics of Udayana University Faculty of Medicine/Sanglah General Hospital Denpasar and formal letter of permission obtained from the education & research division in Sanglah General Hospital Denpasar. All the data information which collected from the medical record will be kept confidential.

In this study, we were using univariate and bivariate analysis. Variable in this study including age, sex, NLR, diabetic status (A1C), comorbidity, and stage of chronic kidney disease (CKD). Numeric data such as A1C, age and NLR was tested using normality test and categorical by cut-off that were taken from the consensus and latest research. Numeric data were presented using means and standard deviations (SD) for normally distributed data, while abnormally distributed data using median and interquartile range (IQR). Normality and frequency test were used as univariate analysis to describe characteristic, proportion of sample and to determine the type of test that will be used in bivariate analysis. Comparing NLR between case and control group and find association of NLR and DN is the aims of bivariate analysis. Normality test were found abnormal for NLR, therefore non parametric test is used.

Mann Whitney test had been used to compare NLR level median between case and control group, Chi square test was used to describe the association between NLR with DN and control group other variables such as age, sex, diabetic status, and cardiovascular status. The results for another variable is not statistically significant (p>0.25) and can't be continued to multivariate analysis, this variable is not confounding in this study. Statistical analysis was performed by using SPSS 23.

Result

The characteristic of this study described in Table 01 and 02 Mean age is 62 years old (S.D 11.5), case group 60±11.75 and control 60.48±9.45. Gender ratio is nearly 2:1 for male and female sex in case and control group. Mean A1C between both group is almost the same, about 8 (Case: 8.14±2.13 and 8.08±2.32 for control group). Cardiovascular disease as comorbidity was founded the most in both groups and CKD stage 3 also was founded the highest overall stage. Other variable such as age, sex, diabetic status and CVD is analysed using chi square test, the result for each variable is above 0.05 (0.081; 0.0813; 0.578; 0.981). There is significant association between NLR with diabetic nephropathy while the others is not statistically significant.

Table 01: Baseline characteristic of sample (n=143)

Variable	Group		P-value
	Case (n=72)	Control (n=71)	
Age	60±11.75	60.48±9.45	NS
Sex*			
Male	27 (37.5)	29 (40.8)	NS
Female	45 (62.5)	42 (59.1)	
A1C (%)	8.14±2.13	8.08±2.32	NS
NLR	2.17 (0.82-15.5)	3.19 (1.19-29.2)	0.002†
Neutrophils (109 /l)	6.57(2-23.4)	4.82 (1.16-15.5)	0.001†
Lymphocytes (109 /l)	2.17(0.51-6.46)	2.13 (0.67-3.58)	NS
Comorbid*			
Cerebrovascular	2 (2.7)	1(1.4)	
Retinopathy	3(4.1)	9(12.6)	NS
Neuropathy	2(2.7)	6(8.4)	
PAD	7(9.7)	4(5.6)	

Diabetic status*			
Uncontrolled	36 (50)	46 (64.7)	NS
Controlled	36 (50)	25 (35.2)	
Prior CVD*			
Yes	40 (55.5	39 (54.9)	NS
No	32 (44.4)	32 (45.07)	

All parameters are expressed as mean standard deviation, unless otherwise stated. Significance level was set at P < 0.05. *Data are expressed as number (%). †Significant different between the two groups. CVD, Cardiovascular disease; NLR neutrophil–lymphocyte ratio; NS, not significant; PAD, peripheral artery disease

CKD stage 3 also was founded the highest overall stage. Other variable such as age, sex, diabetic status and CVD is analysed using chi square test, the result for each variable is above 0.05 (0.081; 0.0813; 0.578; 0.981).

There is significant association between NLR with diabetic nephropathy while the others is not statistically significant. Comparing median NLR between case and control group was done by using mann whitney test. Median NLR in case group is significantly higher than control group (p=0.000). Comparing median NLR between case and control group was done by using mann whitney test. Median NLR in case group is significantly higher than control group (p=0.000). Proportion NLR ≥2.5 is high in case group, while in control group is majority below 2.5 with p value = 0.002. Risk analysis between NLR and diabetic nephropathy obtained OR 2.91 and 95%CI: 1.45-5.76.
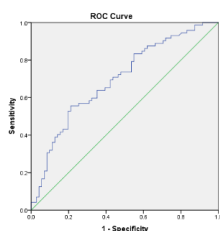
Table 02: Result of Mann Whitney

	Median NLR (minimum - maximum)	P value
Case	3.19 (1.19-29.2)	0.000
Control	2.17 (0.82-15.5)	

Table 03: Odd Ratio of NLR

	Median NLR (minimum - maximum)	P value
Case	3.19 (1.19-29.2)	0.000
Control	2.17 (0.82-15.5)	

The results of AUC was 69.6% with 95%CI within the range (61.1%-78.2%).

ROC Curve

Disscusion

DN is mediated by hyperglycemia that affected activation of polyol pathway, hexokinase pathway, protein kinase C (PKC) and age glycemic end product (AGE). This process induced cytokine inflammatory which leads into enhancement of extracellular matrix. DN is divided into five stages and early stage of DN is reversible with a good control of glycemic status and risk factor could eventually slowing the process. Urine analysis such as UAE and albuminuria were used as standard to DN screening. Except when the phase of hyperfiltration, urine analyses is still found normal, i.e UAE below 30 mg in 24 hours and normal-albuminuria. Also, from the clinical aspect, symptoms appeared after LFG below 30% and 10 to 20 years after diabetes onset [13 14]. While endothelial dysfunction has begun in the process of diabetic nephropathy and increased the risk of cardiovascular disease with acute mortality. Delayed treatment can induce progressivity of DN and precipitate to chronic kidney disease and eventually end-stage renal disease (ESRD). Thus, early detection is important and also to prompt aggressive treatment.[15,16]

NLR potential as a biomarker which represents inflammation process in DN because of the abnormality activation of neutrophil that occurs due to failure deletion of CD11b (a subunit of mac-1). Increasing CD11b has a role in neutrophil migration to the kidney. Also occurs migration of lymphocyte T to the kidney, that is mediated by a receptor which express receptor LFA-1 and ICAM-1 in endothelium, epithelial cells and mesangial cells in kidney. This interaction has a significant role of migration either neutrophil and lymphocyte, which also reflects acute and chronic inflammation. Beside inflammation process, angiogenesis also occurs in DN and related NLR [17,18]. When hyperglycemia occurs, vascular endothelial growth factor (VEGF) increased and reduced Nitric oxide (NO) [19]. Excessive level of VEGF-A in early phase of DN induced a new blood vessel formation and structural changes i.e glomelular hyperthrophy, thickening of glomerular basement membrane (GBM) and expansion mesangial as the initial progression [19,20]. Low NO related to podocyte cells injury and increasing VEGF-A [21]. The end of late phase, apoptosis and severe injury due to inadequate produced VEGF, and low VEGF caused glomerular scar [19].

NLR is calculated by dividing neutrophil and lymphocyte count. Some previous studies showed a significant association between NLR with prevalent chronic and vascular disease specifically DM and hypertension [22]. Shiny et al, stated that NLR is correlated with increasing level of grading of glucose intolerance and insulin resistance and also could be used as a prognostic marker either macrovascular or microvascular complications in patients with glucose intolerance [23]. Increasing NLR is independent predictor microvascular complication and several studies proved association NLR with diabetic nephropathy that may be a predictor for ESRD [12, 24–26] Huang et al stated that there is an association between NLR with diabetic nephropathy with p-value <0.001 and mean NLR 2.48 (SD 0.59).[27] Forget et al found cut off of NLR is 1.65 in normal population [28]. The results of this study is corresponding with previous studies with median NLR for case group is 3.19 and 2.17 for the control group with p-value=0.000. Risk analysis between NLR and diabetic nephropathy obtained OR 2.91 and 95%CI: 1.45-5.76 and other

factors including A1C in table 2.0 doesn't affected NLR titer. A1C is not statistically significant found with NLR. However, based on a study by Park et al, HbA1C is significantly associated with NLR (p<0.05).[24] A1C depends on hemoglobin values and sample were mostly have CKD and may present with anemia, which might be the reason of insignificant association of diabetic status with NLR in this study. Several studies suggested glycated albumin might be a better predictor of progression in diabetic nephropathy in type 2 diabetic patients regardless of HbA1c.[29] Further study needs to establish NLR as biomarkers with determining cut off for each stage of DN.

Limitation

The limitation in this study is that the researcher did not assess the phase of CKD from the early stages to ESRD and also there is confounding factor such as CVD. Researchers suggest that further cohort studies should be carried out by excluding CVD as part of their comorbidity thus, the association between NLR and DN can be clearly defined.

Conclusions

This study demonstrates association between NLR and diabetic nephropathy which potentially reflects the inflammation of diabetic nephropathy process.

Acknowledgements

Statement of competing interests

All authors declare there are no conflict of interest.

References

1. B. Roshan and R. C. Stanton, "A story of microalbuminuria and diabetic nephropathy.," *J. Nephropathol.*, vol. 2, no. 4, pp. 234–40, 2013, doi: 10.12860/JNP.2013.37.
2. World Health Organization, "Diabetes mellitus Fact sheet N°138," 2017, [Online]. Available: http://www.who.int/mediacentre/factsheets/fs138/en/.
3. C. a Carrera Boada and J. M. Martínez-Moreno, "Pathophysiology of diabetes mellitus type 2: beyond the duo 'insulin resistance-secretion deficit'.," *Nutr. Hosp.*, vol. 28 Suppl 2, pp. 78–87, 2013, doi: 10.3305/nh.2013.28.sup2.6717.
4. S.-I. Yamagishi and T. Matsui, "Advanced glycation end products, oxidative stress and diabetic nephropathy.," *Oxid. Med. Cell. Longev.*, vol. 3, no. 2, pp. 101–8, 2010, doi: 10.4161/oxim.3.2.11148.
5. R. Palsson and U. D. Patel, "Cardiovascular Complications of Diabetic Kidney Disease," *Adv. Chronic Kidney Dis.*, vol. 21, no. 3, pp. 273–280, 2015,

doi: 10.1053/j.ackd.2014.03.003.Cardiovascular.
6. S. John, "Complication in diabetic nephropathy," *Diabetes Metab. Syndr. Clin. Res. Rev.*, vol. 10, no. 4, pp. 247–249, 2016, doi: 10.1016/j.dsx.2016.06.005.
7. Z. Cao and M. E. Cooper, "Pathogenesis of diabetic nephropathy," *J. Diabetes Investig.*, vol. 2, no. 4, pp. 243–247, 2011, doi: 10.1111/j.2040-1124.2011.00131.x.
8. S. Toth-Manikowski and M. G. Atta, "Diabetic kidney disease: Pathophysiology and therapeutic targets," *J. Diabetes Res.*, vol. 2015, 2015, doi: 10.1155/2015/697010.
9. A. K. H. Lim, "Diabetic nephropathy ? Complications and treatment," *Int. J. Nephrol. Renovasc. Dis.*, vol. 7, pp. 361–381, 2014, doi: 10.2147/IJNRD.S40172.
10. P. Kruger et al., "Neutrophils: Between Host Defence, Immune Modulation, and Tissue Injury," *PLoS Pathog.*, vol. 11, no. 3, pp. 1–22, 2015, doi: 10.1371/journal.ppat.1004651.
11. X. Li, J. Shen, Z. Lu, M. Chen, X. Fang, and G. Wang, "High neutrophil-to-lymphocyte ratio is associated with increased carotid artery intima-media thickness in type 2 diabetes," *J. Diabetes Investig.*, vol. 8, no. 1, pp. 101–107, 2017, doi: 10.1111/jdi.12541.
12. B. Azab et al., "Neutrophil-to-Lymphocyte Ratio as a Predictor of Worsening Renal Function in Diabetic Patients (3-Year Follow-Up Study)," *Ren. Fail.*, vol. 34, no. 5, pp. 571–576, 2012, doi: 10.3109/0886022X.2012.668741.
13. H. Shahbazian and I. Rezaii, "Diabetic kidney disease; review of the current knowledge," *J. Ren. Inj. Prev.*, vol. 2, no. 2, pp. 73–80, 2013, doi: 10.12861/jrip.2013.24.
14. C. Muhammad and J. Nazar, "Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease," *J. Nephropharmacology J Nephropharmacol*, vol. 3, no. 1, pp. 15–20, 2014, doi: 10.1353/dis.2001.0019.
15. K. Umanath and J. B. Lewis, "Update on Diabetic Nephropathy: Core Curriculum 2018," *Am. J. Kidney Dis.*, vol. 71, no. 6, pp. 884–895, 2018, doi: 10.1053/j.ajkd.2017.10.026.
16. A. Kowalski, A. Krikorian, and E. V. Lerma, "Diabetic nephropathy for the primary care provider: new understandings on early detection and treatment," *Ochsner J.*, vol. 14, no. 3, pp. 369–379, 2014, [Online]. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4171795/pdf/i1524-5012-14-3-369.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25249803.
17. B. Azab et al., "Neutrophil-to-lymphocyte ratio as a predictor of worsening renal function in diabetic patients (3-year follow-up study)," *Ren. Fail.*, vol. 34, no. 5, pp. 571–576, 2012, doi: 10.3109/0886022X.2012.668741.
18. J. Karalliedde and L. Gnudi, "Endothelial factors and diabetic nephropathy," *Diabetes Care*, vol. 34, no. SUPPL. 2, pp. 291–296, 2011, doi: 10.2337/dc11-s241.
19. [19] K. Tanabe, Y. Maeshima, Y. Sato, and J. Wada,

"Antiangiogenic Therapy for Diabetic Nephropathy," *Biomed Res. Int.*, vol. 2017, 2017, doi: 10.1155/2017/5724069.

20. L. Gnudi, "Angiopoietins and diabetic nephropathy," *Diabetologia*, vol. 59, no. 8, pp. 1616–1620, 2016, doi: 10.1007/s00125-016-3995-3.

21. T. Nakagawa, T. Kosugi, M. Haneda, C. J. Rivard, and D. A. Long, "Abnormal angiogenesis in diabetic nephropathy," *Diabetes*, vol. 58, no. 7, pp. 1471–1478, 2009, doi: 10.2337/db09-0119.

22. F. Imtiaz, K. Shafique, S. S. Mirza, Z. Ayoob, P. Vart, and S. Rao, "Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population," *Int. Arch. Med.*, vol. 5, p. 2, 2012, doi: 10.1186/1755-7682-5-2.

23. A. Shiny *et al.*, "Association of Neutrophil-Lymphocyte Ratio with Glucose Intolerance: An Indicator of Systemic Inflammation in Patients with Type 2 Diabetes," *Diabetes Technol. Ther.*, vol. 16, no. 8, pp. 524–530, 2014, doi: 10.1089/dia.2013.0264.

24. B. Afsar, "The Relationship Between Neutrophil Lymphocyte Ratio With Urinary Protein Albumin Excretion in Newly Diagnosed Patients With Type 2 Diabetes," *Am. J. Med. Sci.*, vol. 347, no. 3, pp. 217–220, 2014, doi: 10.1097/MAJ.0b013e31828365cc.

25. E. M. Akbas *et al.*, "Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy," *Int. J. Clin. Exp. Med.*, vol. 7, no. 7, pp. 1794–1801, 2014.

26. Z. A. Öztürk *et al.*, "Is there a link between neutrophil-lymphocyte ratio and microvascular complications in geriatric diabetic patients?," *J. Endocrinol. Invest.*, vol. 36, no. 8, pp. 593–599, 2013, doi: 10.3275/8894.

27. W. Huang *et al.*, "Neutrophil-lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy," *Clin. Endocrinol. (Oxf).*, vol. 82, no. 2, pp. 229–233, 2015, doi: 10.1111/cen.12576.

28. P. Forget, C. Khalifa, J. P. Defour, D. Latinne, and M. C. Van Pel, "What is the normal value of the neutrophil‐to‐lymphocyte ratio ?," *BMC Res. Notes*, pp. 10–13, 2017, doi: 10.1186/s13104-016-2335-5.

29. A. Ake, M. R. Saraswati, and I. G. R. Widiana, "Glycated Albumin Sebagai Penanda Kontrol Glikemik Pada Penderita Diabetes Melitus Tipe 2 Glycated Albumin As a Glycemic Control Marker in Type 2 Diabetes Mellitus Patients," *Udayana J. Intern. Med.*, vol. 1, no. 1, pp. 1–10, 2016.