

## ORIGINAL ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

# Serum microRNA-126 expression as a biomarker of diabetic retinopathy

Ni Made Ayu Surasmiasi<sup>1\*</sup>, Ni Made Ari Suryathi<sup>1</sup>, and Ari Andayani<sup>1</sup>

## ABSTRACT

### BACKGROUND

Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus (DM). Diabetic retinopathy causes permanent blindness in the productive age group and has a multifactorial pathogenesis. MicroRNA-126 (miRNA-126) regulates the expression of the vascular endothelial growth factor (VEGF) gene at the post-transcriptional level, VEGF being an important angiogenic protein regulating inflammation in DR development. This study aimed to determine serum miRNA-126 expression as a biomarker in DM patients with DR.

### METHODS

This was a cross-sectional study involving 4 healthy persons and 21 type 2 DM patients. Subjects consisted of 4 groups: i) healthy controls, ii) DM patients without diabetic retinopathy (NDR), iii) DM patients with non-proliferative DR (NPDR) and iv) DM patients with proliferative DR (PDR). Venous blood was collected from subjects for miRNA-126 examination by real-time polymerase chain reaction (PCR). MiRNA-126 in each group was analyzed using the One-Way Anova test and  $p < 0.05$  was considered to be statistically significant.

### RESULTS

Mean miRNA-126 expression was significantly decreased in PDR ( $1.86 \pm 1.03$ ) and NPDR ( $1.01 \pm 0.43$ ) groups when compared to healthy control ( $2.44 \pm 1.29$ ) and NDR groups ( $2.15 \pm 0.48$ ) ( $p = 0.027$ ). MiRNA-126 values of less than 1.81 can differentiate NDR from the control group (sensitivity 83%, specificity 75%) and miRNA-126 of less than 1.56 can be used to predict NPDR when compared to the control group (sensitivity 86%, specificity 75%).

### CONCLUSION

Serum miRNA-126 is a potential biomarker for screening of NPDR and NDR in type 2 DM patients, and could be considered a non-invasive diagnostic parameter.

**Keywords:** microRNA-126, diabetes mellitus, diabetic retinopathy, biomarker

<sup>1</sup>Ophthalmology Department,  
Faculty of Medicine,  
Udayana University, Bali, Indonesia

### \*Correspondence:

Ni Made Ayu Surasmiasi  
Sekretariat Ilmu Kesehatan Mata  
RSUP Prof I.G.N.G Ngoerah  
Gedung Cempaka lantai IV  
Jalan Diponegoro Denpasar Bali  
Indonesia 80113  
Email: surasmiasi@unud.ac.id  
ORCID: 0000-0001-7835-224X

Date of first submission, March 2, 2023

Date of final revised submission,  
June 13, 2023

Date of acceptance, June 19, 2023

This open access article is distributed  
under a Creative Commons Attribution-  
Non Commercial-Share Alike 4.0  
International License

Cite this article as: Surasmiasi NMA,  
Suryathi NMA, Andayani A. Serum  
microRNA-126 expression as a  
biomarker of diabetic retinopathy. Univ  
Med 2023;42:121-7. doi: 10.18051/  
UnivMed.2023.v42:121-127



## INTRODUCTION

Diabetic retinopathy (DR) is a form of microvascular complication of diabetes mellitus (DM) caused by prolonged hyperglycemia. The prevalence of DR is predicted to increase along with the increase in DM patients. Diabetic retinopathy is the leading cause of vision loss in adults aged 20–74 years.<sup>(1)</sup> According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), in patients with a DM duration of 20 years, the incidence of DR reaches 99% in type 1 DM and 60% in type 2 DM.<sup>(2)</sup>

Diabetic retinopathy can be clinically classified into non-proliferative DR (NPDR) and proliferative DR (PDR). Retinal microvascular changes in the NPDR stage do not cross the internal limiting membrane (ILM). Clinical features of the retina may include microaneurysms, non-perfused capillary areas, nerve fiber layer (NFL) infarction, intraretinal microvascular abnormalities (IRMAs), intraretinal dot and blot hemorrhages, retinal edema, hard exudates, arterial abnormalities, retinal vein dilation and beading. Proliferative DR consists of extra-retinal fibrovascular tissue proliferation, such as the formation of new blood vessels, with minimal fibrous tissue crossing the ILM, with the blood vessels changing in size to become larger in diameter and thinner, accompanied by an increase in fibrous tissue. When the new vessels regress, the fibrovascular tissue remnants will proliferate along the posterior hyaloids. The proliferation of new blood vessels around the optic nerve is called neovascularization at the disc (NVD), whereas proliferation of new vessels in other places is called neovascularization elsewhere (NVE). Decreased visual acuity and blindness occur in severe cases of DR with complications such as vitreous hemorrhage, rhegmatogenous retinal detachment, and fibrovascular proliferation.<sup>(3,4)</sup>

One of the main factors which plays an essential role in angiogenesis in the hyperglycemic condition is vascular endothelial growth factor (VEGF). Intravitreal anti-VEGF injection has been used for DR treatment but the clinical responses have been very diverse.<sup>(5,6)</sup>

Research in microRNA (miRNA) is currently being developed. MicroRNA is a short chain non-coding RNA segment that inhibits gene expression in the post-transcriptional stage. MicroRNA-126 binds to the 3' untranslated region (UTR) of the VEGF messenger RNA and also plays a role in suppressing two negative regulators of VEGF, i.e. sprout-related EVH1 domain-containing protein 1 (SPRED1) and phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2). It has been found that in the hyperglycemic state miRNA-126 expression is low in endothelial cell culture,<sup>(7)</sup> plasma,<sup>(8)</sup> and serum.<sup>(9–12)</sup> The low expression of miRNA-126 in both hyperglycemic endothelial cells (representing intracellular miRNA) and in serum could make miRNA-126 a biomarker for the screening of retinal endothelial cell damage and the early diagnosis of the proliferative phase of DR.<sup>(11,12)</sup>

Many studies about the lower level of miRNA-126, both in vitro and in vivo, have been done in the hyperglycemic state. MicroRNA-126 is a stable molecule in the circulation and its serum level is equivalent to its level in the retinal endothelium, making it a good marker for tissue damage in hyperglycemia. Most of the studies in human subjects aimed to identify markers for the late stage of DR, such as PDR.<sup>(13–16)</sup> The ability to distinguish NPDR as the early stage of DR from non-DR (NDR) in type 2 DM based on the level of unregulated miRNAs, remains unknown. Many studies showed that miRNAs have key roles in the development of many diseases including DR. Therefore, assessment of miRNA levels in plasma may be an important tool for diagnosing diseases and following their progression.<sup>(17)</sup> The present study was conducted to obtain data on serum miRNA-126 expression as a biomarker for the discrimination of NDR, NPDR and PDR in type 2 DM patients.

## METHODS

### Research design

This study was an observational cross-sectional study to determine serum miRNA-126 expression in DM patients with DR divided into

NPDR and PDR. The research was conducted at the Sanglah General Hospital, Bali, from March to August 2021.

### Research subjects

The study involved 25 subjects consisting of 4 healthy non-DM patients and 21 patients with type 2 DM, namely 8 type 2 DM patients without DR (NDR), and 13 DM patients with DR which were subdivided into NPDR and PDR groups of 7 and 6 patients, respectively. The inclusion criterion for DM subjects was type 2 DM patients who were willing to have their venous blood collected and in whom retinal evaluation under dilated pupil was feasible. Exclusion criteria were patients with a history of malignancy, under corticosteroid treatment in the last 3 months, having undergone vitrectomy, and patients with a history of bevacizumab injections in the last 3 months.

### Data collection

History taking was done by a research assistant collecting age, length of DM diagnosis, and DM treatment. The ophthalmological examination included visual acuity, intraocular pressure, and fundus examination under dilated pupil (with instillation of 1% tropicamide). The fundus examination was done using a slit lamp biomicroscope and 78 D condensing lens by an external vitreoretinal consultant. Some patients underwent optical coherence tomography for macular edema.

### Laboratory analysis

All subjects were instructed to fast for 8-10 hours. Six milliliter of venous blood was drawn from the antecubital vein of the fasting subjects under aseptic conditions. The blood samples were then divided into two parts that were placed into an ethylenediaminetetraacetic acid (EDTA) tube for HbA1C examination and a plain red tube for miRNA examination.

### Serum miRNA-126 examination

A total of 3 mL venous blood in a plain red tube was centrifuged at 3000 rpm for 15 minutes

to obtain the serum, which was then stored at minus 80 degrees Celsius. From the serum samples, 400 miRNAs were isolated from RNA by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Total RNA including miRNA was extracted using *mirVana*™ PARISTM RNA reagent. Reverse transcription was done with the TaqMan MicroRNA Reverse Transcription Kit on a TAKARA Thermal Cycler PCR instrument. Expression was detected by real time PCR with TaqMan Fast Advanced Master Mix and TaqMan MicroRNA assay reagents. Gene expression was calculated by relative quantification (comparative delta  $CT=2^{-(\Delta\Delta CT)}$ <sup>(18)</sup> and required comparator genes in the form of endogenous control and reference/calibrator gene samples. The endogenous control used was miRNA-328-3p, which normalizes the number of target miRNAs to the total number of miRNAs in the sample.<sup>(19)</sup> The calibrator sample was a comparison sample from healthy individuals. Relative quantification (RQ) is a value that represents the ratio of miRNA expression in the target sample to that in the calibrator sample. This result is then made the cut-off point, where the values below the cut-off point are classified as low. The cut-off point is obtained from the calculation of the statistical magnitude using the ROC curve.

### Statistical analysis

Data was analyzed using the computer program Statistical Package for the Social Sciences (SPSS) version 22.0 from IBM Corp., Armonk, NY, USA. Numerical data were tested by the Shapiro-Wilk test. Comparison of variables between groups was done using the Kruskal-Wallis and One Way Anova tests. Post-hoc LSD analysis was used for further analysis of between-group differences. A p-value of <0.05 was considered statistically significant. Diagnostic accuracy was presented using the terms sensitivity and specificity. Receiver operator characteristics (ROC) analysis was used to determine the optimum cut-off value for miRNA-126 in differentiating NDR, NPDR, and PDR from the healthy controls.

Table 1. Characteristics of Research Subjects

Parameter	Healthy controls (n=4)	NDR (n=8)	NPDR (n=7)	PDR (n=6)	p value
Sex					
Male	2 (25.0)	1 (12.5)	2 (25.0)	3 (37.5)	0.422*
Female	2 (11.8)	7 (41.2)	5 (29.4)	3 (17.6)	
Age (years)	58.50 ± 9.68	56.88 ± 9.13	61.14 ± 5.24	54.67 ± 4.84	0.462**
DM treatment					
Insulin	0	1 (12.5)	2 (25.0)	5 (62.5)	0.001*
OAD	0	7 (53.8)	5 (38.5)	1 (7.7)	
No other treatment	4 (100)	0	0	0	
Duration of DM (years)					
< 10	0	8 (66.7)	2 (16.7)	2 (16.7)	0.001*
≥ 10	0	0	5 (55.6)	4 (44.4)	
HbA1C (%)		7.38 ± 1.54	7.91 ± 1.40	8.53 ± 1.48	0.539**
miRNA-126	2.44 ± 1.29	2.15 ± 0.48	1.01 ± 0.43	1.86 ± 1.03	0.027**

Notes: Data presented as n (%), except for age, HbA1C and miRNA-126 (mean ± SD); OAD= oral antidiabetic drugs; NDR= non diabetic retinopathy; NPDR= non-proliferative diabetic retinopathy; PDR= proliferative diabetic retinopathy; DM= diabetes mellitus; HbA1C= hemoglobin A1C; miRNA= micro RNA; \*Analyzed using Kruskal-Wallis test; \*\*One Way Anova analysis was done to compare means between groups

### Ethical clearance

Ethical clearance for the study was obtained from the Research Ethics Committee, Faculty of Medicine, Udayana University, under registration number 752/UN14.2.2.VII.14/LT/2021.

### RESULTS

Most of the subjects were women, consisting of 17 persons (68%). The mean age of the subjects was 58.10±7.29 years. Most of the 21 patients with type 2 DM received oral anti-diabetic therapy (61.9%) and had DM for less than 10 years (57.1%). Mean HbA1C was 7.89 ± 1.48% (Table 1). Mean miRNA-126 expression was lower in the NPDR group than in the PDR and NDR groups and was highest in the healthy controls (p=0.027) (Table 1). Post-Hoc analysis using LSD showed that there were significant differences between the healthy control group and the NPDR group (p=0.009) and between the NPDR and NDR groups (p=0.011) (Table 2).

The cut-off point for NDR compared to the healthy group was 1.81 (sensitivity 83%, specificity 75%). NPDR can be distinguished from the healthy group by a miRNA-126 level of less than 1.56 (sensitivity 86%, specificity 75%) (Table 3). The best cut-off point of miRNA-126

was 1.81 for NDR and 1.56 for NPDR. (Table 3, Figure 1)

Based on the ROC analysis, miRNA-126 level in the PDR group showed a tendency to cluster around and above the diagonal. Thus, miRNA-126 cannot be used as a biomarker to differentiate between the PDR group and the healthy control group.

### DISCUSSION

In this study, it was found that 17 (68%) of the 25 study subjects consisted of women. Based on the 2018 Riskesdas report, the prevalence of DM in people aged more than 15 years in Bali

Table 2. Post-Hoc Analysis of miRNA-126 using LSD

		Mean Difference	p value
Healthy	NDR	0.288	0.558
	NPDR	1.429*	0.009
	PDR	0.585	0.263
NDR	NPDR	1.141*	0.011
	PDR	0.297	0.492
NPDR	PDR	-.844	0.068

Note : NDR: non diabetic retinopathy; NPDR : non-proliferative diabetic retinopathy; PDR : proliferative diabetic retinopathy

Table 3. ROC curve analysis of miRNA-126

	AUC	Cut-off point	Sensitivity	Specificity
Healthy vs NDR	0.250	1.81	0.83	0.75
Healthy vs NPDR	0.036	1.56	0.86	0.75
Healthy vs PDR	0.563	1.83	0.75	0.50

Note: NDR= non diabetic retinopathy; NPDR= non-proliferative diabetic retinopathy; PDR= proliferative diabetic retinopathy

Province was 1.7%. The prevalence of DM in Indonesia based on gender was found to be higher in women (2.4%) than in men (1.7%).<sup>(20)</sup>

The present study found that most of the subjects with DM used oral antidiabetic agents rather than insulin injections. This is in line with the 2018 Riskesdas data where the proportions of DM treatments in the province of Bali were 64.6% for oral antidiabetic drugs, 15.7% for insulin injections, and 15.3% for combinations of oral and injected drugs, with 4.4% for the untreated.<sup>(20)</sup>

MicroRNA (miRNA) is an endogenous, single-stranded RNA sequence of about 19-24 nucleotides that interacts with the target RNA and regulates gene expression at the post-transcriptional level. MicroRNA-126 is the most widely expressed miRNA in endothelial cells and plays an important role in angiogenesis and vascular integrity. Under conditions of hypoxia and hyperglycemia, miRNA-126 plays a role in regulating the process of angiogenesis. miRNA-126 inhibits VEGF expression by binding to the 3' untranslated region of the VEGF-A messenger RNA (mRNA).<sup>(21-25)</sup> MicroRNA-126 is one of the miRNAs which plays a role in the DR angiogenesis and inflammation pathways.<sup>(8,10-12)</sup>

Diabetic retinopathy (DR) is one of the microvascular complications of DM causing changes in the retinal vascular endothelium. The early phase of these changes is marked by the loss of endothelial pericytes resulting in disruption of vascular permeability. Disruption of endothelial cells is manifested as microaneurysms as a marker of the NPDR phase, whereas retinal neovascularization is a sign of the PDR phase.<sup>(3,4)</sup> The retinal VEGF molecule is an important molecule as a marker of PDR, making anti-VEGF a major treatment for this condition. As the circulating VEGF molecule cannot be used as a biomarker for DR and PDR, other biomarkers are needed for diagnostic purposes.

Research conducted in type 2 DM patients found lower expression of miRNA-126 compared to non-DM controls,<sup>(9,11)</sup> similar to that found in type 1 DM patients. Patients with pre-diabetes also showed lower miRNA-126 levels than did healthy controls.<sup>(9,12)</sup> Low miRNA-126 is also seen in patients with other vascular diseases such as coronary artery disease<sup>(26)</sup> and intracerebral hemorrhage.<sup>(27)</sup> Our study also found a similar condition, in which the mean miRNA-126 levels in the healthy controls was higher compared to the DM groups. We also found lower miRNA-

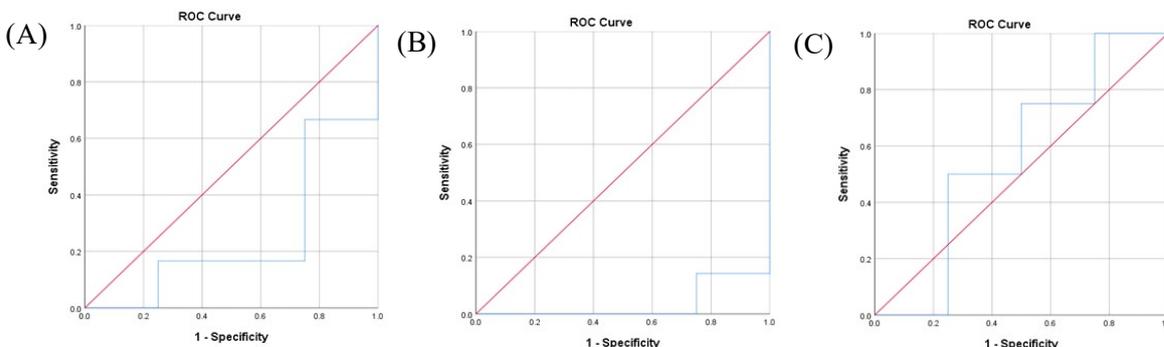


Figure 1. ROC curve analysis of plasma miRNA-126 for discriminating: A. Healthy controls from NDR; B. Healthy controls from NPDR; and C. Healthy controls from PDR

126 expression in the DR group than in the non-DR group.

The present study found that the mean miRNA-126 was higher in the PDR group and therefore does not reflect DR severity. However, if we compare the NPDR and NDR groups with the healthy control group, the ROC area under the curve is below the diagonal curve. This shows that miRNA-126 can be used as predictor for NDR and NPDR but not for PDR. An endogenous control functions to normalize the number of target miRNAs to the total number of miRNAs. The requirement for an endogenous control is stable expression and not being affected by the target miRNA expression, the environment, or the treatment given to the study subjects. The endogenous control used in this study was miRNA-328-3p, although a great number of other studies use miR-39 and U6 snRNA.<sup>(11,19)</sup>

In this study, we conclude that miRNA-126 is expressed in serum, for which the endogenous control miRNA-328-3p can be used in DR subjects. There are differences in mean miRNA-126 expression in the healthy control, type 2 DM without DR, and type 2 DM with DR groups. In the PDR group there was a decrease in miRNA-126 expression compared to the healthy group such that miRNA-126 can be used to predict DR in type 2 DM patients.

This study did not consider the other microvascular complications of DM such as neuropathy and nephropathy. A cohort study on diabetes patients needs to be done to further evaluate the role of serum miRNA-126 on DR progressivity and microvascular improvement with DM treatment.

## CONCLUSION

This study showed serum miRNA-126 to be a biomarker for identifying NPDR and NDR in type 2 DM patients.

## CONFLICT OF INTEREST

Competing interests: No relevant disclosure.

## ACKNOWLEDGEMENT

The author would like to thank the Biomolecular Laboratory, Faculty of Medicine and Health Sciences, Warmadewa University, and the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, for assisting in the molecular testing for this research.

## FUNDING

This research was funded by DIPA PNPB Udayana University TA-2021 in accordance with the Research Implementation Assignment Agreement No. B/5168/UN14.2.2.VII.1/PT.01.03/2021 of June 7, 2021.

## AUTHOR CONTRIBUTION

NMASm contributed to the study concept and design, statistical analysis, and manuscript writing. AA contributed to data collection and assembly. AA and NMASy contributed to data analysis and interpretation. All authors reviewed the manuscript, approved the final manuscript, and take public responsibility for the content of the manuscript submitted to *Universa Medicina*. 

## REFERENCES

1. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis* 2015;2:1–25. doi: 10.1186/s40662-015-0026-2
2. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017;128:40–50. DOI: 10.1016/j.diabres.2017.03.024.
3. Wiley H, Chew E, Ferris FL. Nonproliferative diabetic retinopathy and diabetic macular edema. In: Schachat AP, Wilkinson CP, Hinton DR, Sarda SR, Wiedemann P, editors. *Ryan's Retina*. 6th ed. London: Elsevier; 2018. p. 3210–304.
4. Sun J, Silva P, Cavallerano J, et al. Proliferative diabetic retinopathy. In: Schachat AP, Wilkinson CP, Hinton DR, Sarda SR, Wiedemann P, editors. *Ryan's Retina*. 6th ed. London: Elsevier; 2018. p. 3305–48.

5. Wells JA, Glassman AR, Ayala AR, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema: two-year results from a comparative effectiveness randomized clinical trial. *Ophthalmology* 2016;123:1251–359. doi: 10.1016/j.ophtha.2016.02.022.
6. Zhao Y, Singh RP. The role of anti-vascular endothelial growth factor (anti-VEGF) in the management of proliferative diabetic retinopathy. *Drugs Context* 2018;7:1–10. doi: 10.7573/dic.212532.
7. Wang Y, Yan H. MicroRNA-126 contributes to Niaspan treatment induced vascular restoration after diabetic retinopathy. *Sci Rep* 2016;6:26909. DOI: 10.1038/srep26909.
8. Zampetaki A, Willeit P, Burr S, et al. Angiogenic microRNAs linked to incidence and progression of diabetic retinopathy in type 1 diabetes. *Diabetes* 2016;65:216–27. doi: 10.2337/db15-0389.
9. Liu Y, Gao G, Yang C, et al. The role of circulating microRNA-126 (miR-126): a novel biomarker for screening prediabetes and newly diagnosed type 2 diabetes mellitus. *Int J Mol Sci* 2014;15:10567–77. doi: 10.3390/ijms150610567.
10. Barutta F, Bruno G, Matullo G, et al. MicroRNA-126 and micro-/macrovascular complications of type 1 diabetes in the EURODIAB Prospective Complications Study. *Acta Diabetol* 2017;54:133–9. DOI: 10.1007/s00592-016-0915-4.
11. Qin LL, An MX, Liu YL, Xu HC, Lu ZQ. MicroRNA-126: a promising novel biomarker in peripheral blood for diabetic retinopathy. *Int J Ophthalmol* 2017;10:530–4. doi: 10.18240/ijo.2017.04.05.
12. Rezk NA, Sabbah NA, Saad MSS. Role of microRNA 126 in screening, diagnosis, and prognosis of diabetic patients in Egypt. *IUBMB Life* 2016;68:452–8. doi: 10.1002/iub.1502.
13. Kamel N, Ayoub N, Ibrahim R, Ghalwash A, Khalaf N. Clinical significance of microRNA 126 in diabetic retinopathy in type 2 diabetes mellitus. *J Recent Adv Med* 2020;1:128–35. doi: 10.21608/JRAM.2020.112534.
14. Pramanik S, Saha C, Chowdhury S, Bose C, Bhattacharyya NP, Mondal LK. Decreased levels of miR-126 and miR-132 in plasma and vitreous humor of non-proliferative diabetic retinopathy among subjects with type-2 diabetes mellitus. *Diabetes Metab Syndr Obes* 2022;15:345–58. doi: 10.2147/DMSO.S346097.
15. Shaker OG, Abdelaleem OO, Mahmoud RH, et al. Diagnostic and prognostic role of serum miR-20b, miR-17-3p, HOTAIR, and MALAT1 in diabetic retinopathy. *IUBMB Life* 2019;71:310–20. doi: 10.1002/iub.1970.
16. Qing S, Yuan S, Yun C, et al. Serum miRNA biomarkers serve as a fingerprint for proliferative diabetic retinopathy. *Cell Physiol Biochem* 2014;34:1733–40. doi: 10.1159/000366374.
17. Rawlings-Goss RA, Campbell MC, Tishkoff SA. Global population-specific variation in miRNA associated with cancer risk and clinical biomarkers. *BMC Med Genomics* 2014;7:53. <https://doi.org/10.1186/1755-8794-7-53>.
18. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative  $C_t$  method. *Nat Protoc* 2008;3:1101–8. doi: 10.1038/nprot.2008.73.
19. Prado MSG, de Goes TC, de Jesus ML, Mendonça LSO, Nascimento JS, Kaneto CM. Identification of miR-328-3p as an endogenous reference gene for the normalization of miRNA expression data from patients with diabetic retinopathy. *Sci Rep* 2019;9:19677. doi: 10.1038/s41598-019-56172-w.
20. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia. Laporan Riset Kesehatan Dasar 2018. Lembaga Penerbit Balitbangkes. Jakarta; 2018.
21. Ye P, Liu J, He F, Xu W, Yao K. Hypoxia-induced deregulation of miR-126 and its regulative effect on VEGF and MMP-9 expression. *Int J Med Sci* 2014;11:17–23. doi: 10.7150/ijms.7329.
22. Shi L, Kim AJ, Chang RCA, et al. Deletion of miR-150 exacerbates retinal vascular overgrowth in high-fat-diet induced diabetic mice. *PLoS One* 2016;11:e0157543. doi: 10.1371/journal.pone.0157543.
23. Li EH, Huang QZ, Li GC, Xiang ZY, Zhang X. Effects of miRNA-200b on the development of diabetic retinopathy by targeting *VEGFA* gene. *Biosci Rep* 2017;37:BSR20160572. doi: 10.1042/BSR20160572.
24. Chen N, Wang J, Hu Y, et al. MicroRNA-410 reduces the expression of vascular endothelial growth factor and inhibits oxygen-induced retinal neovascularization. *PLoS One* 2014;9:e95665. DOI: 10.1371/journal.pone.0095665.s002.
25. Fang S, Ma X, Guo S, Lu J. MicroRNA-126 inhibits cell viability and invasion in a diabetic retinopathy model via targeting IRS-1. *Oncol Lett* 2017;14:4311–8. DOI: 10.3892/ol.2017.669526.
26. Mishra S, Rizvi A, Pradhan A, Perrone MA, Ali W. Circulating microRNA-126 & 122 in patients with coronary artery disease: correlation with small dense LDL. *Prostaglandins Other Lipid Mediat* 2021;153:106536. doi: 10.1016/j.prostaglandins.2021.106536.
27. Fu X, Niu T, Li X. MicroRNA-126-3p attenuates intracerebral hemorrhage-induced blood-brain barrier disruption by regulating VCAM-1 expression. *Front Neurosci* 2019.13:866. doi: 10.3389/fnins.2019.00866.