Original Article

Association of Serum Vascular Endothelial Growth Factor Levels with Nephropathy and Retinopathy in Type 2 Diabetic Patients

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Abstract

Introduction: Vascular endothelial growth factor (VEGF), protein originally referred to as vascular permeability factor, maybe a signal protein produced by cells that stimulate the formation of blood vessels and a sub-family of growth factors. They are important signaling proteins engaged in both vasculogenesis and angiogenesis. It is a part of the system that restores the oxygen supply to tissues when blood circulation is insufficient like in hypoxic conditions. The serum concentration of VEGF is high in asthma and DM. The aim of this study to explore the relevance of VEGF level with the complication of diabetes mellitus (diabetic retinopathy [DR] and diabetic nephropathy (DN)). **Methodology:** Case–control study carried out in Khartoum–Sudan from August 2018 to August 2020. One hundred and four patients divided into four groups, 3 ml of blood were collected from each subject in sterile plain vacationer tube (3 ml) then was centrifuged, and the serum was stored at −20°C to measure VEGF level by using ELISA kits (E-EL-H0111, 96T). **Results:** We have confirmed previous observations of increased plasma VEGF Levels in patients with DR and DN. A total of 103 quality controls divided into four groups (diabetics with DR, diabetics with DN diabetics without DR or DN, and health individuals) were observed to determine the relationship between serum of VEGF levels and diabetes complication DR and DN. **Conclusions:** High level of VEGF plays a major role in the pathogenesis of DR and DN.

Keywords: Diabetes, nephropathy, vascular

Introduction

Diabetes mellitus (DM) commonly mentioned as diabetes, could also be a gaggle of metabolic disorders characterized by a high blood sugar level over a protracted period of sometime.[1]

Diabetes can cause many complications if left untreated.[2] severe complications can involve diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death.[3,4] Serious long-term complications include disorder stroke, chronic renal disorder, foot ulcers, damage to the nerves, damage to the eyes, and cognitive impairment.[2,5]

Diabetes can be managed well but the potential complications are the same for Type 1 and Type 2 diabetes including heart attack, stroke, kidney disease, limb amputation, depression, anxiety, and blindness.

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Diabetic retinopathy (DR) may be a common microvascular complication of diabetes and is that the leading explanation for blindness in adults aged 20–74 years in developed countries.[6,7] It occurs in more than 60% of patients with Type 2 diabetes mellitus (T2DM) it is any damage to the retina of the eyes, which may cause vision impairment.[8] Retinopathy predominately refers to retinal vascular disease, or defacement to the retina due to abnormal blood flow. Frequently, it's an ocular manifestation of systemic disease as seen in diabetes or hypertension.[9] The development of retinopathy is often

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weakened into proliferative and nonproliferative types. Both types cause disease by altering the traditional blood flow to the retina through different mechanisms.

Diabetic nephropathy (DN) develops in approximately 30% of diabetic patients, representing the leading cause of end-stage renal disease worldwide.[10]

It is the chronic loss of kidney function occurring in those with diabetes mellitus.[11] Protein loss within the urine thanks to damage to the glomeruli may become massive, and cause coffee albumin with resulting generalized body swelling (edema) and end in the nephrotic syndrome. The estimated glomerular filtration rate (eGFR) may progressively fall from a traditional of over 90 ml/min/1.73 m^2 to but 15, at which point the patient is claimed to possess end-stage kidney disease (ESKD).[12] It usually is slowly progressive over years.[13]

Vascular endothelial growth factor (VEGF) is an important organizer of angiogenesis and has been examined as a candidate gene in a number of conditions, inclusive diabetes and its microvascular complications (e.g., retinopathy and nephropathy). It's a substance made by cells that stimulate the formation of new blood vessels, a process called angiogenesis. VEGF also works as a mitogen for vascular endothelial (vessel lining) cells, activating these cells to divide and multiply It can stimulate angiogenesis, enhance collateral vessel formation, and increase the permeability of the microvasculature.[14,15] Diabetic microvascular changes in the retina lead to hypoxia, which stimulates the production of VEGF, a multifunctional cytokine that promotes angiogenesis and is a potent mediator of microvascular permeability.[16] VEGF is believed to play a significant role in the development of DR by inducing hyperpermeability of retinal vessels, breakdown of the blood-retinal barrier and neovascularization[17,18] Complications can appear as a result of the abnormal barrier function of new vessels, guiding to intraregional hemorrhage and exudation. New blood vessels have increased fragility resulting in sudden severe loss of vision thanks to vitreous hemorrhage.

VEGF is involved in the evolution of DN for neutralization with anti-VEGF antibodies in experimental models significantly reduces hyperfiltration, albuminuria, and glomerular hypertrophy.[19] VEGF is a secreted mitogen highly specific for the vascular endothelial cell which have been implicated in endothelial cell proliferation and migration.

At variance from other tissues that stop expressing VEGF-A at the cessation of development, kidney podocytes and tubular cells express VEGF-A throughout life.[20,21] Podocytes are the major source of VEGF-A in renal glomeruli.

VEGF-A binds VEGF receptors 1 and a couple of (VEGFR1 and VEGFR2), and co-receptors neuropilin 1 and a couple of (NRP1 and NRP2). VEGF-A signals through VEGFR2, NRP1 and NRP2 amplify VEGFR2 signals, while VEGFR1 functions mostly as a decoy.[22] All VEGF-A receptors are most predominant in endothelial cells but they are expressed by variant cells, including podocytes and tubular cells Hypoxia and high glucose upregulate podocyte VEGF-A protein expression.[23,24]

In addition, genetic variations within the VEGF gene might cause high–level expression of VEGF. However, high expression of VEGF may alter intracellular signal transduction, promote extracellular matrix synthesis, and stimulate renal hypertrophy, which are thought to be key factors within the increase of susceptibility to DN.

DM is a large problem worldwide. A total of 424.9 million adults have been estimated to have had DM, and this is estimated to rise to 628.6 million patients.[24] The WHO Eastern Mediterranean region has the very best prevalence of DM within the world. Seven countries in this area have a high propagation of DM and a further seven countries (including Sudan) have a middle prevalence (9%–12%) of DM[25] T2DM is the major type of DM, accounting for approximately 90% of all cases. The estimated prevalence of DM in Africa in 2017 was 3.3%, and Sudan was among the countries that had a prevalence of DM of more than 12%.[24]

Methods

The Institutional Review Board of Al-neelin University in August 30, 2018 (IRB serial no. NU-IRB-18-8-8-40) approved this study and a waiver of informed consent was received.

Case–control study carried out in Khartoum–Sudan from August 2018 to August 2020. A total of 104 subjects were divided into four groups, first group (DR) were enrolled from the Ophthalmological Clinic and had submit complete ophthalmological examination, this attended from Makaa Hospital. The second group (DN) was enrolled from Selma Centre for Kidney Diseases. The third group (diabetic control [DC]) without DN and retinopathy, attended from Zenam Hospital. The fourth group was health individuals (normal control [NC]).

The practical side of the study was performed at the laboratory of the Biochemistry Department in the College of Medical Laboratory in Alnileein, Alribat, National University and Selma center of kidney disease. Each individual submit to full medical examination which include:

1. Full personal, family, and medical history including a standardized questionnaire for any chronic diseases, gender, age, age of onset of diabetes, duration of diabetes and Medical examination which include (ophthalmological examination for DR, all renal test for DN, fasting glucose level and HBA1c)
2. ELISA KITS which include serum level of VEGF in all groups under certain conditions.

Sample collection

A volume of 3 ml of blood were collected in sterile plain vacationer tube (3 ml) then was centrifuged, and the serum was stored at −20°C to measure VEGF level by using ELISA kits (E-EL-H0111, 96T).

ELISA kits

- 100 micro standard or sample was added to each well
- Incubated for 90 min at 37C
- Liquid was removed, 100 micro of biotinylated detection Ab/Ag was added, and incubated for 1 h at 37C
- Aspirated and washed for 3 times
- 100 micro of HRP conjugate was added. Incubated for 30 min at 37C
- Aspirated and washed for five times
- 90 micro of substrate reagent was added; incubate for 15 min at 37 C
- 50 micro of stop solution determine was added to the OD value at 450 nm immediately
- The results were calculated.

Statistical analysis

Data were examined using Statistical Package of Social Science (IBM SPSS version 20.0) (IBM SPSSInc., Chicago, IL) for Windows software package. A $P \leq 0.05$ was interpreted as statistically significant. Comparison of groups was made by the Welch test and the *post-hoc* was done using the Games-Howell test. Correlation between quantitative variables was done by Spearman's rank correlation test.

Results

Descriptive

One hundred and four subjects were categorized into four groups 26 each Subject NC, DC, DR, and DN. Regarding the gender in the present study, there was 46.2%, 42.30%, 57.7%, and 53.8% of males, and 53.8%, 57.7%, 42.3%, and 46.2% of females in NC, DC, DR, and DN groups, respectively.

The age mean for the NC group was 65.88±2.61, for the DC group was 64.32±2.50, for DR group was 68.38 ± 1.50 and the DN group was 60.35± 2.80. The VEGF levels had the highest mean (198.02 ± 9.60) in the DR group followed by DN (136.33 ± 3.97) DC (84.96 ± 2.08) and NC (35.72 ± 2.30) groups, respectively [Table 1].

Vascular endothelial growth factor level between four groups

There was a significant difference within all four groups (Welch test, $P < 0.001$). Similarly, a significant difference was discovered between groups (Games-Howell *post-hoc* test), (NC-DC. $P < 0.001$) (NC-DR $P < 0.001$), (NC-DN $P < 0.001$), (DC-DR $P < 0.001$), (DC-DN $P < 0.001$) (DR-DN $P = 0.000004$). The DR group showed the highest level of VEGF (198.48 ± 9.43) followed by the DN group (136.33 ± 3.97), DM group (85.50 ± 2.07), and the NC group (35.72 ± 2.30), respectively [Figure 1].

Discussion

Chronic hyperglycemia has been reported to activate the synthesis and secretion of VEGF-A. It triggers a chain reaction that contributes to VEGF-A accumulation and then leads to DM microvascular complications.[26] The major physiological stimulus for VEGF production is cellular hypoxia and hyperglycemia. Hyperglycemia act as toxin to the endothelium through rising oxidative exertion. The high concentration of blood glucose increases the production of vasoconstrictor substances, particularly endothelin-1.[27] Hyperglycemia-stimulate pathological mechanism affects the manifestation of VEGF and its receptors VEGFR1 and VEGFR2. VEGF-A polymorphisms are associated with DR and DN as well.[26]

In DR may progress through several stages, including early nonproliferative DR (NPDR), moderate NPDR, severe NPDR, and finally advanced proliferative, DR (PDR).[28,29] The distinctive features of PDR include increased vascular permeability, tissue ischemia, and neovascularization that cause fibrovascular changes, vitreoretinal traction, and detachment

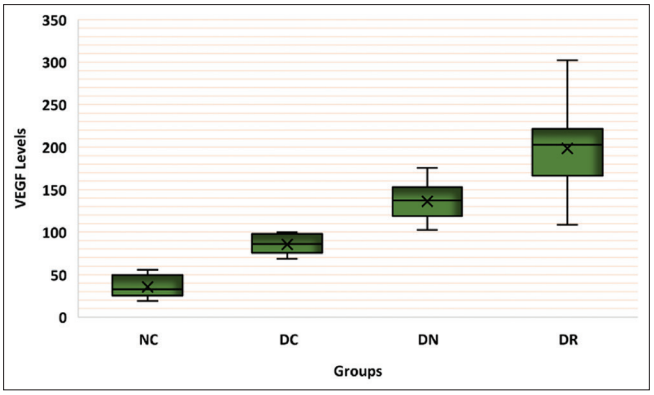

Figure 1: Vascular endothelial growth factor levels according to study group. NC: Normal Control, DC: Diabetic control without complications, DR: Diabetic with retinopathy, DN: Diabetic with nephropathy

Table 1: Descriptive analysis

Variables	Descriptive	NC	DC	DR	DN
Gender (%)	Males	46.2	42.3	57.7	53.8
	Females	53.8	57.7	42.3	46.2
Age	Mean±SE	65.88±2.61	64.35±2.4	66.92±1.74	60.35±2.80
Age	Mean±SD	65.88±13.32	64.35±12.24	66.92±8.87	60.35±14.30
VEGF levels	Mean±SE	35.72±2.30	85.50±2.07	198.48±9.43	136.33±3.97
Total number (*n*)	-	26	26	26	26

SE: Standard error, SD: Standard deviation, VEGF: Vascular endothelial growth factor, NC: Normal control, DC: Diabetic control, DR: Diabetic retinopathy, DN: Diabetic nephropathy

of the retina, eventually leading to blindness.[30-32] In the retina VEGF is cerated by different cell types, overall the retinal pigment epithelium (RPE), pericytes, endothelial, glial, Muller, and ganglion cells.[33,34] Among them, Müller cells and RPE are believed to be the major source of VEGF in the retina, and endothelial cells to be the primary target of VEGF.[34,35] In DN several pathophysiological mechanisms have been proposed to explain the dysfunction of the glomerular filtration barrier, which leads to diabetic microalbuminuria and eventually proteinuria. Synergistic interaction of hyperglycemia and raised VEGF-A in diabetic glomerulopathy could even be expounded by the unique hypothesis of "uncoupling of VEGF-A with gas (NO)."[36,37] Normally, VEGF-A catalyze endothelial NO release, and its required for the act of VEGF-A on endothelial cells. When hyperglycemia impairs normal endothelial function and reduces NO production, elevated levels of glomerular VEGF-A noted in diabetes could exert deleterious effects on endothelial cells, resulting in diabetic glomerulopathy.

On the opposite hand, Veron *et al*.[38] Suggested that a "normal" level of VEGF-A is important for maintaining the glomerular capillary structure, including the glomerular filtration barrier within the adult kidneys, and both an excessive amount of and insufficient VEGF-A in glomeruli can cause significant renal pathology.

The results of our project are in formation with the study of Zhang Q. *et al*.,[39] and Mahdy *et al*.[40] which suggests serum VEGF levels are a reliable biomarker for evaluating the development and progression of DR and DN.

On the other hand, Veron *et al*.[38] proposed that a "normal" level of VEGF-A is fundamental for maintaining the glomerular capillary structure, inclusive the glomerular filtration barrier in the adult kidneys, and both too much and too little VEGF-A in glomeruli can progress to significant renal pathology VEGF may be a predominant mediator of pathologic angiogenesis in DR and DN.

Moreover, the American Academy of Ophthalmology (AAO) preferred practice pattern committee now stated that there's sufficient evidence for the treatment of DR with anti-VEGF treatment (AAO[41]).

The reality that the surplus expression of VEGF-A in podocytes related to hyperglycemia commands to special glomerular alterations provides the rationale for anti-VEGF therapy against DN. The trial detects that the management of neutralizing monoclonal anti-VEGF antibodies to Type 1 and sort 2 diabetic relief the albuminuria and glomerular hypertrophy,[42] mentioning the activity of anti-VEGF therapy against DN. Then, SU5416, a pan-VEGF receptor tyrosine kinase inhibitor, was also conveying to attenuate albuminuria in Type 2 diabetic.[43]

Conclusions

High level of VEGF in DR and nephropathy patients was observed in this study suggesting that high levels of VEGF are associated with the progression of DN and DR.

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Conflicts of interest

There are no conflicts of interest.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009;32 Suppl 1:S62-7.
2. Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, *et al.* Causes of vision loss worldwide, 1990-2010: A systematic analysis. Lancet Glob Health 2013;1:e339-49.
3. Awuchi CG, Echeta CK, Igwe VS. Diabetes and the nutrition and diets for its prevention and treatment: A systematic review and dietetic perspective. Health Sci Res 2020;6:5-19, 136-7.
4. B. B. Tripathy, HB Chandalia and et al, RSSDI Textbook of Diabetes Mellitus. Revised 2nd ed., New Delhi, India: Jaypee Brothers Medical Publishers; 2012. p. 235.
5. Li W, Huang E, Gao S. Type 1 diabetes mellitus and cognitive impairments: A systematic review. J Alzheimers Dis 2017;57:29-36.
6. Sayin N, Kara N, Pekel G. Ocular complications of diabetes mellitus. World J Diabetes 2015;6:92-108.
7. Jeganathan VS. Anti-angiogenesis drugs in diabetic retinopathy. Curr Pharm Biotechnol 2011;12:369-72.
8. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol 1984;102:520-6.
9. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular complications of type 2 diabetes mellitus. Curr Vasc Pharmacol 2020;18:117-24.
10. Saran R, Robinson B, Abbott KC, Agodoa LY, Albertus P, Ayanian J, *et al.* US renal data system 2016 annual data report: Epidemiology of kidney disease in the United States. Am J Kidney Dis 2017;69:A7-8.
11. Rao M, Pallavi S, Khan A, Mathai D, Verma M, Vasudevan A. Sugar disease and its ramification on kidneys. J Clin Med Res 2020;2:1-11.
12. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Manual of Medicine. 18th ed. New York: McGraw-Hill Medical; 2013. p. 2982.
13. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, *et al.* Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. JAMA 2016;316:602-10.
14. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol 2001;106:148-56.
15. Roy H, Bhardwaj S, Ylä-Herttuala S. Biology of vascular endothelial growth factors. FEBS Lett 2006;580:2879-87.
16. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, *et al.* Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480-7.
17. Ferrara N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am J Physiol Cell Physiol 2001;280:C1358-66.
18. Ribatti D. The crucial role of vascular permeability factor/vascular endothelial growth factor in angiogenesis: A historical review. Br J Haematol 2005;128:303-9.
19. Flyvbjerg A, Dagnaes-Hansen F, De Vriese AS, Schrijvers BF, Tilton RG, Rasch R. Amelioration of long-term renal changes in obese Type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. Diabetes 2002;51:3090-4.
20. Simon M, Gröne HJ, Jöhren O, Kullmer J, Plate KH, Risau W, *et al.* Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney. Am J Physiol 1995;268:F240-50.
21. Tufro A, Norwood VF, Carey RM, Gomez RA. Vascular endothelial growth factor induces nephrogenesis and vasculogenesis. J Am Soc Nephrol 1999;10:2125-34.
22. Weis SM, Cheresh DA. Pathophysiological consequences of

VEGF-induced vascular permeability. Nature 2005;437:497-504.
23. Kim BS, Chen J, Weinstein T, Noiri E, Goligorsky MS. VEGF expression in hypoxia and hyperglycemia: Reciprocal effect on branching angiogenesis in epithelial-endothelial co-cultures. J Am Soc Nephrol 2002;13:2027-36.
24. Atlas D. International diabetes federation. IDF Diabetes Atlas. 7th ed. Brussels, Belgium: International Diabetes Federation; 2015.
25. Abdesslam Boutayeb, Mohamed Lamlili, Wiam Boutayeb, *et al*. The rise of diabetes prevalence in the Arab region. Open J Epidemiol 2012;2:55-60.
26. Tufro A, Veron D, editors. VEGF and podocytes in diabetic nephropathy. Seminars in Nephrology. Semin Nephrol: Elsevier; 2012:32:385-93
27. Ruszkowska-Ciastek B, Sokup A, Socha MW, Ruprecht Z, Hałas L, Góralczyk B, *et al*. A preliminary evaluation of VEGF-A, VEGFR1 and VEGFR2 in patients with well-controlled type 2 diabetes mellitus. J Zhejiang Univ Sci B 2014;15:575-81.
28. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, *et al.* Diabetic retinopathy: Seeing beyond glucose-induced microvascular disease. Diabetes 2006;55:2401-11.
29. Mitchell P, Wong TY. DIRECT new treatments for diabetic retinopathy. Lancet 2008;372:1361-3.
30. Wong TY, Klein R, Islam FM, Cotch MF, Folsom AR, Klein BE, *et al.* Diabetic retinopathy in a multi-ethnic cohort in the United States. Am J Ophthalmol 2006;141:446-55.
31. Jeganathan SE. Anti-angiogenesis drugs in diabetic retinopathy. Curr Pharm Biotechnol 2011;12:369-72.
32. Balasubramanyam M, Rema M, Premanand C. Biochemical and molecular mechanisms of diabetic. Curr Sci 2002;83:12.
33. Pe'er J, Shweiki D, Itin A, Hemo I, Gnessin H, Keshet E. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. Lab Invest 1995;72:638-45.
34. Dorey CK, Aouididi S, Reynaud X, Dvorak HF, Brown LF. Correlation of vascular permeability factor/vascular endothelial growth factor with extraretinal neovascularization in the rat. Arch Ophthalmol 1996;114:1210-7.
35. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci U S A 1995;92:905-9.
36. Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: An explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 2007;292:F1665-72.
37. Nakagawa T, Sato W, Kosugi T, Johnson RJ. Uncoupling of VEGF with endothelial NO as a potential mechanism for abnormal angiogenesis in the diabetic nephropathy. J Diabetes Res. 2013;2013:184539. doi: 10.1155/2013/184539.
38. Veron D, Reidy KJ, Bertuccio C, Teichman J, Villegas G, Jimenez J, *et al.* Overexpression of VEGF-A in podocytes of adult mice causes glomerular disease. Kidney Int 2010;77:989-99.
39. Zhang Q, Fang W, Ma L, Wang ZD, Yang YM, Lu YQ. VEGF levels in plasma in relation to metabolic control, inflammation, and microvascular complications in type-2 diabetes: A cohort study. Medicine (Baltimore) 2018;97:e0415.
40. Mahdy R, Nada W, Hadhoud K, El-Tarhony S, The role of vascular endothelial growth factor in the progression of diabetic vascular complications. cEye (Lon) 2010;24:1576-84.
41. Al-Hilali KA, Mosa MJ, Hussein AA. The role of hyperglycemia and coexisting hypertension in the development of diabetic nephropathy in Type II diabetes mellitus. Medico Legal Update 2020;20:1161-7.
42. Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol 2001;12:993-1000.
43. Sung SH, Ziyadeh FN, Wang A, Pyagay PE, Kanwar YS, Chen S. Blockade of vascular endothelial growth factor signaling ameliorates diabetic albuminuria in mice. J Am Soc Nephrol 2006;17:3093-104.