SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS AND SOME CHARACTERISTICS OF DIABETIC RETINOPATHY PATIENTS

Vu Van Nga[1,2], Nguyen Thi Binh Minh[3], Dinh Thi My Dung[3]
Nguyen Thi Lan Anh[3], Hoang Thi Thu Ha[4], Le Thi Minh Phuong[2]
Do Thi Le Hang[2], Do Thi Quynh[2], Pham Thi Thuy An[2]
Le Ngoc Thanh[2,3], Vu Thi Thom[2] and Tran Van Khanh[1,✉]

[1]Hanoi Medical University
[2]University of Medicine and Pharmacy, Vietnam National University Hanoi
[3]E hospital
[4]Vietnam National Institute of Ophthalmology

Diabetic retinopathy (DR) is a dangerous microvascular complication of diabetes mellitus (DM). The escalating prevalence of diabetes increases the rate of DR patients in Vietnam. Numerous studies have indicated that serum vascular endothelial growth factor (VEGF) can be used as a marker for monitoring the progression of this disease. Our study included 150 diabetic patients and 75 healthy individuals in the control group. Our study included 150 diabetic patients and a control group of 75 healthy individuals. We investigated the association between serum VEGF levels and some characteristics in diabetic retinopathy patients. Clinical data (pulse, blood pressure, medical history, and body mass index) and paraclinical data (Glucose, HbA1c) were collected. Serum samples were analyzed to determine VEGF concentrations. Our study fond that serum VEGF levels were higher in the group with DR compared to the other two groups and exhibited a correlation with glucose levels in diabetic patients ($p < 0.05$).

Keywords: Diabetic retinopathy, diabetes mellitus, VEGF.

I. INTRODUCTION

Diabetic retinopathy (DR) is a microvascular complication that threatens the vision of individuals with diabetes. It is the leading cause of preventable blindness in working-age adults. This complication annually causes the loss of vision for thousands of people.[1]

Currently, the rate of diabetic patients in Vietnam is increasing rapidly, concomitant with a rise in the incidence of DR, greatly impacting patients' quality of life and imposing burdens on the healthcare system.[2] However, clinically, the majority of patients with DR are detected in the late stages, when this complication is difficult or impossible to recover. This situation raises the imperative of devising research methodologies for early and accurate diagnosis.

Many studies have explored disease cohorts comprising many subgroups to find biomarkers for the progression of DR across its various stages (no DR, non-proliferative diabetic retinopathy, proliferative diabetic retinopathy). Among these biomarkers, vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR) in serum are the most important markers related to DR, clearly indicating the extent of neovascularization proliferation in DR, as evidenced by various studies. Consequently, serum VEGF can be used as a biomarker to monitor the progression of DR. VEGF is a member of the growth factor

Corresponding author: Tran Van Khanh
Hanoi Medical University
Email: tranvankhanh@hmu.edu.vn
Received: 08/03/2024
Accepted: 19/03/2024

subfamily and functions as a signaling protein pivotal to angiogenesis. VEGF is biosynthesized and secreted from retinal pigment epithelial cells, pericytes, astrocytes, Muller cells, glial cells, and endothelial cells. The equilibrium between VEGF and angiogenesis inhibitors critically regulates angiogenesis and proliferation in DR.[3,4] Many studies have shown that serum VEGF levels are associated with DR, increased macular thickness, and disruption of the retinal photoreceptor ellipsoid zone.[5] Therefore, research cohorts worldwide have assessed that serum VEGF can be chosen as a biomarker to monitor the progression of DR.

However, in Vietnam, there are currently not many studies evaluating serum VEGF levels in diabetic retinopathy patients. Thus, we carried out this project to determine serum VEGF levels and their relationship with some characteristics of these patients.

II. METHODS

1. Objectives

Type 2 diabetes patients who came for examination and treatment at E Hospital and Vietnam National Eye Hospital from January 2020 to March 2023 were selected based on selection criteria and voluntarily participated in the study. Diabetic patients were divided into two groups: those with and without diabetic retinopathy.

The control group is healthy people without diabetes and retinal disease.

Selection criteria

Diabetes group: Patients diagnosed with type 2 diabetes according to the standards of the American Diabetes Association (ADA) 2019 [6] and the classification of diabetic retinopathy of the International Council of Ophthalmology (2017).[7] Control group: Healthy people without diabetes and retinal disease.

Exclusion criteria

Patients were excluded if they had a diagnosis of cancer, rheumatoid arthritis, or severe cataracts precluding fundus examination.

2. Methods

Research design: cross-sectional description.

Sample size: use the sample size formula

$$n = Z^2_{1-\alpha/2} \frac{p\,(1 - p)}{(p.\varepsilon)^2}$$

In which:

n: research sample size, p: 0.425,[8]

$Z_{1-\alpha/2}$: 1.96 and ε: 0.28. Substituting these values into the formula yields n = 66.29. During the study, we collected 225 patients in two groups of type 2 diabetes with and without DR and a control group of healthy people (75 individuals in each group).

Research steps: Research subjects were given a general examination, eye examination, and medical interviews to collect clinical information according to research medical records. Venous blood samples were collected in the morning after an overnight fast of at least 8 hours. These samples, totaling 4mL each, were collected into two test tubes containing silica gel particles. Following collection, the test tubes were maintained upright for 60 minutes at room temperature to facilitate clot formation. Subsequently, the tubes were centrifuged at 4000-5000 rpm for 10 minutes at room temperature to separate serum from blood cells. The serum was then transferred to Eppendorf tubes and stored at -80°C until further analysis.

Research variables and indicators: Information on age, gender, patient history, pulse, blood pressure, and body mass index (BMI):

BMI = weight/ $(\text{height})^2$ (kg/m^2).

Being overweight is a BMI of 23 - 24.9 kg/

m2; obesity is a BMI ≥ 25 kg/m^2 [9]. Glucose concentration was analyzed using the hexokinase method (AU5800). HbA1C concentration was analyzed using boronate particle affinity high-performance liquid chromatography (Premier 9210). Serum VEGF concentrations were measured by the sandwich ELISA technique using the MyBioSource Human-VEGF ELISA analysis kit (MBS355343) following the manufacturer's instructions at the laboratory of University of Medicine and Pharmacy, Vietnam National University, Hanoi. The absorbance from each sample was measured by a spectrophotometric microplate reader at a wavelength of 450 nm (Epoch 2 machine, Biotek instruments, USA). Absorbance from each sample has been measured in duplicate.

3. Data analysis

Data was collected following the prescribed research medical record form and analyzed using statistical methods employing SPSS 22.0 software. The association between two qualitative variables was assessed using the Chi-square (x^2) test, while the Kruskal-Wallis and Mann-Whitney tests were employed to evaluate relationships between two quantitative variables. Correlation analysis between two quantitative variables was conducted using Spearman analysis. A significance level of $p \leq 0.05$ was considered statistically significant.

4. Research ethics

The study was approved by the Ethics Council in Biomedical Research, Hanoi Medical University, number IRB-VN01.001/IRB00003121/FWA 00004148, on March 18, 2020.

III. RESULT

The general characteristics of the subjects in the study are shown in Table 1.

Table 1. Some characteristics of the research objects

Characteristic		Control group n,%	No diabetic retinopathy n,%	Diabetic retinopathy n,%	p
Gender	Male	21 (23.3)	38 (42.2)	31 (34.4)	0.017[a]
	Female	54 (40.0)	37 (27.4)	44 (32.6)	
Age (years)	< 60	50 (54.3)	29 (31.5)	13 (14.1)	< 0.001[a]
	≥ 60	25 (18.8)	46 (34.6)	62 (46.6)	
BMI (kg/m^2)	< 23	45 (37.2)	31 (25.6)	45 (37.2)	0.040[a]
	23-25	21 (35.6)	22 (37.3)	16 (27.1)	
	≥ 25	9 (20.0)	22 (48.9)	14 (31.1)	
Duration of diabetes (years)	< 10		60 (69.0)	27 (31.0)	< 0.001[a]
	≥ 10		15 (23.8)	48 (76.2)	
History of hypertension	No	72 (56.7)	31 (24.4)	24 (18.9)	< 0.001[a]
	Yes	3 (3,1)	44 (44.9)	51 (52.0)	

Characteristic		Control group n,%	No diabetic retinopathy n,%	Diabetic retinopathy n,%	p
Pulse (Beat/minute)	$\bar{X}$ ± SD	75.16 ± 9.75	81.15 ± 7.95	83.47 ± 8.59* ,#	< 0.001[b]
	Median	78.00	80.00	82.00	
SBP (mmHg)	$\bar{X}$ ± SD	119.73 ± 13.35	130.80 ± 20.40*	130.19 ± 22.13*	< 0.001[b]
	Median	120.00	130.00	130.00	
DBP (mmHg)	$\bar{X}$ ± SD	75.67 ± 7.60	76.64 ± 10.08	76.27 ± 9.69	0.912[b]
	Median	75.00	75.00	80.00	

*a: Chi-square test; b: Kruskal wallis test; *: different from the control group (p < 0.05),*

#: different from the diabetic group with complications (p < 0.05)

Patients with diabetes had a higher rate of age over 60 compared to the control group (p < 0.001). The proportion of overweight patients (BMI ≥ 25 kg/m^2) was predominantly observed in the diabetic cohort (80%). Additionally, a greater proportion of patients in the diabetic group presented with hypertension. The DR patients demonstrated a higher prevalence of individuals with diabetes for over ten years compared to the no-DR group. Systolic blood pressure and pulse in the diabetes patients were higher than in the control group.

After analyzing the VEGF concentration in the serum samples of the study subjects, the results are shown in Figure 1.

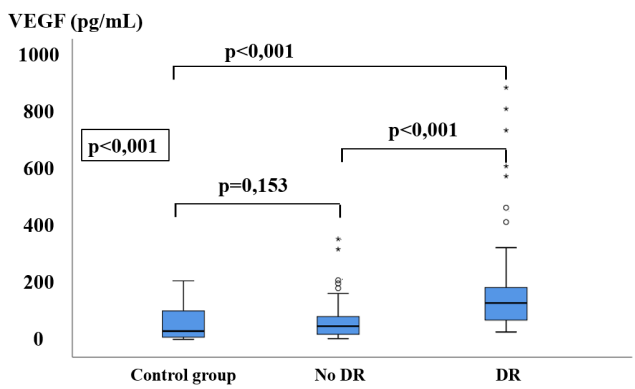


Figure 1. Serum VEGF concentration in the study

Median (Min-Max) of VEGF concentrations in the groups were: Control group: 35.53 (6.3-210.94) pg/mL, no DR: 52.5 (9.18-356.82) pg/mL, DR: 133.29 (32.12-858.38) pg/mL. VEGF concentrations in the DR group were higher than in the other two groups (p < 0.001).

We continued to analyze VEGF concentrations according to some clinical characteristics of the study subjects, and the results are shown in Table 3.

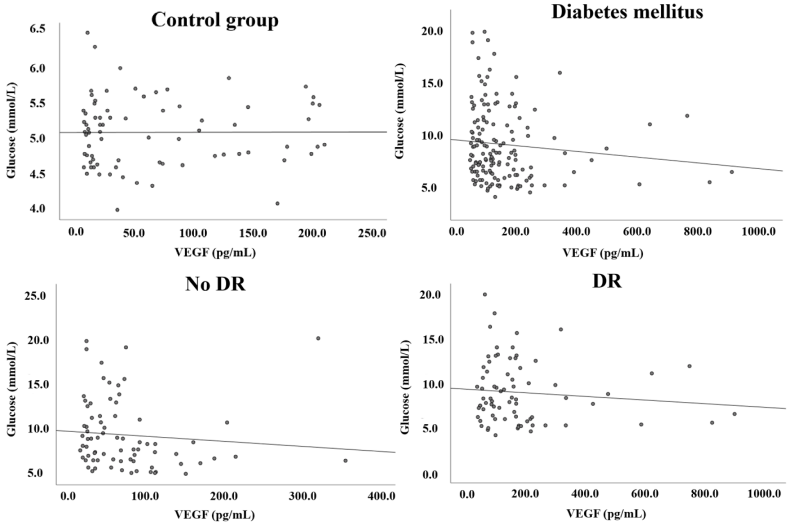
Table 3. Distribution of VEGF concentrations according to some clinical characteristics

Characteristic		Gender		p	Age (years)		p	Duration of diabetes (years)		p
		Male	Female		< 60	≥ 60		< 10	≥ 10	
Control group (pg/mL)	Median (Min-Max)	57.5 (8.00-210.94)	31.34 (6.30-206.63)	0.040	27.72 (6.30-210.94)	50.38 (9.76-206.63)	0.286			
No DR (pg/mL)	Median (Min-Max)	39.88 (9.18-356.82)	59.1 (12.12-201.63	0.424	60.35 (9.18-356.82)	51.13 (12.50-212.88)	0.998	56.31 (9.18-356.82)	36.30 (17.41-107.10)	0.534
DR (pg/mL)	Median (Min-Max)	100.94 (32.12-811.00)	147.99 (34.47-885.38)	0.533	100.94 (42.50-177.50)	136.20 (32.12-885.38)	0.234	161.53 (34.47-885.38)	107.99 (32.12-576.00)	0.197

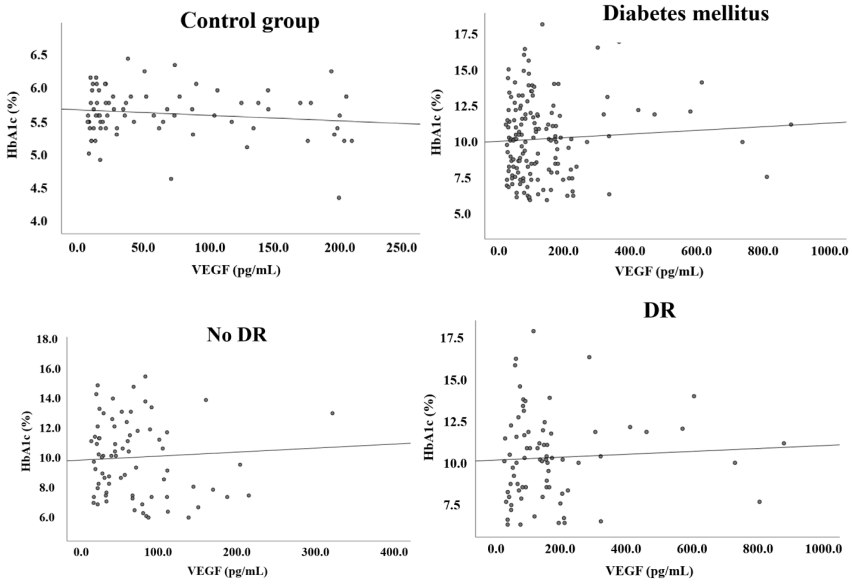
Characteristic		History of hypertension		p	BMI (kg/m^2)			p
		No	Yes		< 23	23-25	≥ 25	
Control group (pg/mL)	Median (Min-Max)	34.47 (6.63 - 210.94)	50.38 (6.30 - 87.25)	0.646	42.12 (6.63 - 210.94)	20.35 (6.3 - 206.63)	26.00 (12.71 - 200.94)	0.685
No DR (pg/mL)	Median (Min-Max)	69.10 (9.18 - 356.82)	48.00 (12.50 - 157.41)	0.349	60.35 (9.18 - 356.82)	39.88 (12.12 - 321.10)	44.18 (12.50 - 212.88)	0.437
DR (pg/mL)	Median (Min-Max)	121.10 (34.47 - 885.38)	133.29 (32.12 - 811.00)	0.856	141.25 (32.12 - 885.38)	122.30 (42.12 - 811.00)	121.84 (53.30 - 226.00)	0.816

Mann-Whitney test

In the control group, VEGF concentrations were higher in men than in women ($p < 0.05$). However, the study did not observe statistically significant differences in VEGF concentrations based on gender, age group, BMI, duration of diabetes, history of hypertension, or BMI subgroups within each group.



A. Correlation between serum VEGF and glucose concentrations



B. Correlation between serum VEGF and HbA1C concentrations.

Figure 2. Correlation between serum VEGF concentrations and glucose (A) and HbA1C concentrations (B).

Spearman correlation analysis between serum VEGF and glucose concentrations in patients with diabetes and no DR patients revealed a negative correlation ($r = -0.188$; $r = -0.301$; $p < 0.05$). Conversely, no significant correlation was observed between serum VEGF and HbA1C levels within the groups ($p > 0.05$).

IV. DISCUSSION

Research findings indicate a higher prevalence of individuals aged over 60 among patients with diabetes compared to the control group, particularly evident in the DR group, where over 80% fall within this age bracket. As

age increases, the likelihood of chronic diseases such as diabetes and associated complications escalates. Research in Iran (2017) showed that the prevalence of DR increases with age from 55 to 74 years, with a peak of 8.2% in the 70-74 age group, and the rate decreases to 3.4% in individuals aged 75 years and older.[10]

People with metabolic disorders like diabetes are often at risk of high blood pressure, which is a major risk factor for complications such as cardiovascular disease, kidney disease, stroke, and microvascular problems. Studies showed that about 50% to 80% of patients with type 2 diabetes exhibit hypertension.[11] Our study also recorded that the average systolic blood pressure and the rate of patients with hypertension in the diabetic group were higher than in the control group.

Our research focuses on evaluating serum VEGF levels in patients with type 2 diabetes and the relationship of this factor with DR. VEGF assumes a significant function in angiogenesis and the permeability of blood vessels, which is associated with DR. VEGF governs alterations in retinal vascular permeability, which affects the phosphorylation of proteins.[3] VEGF regulates neovascularization, activates endothelial cells, and causes basement membrane damage. High levels of VEGF lead to retinal neovascularization and proliferative retinal complications. Elevated VEGF has an effect even before signs of angiogenesis appear, and this effect is related to disease duration.[3] VEGF is a dimeric glycoprotein with heparin-binding activity, and its main role is to promote the proliferation of endothelial cells and induce the formation of new blood vessels. The blood supply to the retina plays an important role in maintaining and protecting the function of retinal ganglion cells. VEGF can specifically bind to vascular endothelial cell receptors, increasing vascular permeability and exacerbating ischemia and hypoxia in local retinal tissues.[12] Several studies showed that VEGF concentrations in the vitreous and serum are significantly higher in nonproliferative and proliferative DR cases than in controls. Moreover, analyses highlight a correlation between vitreous and serum VEGF concentrations.[13] Thus, assessing serum VEGF aids in gauging the onset and progression of diabetic retinopathy.

Our study showed no difference in VEGF concentration between the control group and the no DR group ($p = 0.381$). However, serum VEGF concentrations were notably higher in the DR group compared to both other groups ($p < 0.001$), consistent with findings from numerous previous studies.[3] Our analysis of VEGF concentrations by age group, gender, BMI subgroup, history of hypertension, and duration of diabetes found no differences within the same group. This result is similar to some previous research.[13]

Serum hemoglobin A1c (HbA1c) is a clinical indicator to evaluate blood sugar control in diabetic patients. HbA1c correlates well with mean plasma glucose concentrations and provides a moving average of blood glucose levels over the preceding three months.[14] HbA1c concentration has an inverse association with the rate of developing DR. High blood glucose levels and the formation of advanced glycation products (AGEs) have been shown to increase VEGF gene transcription and mRNA production.[15] In our study, while a distinct correlation between VEGF and glucose was noted in the diabetic group, no notable difference was observed concerning HbA1C. This may be because our study sample size was small, with a high proportion of patients exhibiting elevated HbA1c levels, so the correlation with VEGF levels was unclear.

V. CONCLUSION

Serum VEGF levels were increased in the group of DR group compared to the no DR group ($p < 0.001$). This study found a correlation between serum VEGF and glucose levels in diabetic patients ($r = -0.188$; $p < 0.05$).

ACKNOWLEDGMENTS

We would like to thank the sponsorship of the Ministry of Science and Technology and the Protocol Program for funding the project code NDT.69/CHN/19. We sincerely thank the Administrator of the University of Medicine and Pharmacy - VNU, Hanoi, and the staff of the Department of Basic Science in Medicine and Pharmacy - University of Medicine and Pharmacy, VNU, Hanoi; The Board of Directors and staff of Hospital E, the Board of Directors and staff of the Vietnam National Eye Hospital, for their supported and facilitation of our research.

REFERENCES

1. Kropp M, Golubnitschaja O, Mazurakova A, et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications-risks and mitigation. *EPMA J*. 2023; 14(1): 21-42. doi: 10.1007/s13167-023-00314-8.

2. Tuyen BP, Trung TN, Huyen TT, et al. Effects of Diabetic Complications on Health-Related Quality of Life Impairment in Vietnamese Patients with Type 2 Diabetes. *Hindawi Journal of Diabetes Research.* 2020; 2020(Article ID 4360804): 8 pages. doi: https://doi.org/10.1155/2020/4360804.

3. Ahuja S, Saxena S, Akduman L, et al. Serum vascular endothelial growth factor is a biomolecular biomarker of severity of diabetic retinopathy. *Int J Retin Vitr.* 2019; 5(29). answer: https://doi.org/10.1186/s40942-019-0179-6.

4. Gupta N, Mansoor S, Sharma A, et al. Kennedy. Diabetic retinopathy and VEGF. *Open Ophthalmol J*. two thousand and thirteen; 7:4–10. phone number : 10.2174/1874364101307010004.

5. Jain A, Saxena S, Khanna VK, et al. Status of serum VEGF and ICAM-1 and its association with external limiting membrane and inner segment-outer segment junction disruption in type 2 diabetes mellitus. *Mol Vis*. two thousand and thirteen; 4(19): 1760-8.

6. American Diabetes Association . Standards of medical care in diabetes – 2019. *The journal of clinical and applied research and education* . 2019; 42(1): S13-S28.

7. International Council of Ophthalmology . "Guide to eye care for diabetic patients". 2017;

8. Binh VT, Anh BH, Sơn NM, Nghi TH. Dac diem ton thuong vong mac mat dai thao duong o benh nhan dai thao duong tuyp 2 tai Benh vien Dai hoc Y Thai Binh. Journal of 108-clinical medicine and pharmacy. 2021. 16: 65-71.

9. Bryan W, Giuseppe M, Wilko S, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH), *European Heart Journal*. 2018; 39(33): 3021–3104. doi: https://doi.org/10.1093/eurheartj/ehy339.

10. Hassan H, Mehdi K, Payam N, et al. The prevalence of age-related eye disease in an elderly population. *Ophthalmic Epidemiol*. 2017; 24 (4): 222–228. doi: 10.1080/09286586.2016.1270335.

11. Jia G, Sowers JR. Hypertension in Diabetes: An Update of Basic Mechanisms and Clinical Disease. *Hypertension*. 2021; 78(5): 1197-1205. doi: 10.1161/

HYPERTENSIONAHA.121.17981.

12. Terasaki H, Ogura Y, Kitano S, et al. Management of diabetic macular edema in Japan: a review and expert opinion. *Jpn J Ophthalmol* . 2018; 62(1): 1–23.

13. Baharivand N, Zarghami N, Panahi F, et al. Relationship between vitreous and serum vascular endothelial growth factor levels, control of diabetes and microalbuminuria in proliferative diabetic retinopathy. *Clin Ophthalmol*. 2012; 6: 185-91. doi: 10.2147/OPTH.S27423.

14. Barr RG, Nathan DM, Meigs JB, Singer DE. Tests of glycemia for the diagnosis of type 2 diabetes mellitus. *Ann Intern Med*. 2002; 137: 263–272. doi: https://doi.org/10.7326/0003-4819-137-4-200208200-00011.

15. Tamarat R, Silvestre JS, Huijberts M, et al. Blockade of advanced glycation end-product formation restores ischemia-induced angiogenesis in diabetic mice. *Proc Natl Acad Sci USA*. 2003; 100(14): 8555-60. doi: 10.1073/pnas.1236929100.