


Effect of glycemic control and dyslipidemia on plasma vascular endothelial growth factor and pigment epithelium-derived factor in diabetic retinopathy patients in Northern Nigeria

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ABSTRACT

Objectives: The disruption of the reciprocal regulation between vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) has been associated with the pathogenesis of diabetic retinopathy (DR). This study assessed the levels of VEGF, PEDF, indices of glycemia, and lipid profile in diabetic patients with retinopathy.

Methods: One hundred fifty participants comprised 50 type 2 diabetic patients with DR, 50 without DR and 50 non-diabetic normotensive controls, aged 30–80 years, were randomly recruited for this case-control study. The study was carried out from November 2017 to December 2018. VEGF, PEDF, glycated hemoglobin (HbA1c), fasting plasma glucose, and lipid profile were determined using standard methods. Blood pressures (BP) and anthropometric indices were measured. Chi-squared test of independence, analysis of variance, and Pearson's correlation were used to analyze data. Statistical significance was set at $P < 0.05$ and 95% confidence interval.

Results: Both diabetic groups had significantly higher ($P = 0.001$) systolic and diastolic BP, VEGF, PEDF, HbA1c, fasting plasma glucose, triglycerides, total, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels and significantly lower ($P = 0.005$) VEGF/PEDF than the controls. However, the diabetics with retinopathy had significantly higher ($P = 0.001$) HDL-C, LDL-C, VEGF, and PEDF levels compared to the diabetics without retinopathy. There were no significant differences ($P > 0.05$) in the levels of VEGF, PEDF, and VEGF/PEDF in both groups of diabetics that had good glycemic control and poor glycemic control. There was also no significant difference ($P > 0.05$) in the levels of VEGF and PEDF between the dyslipidemic and non-dyslipidemic subjects in both diabetic groups.

Conclusion: DR is associated with higher levels of VEGF and PEDF while good glycemic control and dyslipidemia seem not to have a profound effect on VEGF and PEDF levels in diabetics with or without DR. Higher PEDF levels are associated with higher atherogenic risk in the diabetics with retinopathy.

Keywords: Dyslipidemia, glycemic control, pigment epithelium-derived factor, type 2 diabetes mellitus, vascular endothelial growth factor

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Introduction

Diabetic retinopathy (DR) is a complication of diabetes that affects millions of people globally. It was reported that 2.6 million persons globally were visually impaired due to DR in 2015, this figure projected to rise in 2020 to 3.2 million.^[1] The Nigerian national blindness and visual impairment survey reported that in Nigeria, the prevalence of diabetes was 3.25% and more than a tenth of persons

who had diabetes and were 40 years old or more had their eyesight threatened by DR.^[2]

In DR, the retina becomes damaged in a progressive manner, resulting in vision loss and consequently blindness. DR is characterized by increased vascular permeability, tissue ischemia, and neovascularization.^[3] Known risk factors of DR include hyperglycemia, dyslipidemia, and hypertension.^[4] Although tight control of these factors has shown proven

benefits in reducing the incidence and progression of DR, the incidence of DR keeps increasing exponentially.^[4] It has been reported that changes in level of several metabolites may contribute to changes in the way some mediators are produced. This results in an increase in blood flow, capillary permeability, apoptosis, and consequently angiogenesis.^[5]

Vascular endothelial growth factor (VEGF) is a signal protein that promotes normal and pathological angiogenesis and is a proangiogenic factor in DR. VEGF is chemotactic for macrophages and vascular smooth muscle cells; it also initiates the migration as well as proliferation of endothelial cells, microvascular permeability, and blood flow increase.^[6] High levels of VEGF have been linked with the pathogenesis of DR.^[7]

Pigment epithelium-derived factor (PEDF) is a multifunctional protein which has been shown to inhibit DR development through its anti-oxidative properties. It possesses antiangiogenic, antitumorigenic, anti-inflammatory, neurotrophic, antifibrosis, and antivasopermeability properties.^[8] PEDF is an extracellular component of the retina and decreased levels of PEDF have been reported to be associated with DR pathogenesis as PEDF downregulation is associated with the increase of VEGF expression which may contribute to neovascularization of the retina resulting in DR development.^[9]

The balance between VEGF and PEDF is very important in the regulation of vascular permeability and angiogenesis. Studies have shown that there is a reciprocal interaction between both proteins in the retina and it has been suggested that the reciprocal regulation between them may be a factor in angiogenic control.^[10] In the diagnosis and management of diabetes generally and DR in particular, the emphasis has always been on tight control of fasting plasma glucose concentration, percentile glycated hemoglobin (HbA1c), and lipid profile.^[11,12] However, it has been shown that some diabetics may still develop DR even with good glycemic control and absence of dyslipidemia. This has led to studies which are beginning to focus attention on micromolecules such as PEDF and VEGF. There is a paucity of literature on VEGF and PEDF in DR in our population. It is hoped that this study would provide the needed data on the levels of VEGF and PEDF in diabetics with retinopathy in our population and the effect of glycemic control and dyslipidemia on these molecules. This information may be useful in the management of diabetic patients who seem to have good control of DR risk factors but still develop or have progressive DR. This study assessed the levels of VEGF and PEDF and lipid profile in DR to determine the effect of glycemic control and dyslipidemia on VEGF and PEDF in diabetics with retinopathy.

Materials and Methods

This was a case-control study carried out in the Department of Retinal Medicine, National Eye Centre, Kaduna (a

referral Eye Hospital in Northern Nigeria) from November 2017 to December 2018. The study enrolled a total of 150 subjects of both genders consisting of 50 type 2 diabetics with retinopathy, 50 type 2 diabetics without retinopathy, and 50 apparently healthy non-diabetic and normotensive volunteers recruited from around the Kaduna metropolis. They were aged between 30 and 80 years. Sampling was done by simple random sampling. The patients were assessed by the Ophthalmologists of Retinal Medicine Department, National Eye Centre, Kaduna, Nigeria. Classifications of patients were based on patient history and their attendant complications based on the findings of widefield fundus photography. The presence of microaneurysms, dot and blot hemorrhages, flame-shaped hemorrhages, retinal edema and hard exudates, venous loops and venous beading, cotton-wool spots, macular edema, neovascularization, fibrovascular tissue proliferation, and traction retinal detachments were suggestive of DR. Ethical permission was obtained from the National Eye Centre ethics and Research Committee (NECEC/PATH/20170015). Written informed consent was also obtained from each subject before being enrolled in the study. The tenets of the Declaration of Helsinki were adhered to in this study. Treatment modalities included for diabetics with retinopathy, surgical or laser interventions, and vitrectomy. In addition to this, according to hospital protocol, every patient with DR is placed on strict dietary regimens and exercises besides various pharmacologic interventions in the management of diabetes and DR.

Inclusion and exclusion criteria

All subjects were 30 years and above. Both male and female subjects were recruited and only patients with type 2 diabetes were recruited. Any participant with the overt renal disease was excluded from the study. Those on anti-VEGF therapy were excluded from the study. Controls with hypertension were excluded as hypertension is known to also cause increases in VEGF levels.

Measurement of anthropometric indices and blood pressures (BP) and definition of cut-off

Waist circumference (cm), weight (kg), and height (m) of each subject were measured as described elsewhere.^[13] Body mass index (BMI) was computed as the ratio of body weight (kg) to the square of body height (m²). The BP was measured on the right arm with a mercury sphygmomanometer (cuff size 12.5 × 40 cm) with the patient in a seated position and after a 5 min rest. The systolic and diastolic BPs were recorded. Hypertension was defined as BP ≥140/90 mmHg or the use of antihypertensive medications; diabetes as elevated fasting glucose >7.1 mmol/L or the presence of type 2 diabetes and/or the use of antidiabetic medications.^[14] Dyslipidemia as triglycerides ≥1.70 or high-density lipoprotein-cholesterol (HDL-C) <0.90 mmol/L; obesity was defined as BMI ≥30 kg/m²; poor glycemic control as HbA1c >7% and good control as HbA1c ≤7%.^[14-16]

Sample collection and preparation

Six milliliters of venous blood were aseptically obtained by venipuncture, using Terumo Vacutainer (Terumo, Japan), from all participants at 8–10 am after an overnight fast. Two milliliters (2 ml) each of whole blood were dispensed into sodium fluoride oxalate bottle and EDTA bottles for plasma glucose estimation and HbA1c estimation, respectively. Plasma was also obtained by centrifuging an aliquot of the EDTA sample at 1000× g for 15 min and the plasma collected and aliquots were stored at –20°C. The remaining 2 ml were dispensed into plain bottles and serum obtained for lipid profile and urea and creatinine.

Laboratory assay methods

Blood glucose concentration was determined by glucose oxidase colorimetric method (Agape reagent kits, China). HbA1c percentage was estimated by a cation exchange resin chromatography kit by Pointe Scientific Inc., USA. Total cholesterol and triglycerides, urea, and creatinine were analyzed using the enzymatic colorimetric method with kits from Agape Diagnostics, China. HDL-C was carried out in two stages, precipitation and color development stage. The second stage was analyzed using the enzymatic colorimetric method with kit from Agape Diagnostics, China. Low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald equation, $LDL-C = TC - HDL-C - VLDL-C$.^[17]

Assay of plasma VEGF

Plasma level of VEGF was determined by sandwich ELISA methods with kits obtained from PeptoTech Inc. (Rocky Hill, USA). In this assay, monoclonal anti-VEGF antibody is adsorbed to the surface of the microplate well. An aliquot of sample or calibrator containing the “antigen” (VEGF) to be quantitated was added and allowed to bind with a solid phase antibody. After washing, peroxidase-conjugated monoclonal anti-VEGF antibody was added, thus forming an Ab-Ag-Ab-Enzyme sandwich complex. Unbound antibody was then washed away and TMB substrate added; the enzyme catalytically converts the substrate to products. The reaction was stopped and color read at 450 nm. The amount of product formed is proportional to the quantity of VEGF in the sample.

Assay of plasma PEDF

Plasma level of PEDF was determined by Aviscera ELISA kits obtained from Bioscience (Santa Clara, USA). The plate comes pre-coated with an antibody specific for human PEDF which binds human PEDF in the standard and samples. After washing to remove unbound substances, a biotinylated detection antibody was then added to the wells. After washing to remove unbound biotinylated detection antibody, streptavidin-HRP conjugate was added to the wells. It was washed again to remove any streptavidin-HRP conjugate. Substrate solution was then added to the wells and color development occurred and the reaction was stopped and read at 450 nm. The intensity of the color developed is directly

proportional to the amount of human PEDF present in the samples.

Statistical analysis

Data were analyzed using R version 4.0.0 from The R Foundation for Statistical Computing Platform, Vienna, Austria and Statistical package for the Social Science version 18 (SPSS Inc. Chicago IL, USA). Results were expressed as mean ± SD. Chi-squared test of independence was used to test the significant relationship between categorical variables using the R version 4.0.0. The Shapiro–Wilk test was used to test for normality of data. ANOVA was used to compare continuous variables among the three groups and least significant difference for *post hoc* analysis. Pearson’s correlation coefficient (*r*) was used to evaluate the correlations among parameters. Statistical significance was set at $P < 0.05$ and 95% confidence interval.

Results

In Table 1, the sociodemographic characteristics of the study participants are presented. A total of 150 participants (50 type 2 DM patients with DR, 50 type 2 DM patients without DR, and 50 controls) were enrolled in this study. There were 75 males and 75 females in all. DM patients with DR had a higher mean duration of diabetes compared to DM patients without DR. More of the diabetics had poor glycemic control. The frequency of hypertension and obesity was similar among the groups. The diabetics without retinopathy had the highest frequency of dyslipidemia.

Table 2 shows fasting plasma glucose, HbA1c, lipid profile, VEGF, and PEDF in diabetics with retinopathy, diabetics without retinopathy, and non-diabetics. There were significant variations in systolic ($P = 0.0001$) and diastolic BP ($P = 0.001$), fasting plasma glucose, HbA1c, total cholesterol, triglyceride, HDL-C, VEGF, PEDF, LDL-C/HDL-C ($P = 0.0001$), LDL-C, and VEGF/PEDF ($P = 0.001$). *Post hoc* analysis of these indices showed that diabetics with retinopathy had significantly higher ($P = 0.0001$) HDL-C, VEGF, and PEDF and significantly lower LDL-C ($P = 0.031$) and LDL-C/HDL-C ($P = 0.0001$) than the diabetics without retinopathy. Furthermore, the diabetics with retinopathy had significantly higher ($P = 0.0001$) systolic and diastolic BP, fasting plasma glucose, HbA1c, total cholesterol, triglyceride, HDL-C, VEGF, and PEDF and significantly lower ($P = 0.005$) VEGF/PEDF than the controls. Similarly, diabetics without retinopathy had significantly higher ($P = 0.0001$) systolic and diastolic BP, fasting plasma glucose, HbA1c, total cholesterol, triglyceride, VEGF, PEDF as well as LDL-C but significantly lower HDL-C ($P = 0.0001$) and VEGF/PEDF ($P = 0.001$) compared to non-diabetics [Table 3].

The effect of glycemic control on VEGF, PEDF, and VEGF/PEDF levels in diabetics with retinopathy and diabetics without retinopathy was shown in Table 4; there were no differences

Table 1: Sociodemographic characteristics of the study participants

Variables	Diabetics with retinopathy <i>n</i> =50	Diabetics without retinopathy <i>n</i> =50	Non-diabetics <i>n</i> =50	<i>P</i> -value
Age (years) <i>n</i> (SD)	57.0 (9.71)	57.0 (9.11)	53.9 (14.34)	0.288 ^A
BMI (kg/m ²) (SD)	28.7 (3.67)	27.8 (4.75)	27.2 (3.33)	0.173 ^A
Duration of diabetes (year) <i>n</i> (SD)	4.9 (4.75)	2.8 (3.32)	-	0.010 ^C
Kidney function <i>n</i> (SD)				
Urea (mmol/L)	4.9 (2.12)	5.2 (2.40)	4.4 (1.14)	0.103 ^A
Creatinine (μmol/L)	79.0 (26.81)	78.5 (39.03)	69.1 (18.45)	0.169 ^A
Gender (<i>n</i> , %)				
Male	24 (48)	27 (54)	24 (48)	0.786 ^B
Female	26 (52)	23 (46)	26 (52)	
Glycemic control (<i>n</i> , %)				
Poor ^a	39 (78)	38 (76)	-	0.898 ^B
Good ^b	11 (22)	12 (24)	-	
Hypertension ^c (<i>n</i> , %)	21 (42)	19 (38)	-	0.683 ^B
Obesity ^d (<i>n</i> , %)	18 (36)	12 (24)	9(18)	0.113 ^B
Dyslipidemia ^e (<i>n</i> , %)	8 (16)	23 (46) [#]	3 (6)	0.0001 ^{*B}

*Significant at $P \leq 0.05$. Superscripts are defined as: (a) HbA1c >7%, (b) HbA1c \leq 7%, (c) Blood pressure \geq 140/90 mmHg, (d) body mass index \geq 30 kg/m², (e) triglyceride \geq 1.70 mmol/L and/or HDL-C <0.9 mmol/L. ^AAnalysis of variance, ^BChi-square test of independence, ^CStudent's *t*-test. [#]Significantly higher than diabetics with retinopathy and controls

Table 2: Fasting plasma glucose, HbA1c, lipid profile, VEGF, and PEDF in diabetics with retinopathy, diabetics without retinopathy, and non-diabetics

Parameters	Diabetics with retinopathy <i>n</i> =50	Diabetics without retinopathy <i>n</i> =50	Non-diabetics <i>n</i> =50	Calc. F	<i>P</i> -value
Systolic BP (mmHg)	140.2±17.90	135.6±18.68	121.6±10.56	18.124	0.0001*
Diastolic BP (mmHg)	86.4±12.15	85.9±13.40	78.2±7.46	8.243	0.007*
Fasting plasma glucose (mmol/L)	7.93±1.83	7.55±3.53	4.37±0.50	35.654	0.0001*
HbA1c (%)	8.6±2.23	8.6±2.08	5.35±0.77	30.183	0.0001*
T-cholesterol (mmol/L)	4.8±0.93	5.0±1.2	4.0±0.92	12.367	0.0001*
Triglyceride (mmol/L)	1.11±0.42	1.08±0.39	0.82±7.46	9.890	0.0001*
HDL-C (mmol/L)	1.91±0.60	1.09±0.40	1.54±0.59	35.907	0.0001*
LDL-C (mmol/L)	2.67±2.40	3.37±1.10	2.12±0.92	7.642	0.001*
VEGF (μg/ml)	45.1±12.31	36.7±10.05	31.7±6.34	23.034	0.0001*
PEDF (pg/mL)	7.87±1.98	6.69±1.64	4.38±0.91	63.558	0.0001*
VEGF/PEDF(μg/pg)	6.18±2.59	5.86±3.32	7.57±2.43	6.912	0.001*
LDL-C/HDL-C	1.47±1.15	3.92±3.00	1.54±0.96	25.881	0.0001*

*Significant at $P < 0.05$. HbA1c: Glycated hemoglobin, VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor, BP: Blood pressure, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol

($P > 0.05$) in the levels of VEGF, PEDF, and VEGF/PEDF between those with good glycemic control and poor glycemic control in diabetics with or without DR. Furthermore, the effect of dyslipidemia was examined in Table 5 on VEGF, PEDF, and VEGF/PEDF in diabetics with retinopathy and diabetics without retinopathy and similar observations were made.

Figure 1 shows a significant negative correlation between PEDF and HDL-C ($r = -0.318$, $P = 0.025$). There was a significant positive correlation between PEDF and LDL-C/HDL-C ($r = -0.334$, $P = 0.014$) in the diabetics with retinopathy [Figure 2]. There were no other significant correlations with other parameters. There was also no significant correlation of duration of diabetes with VEGF, PEDF, or VEGF/PEDF in both diabetics with retinopathy or without retinopathy.

Discussion

Diabetes mellitus, which is considered by some as the epidemic of the 21st century, is a global health problem. It is associated with microvascular and macrovascular complications such as retinopathy which could lead to blindness.^[18-21] Many biochemical factors have been reported to play contributory roles in DR; these include VEGF and PEDF.^[22] Reports on the role of glycemic control and dyslipidemia on VEGF and PEDF are controversial.^[23,24]

In this study, we found that VEGF levels were higher in all diabetic groups (with or without retinopathy) compared to the controls. Many mechanisms to explain the elevation of VEGF in vascular diseases have been proposed. First, a wide array of

Table 3: Comparison of fasting plasma glucose, HbA1c, blood pressures, lipid profile, VEGF, and PEDF in diabetics with retinopathy and diabetics and non-diabetics without retinopathy using *post hoc* analysis

Parameter	Groups		Mean difference	P-value
	Diabetics with retinopathy (n=50)	Diabetics without retinopathy (n=50)		
HDL-C (mmol/L)	1.91±0.60	1.09±0.40	0.817	0.0001*
LDL-C (mmol/L)	2.67±2.40	3.37±1.10	-0.705	0.031*
VEGF (µG/ml)	45.1±12.31	36.7±10.05	8.420	0.0001*
PEDF (pg/mL)	7.87±1.98	6.69±1.64	1.181	0.0001*
LDL-C/HDL-C	1.47±1.15	3.92±3.00	-2.451	0.0001*
Diabetics with retinopathy (n=50)		Non-diabetics (n=50)		
Systolic BP (mmHg)	140.2±17.90	121.6±10.56	18.640	0.0001*
Diastolic BP (mmHg)	86.4±12.15	78.2±7.46	8.220	0.0001*
Fasting plasma glucose (mmol/L)	7.93±1.83	4.37±0.50	3.558	0.0001*
HbA1c (%)	8.6±2.23	5.35±0.77	3.202	0.0001*
Total cholesterol (mmol/L)	4.8±0.93	4.0±0.92	0.745	0.0001*
Triglyceride (mmol/L)	1.11±0.42	0.82±7.46	0.292	0.0001*
HDL-C (mmol/L)	1.91±0.60	1.54±0.59	0.375	0.0001*
VEGF (µG/ml)	45.1±12.31	31.7±6.34	13.420	0.0001*
PEDF (pg/mL)	7.87±1.98	4.38±0.91	3.489	0.0001*
VEGF/PEDF(µg/pg)	6.18±2.59	7.57±2.43	-1.031	0.005*
Diabetics without retinopathy (n=50)		Non-diabetics (n=50)		
Systolic BP (mmHg)	135.6±18.68	121.6±10.56	14.060	0.0001*
Diastolic BP (mmHg)	85.9±13.40	78.2±7.46	7.640	0.001*
Fasting blood sugar (mmol/L)	7.55±3.53	4.37±0.50	3.182	0.0001*
HbA1c (%)	8.6±2.08	5.35±0.77	3.284	0.0001*
Total cholesterol (mmol/L)	5.0±1.2	4.0±0.92	0.978	0.0001*
Triglyceride (mmol/L)	1.08±0.39	78.2±7.46	0.255	0.0001*
HDL-C (mmol/L)	1.09±0.40	1.54±0.59	-0.442	0.0001*
LDL-C (mmol/L)	3.37±1.10	2.12±0.92	1.260	0.0001*
VEGF (µG/ml)	36.7±10.05	31.7±6.34	5.000	0.013*
PEDF (pg/mL)	6.69±1.64	4.38±0.91	2.308	0.0001*
VEGF/PEDF(µg/pg)	5.86±3.32	7.57±2.43	-1.713	0.001*
LDL-C/HDL-C	3.92±3.00	1.54±0.96	2.378	0.0001*

*Significant at P<0.05. HbA1c: Glycated hemoglobin, VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor, BP: Blood pressure, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol

Table 4: Effect of glycemic control on VEGF and PEDF and VEGF/PEDF ratio in diabetics with retinopathy

Parameter	Diabetics with retinopathy		Calc. t	P-value
	Good glycemic control (n=11)	Poor glycemic control (n=39)		
VEGF (µg/ml)	50.3±8.78	42.62±12.86	1.609	0.114
PEDF (pg/mL)	7.44±1.91	8.00±2.01	0.825	0.413
VEGF/PEDF	7.1±2.14	5.9±2.66	1.443	0.155
Diabetics without retinopathy				
Good glycemic control (n=12)		Poor glycemic control (n=38)		
VEGF (µg/ml)	38.3±14.44	36.1±5.41	0.502	0.624
PEDF (pg/mL)	6.65±1.71	6.71±1.64	0.100	0.921
VEGF/PEDF	6.3±3.33	5.7±1.94	0.760	0.451

VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor

Table 5: Effect of dyslipidemia on VEGF and PEDF level in diabetics with retinopathy

Parameter	Diabetics with retinopathy		Calc. t	P-value
	Dyslipidemic (n=11)	Not dyslipidemic (n=39)		
VEGF (µg/ml)	43.09±11.89	45.6±12.53	0.603	0.550
PEDF (pg/mL)	6.91±1.86	8.15±1.95	1.879	0.066
VEGF/PEDF	6.6±2.64	6.1±2.60	0.601	0.551
Diabetics without retinopathy				
Dyslipidemic (n=23)		Not dyslipidemic (n=27)		
VEGF (µg/ml)	38.3±10.01	35.3±10.08	1.040	0.304
PEDF (pg/mL)	6.87±1.86	6.54±1.44	0.723	0.473
VEGF/PEDF	6.1±2.67	5.7±2.02	0.610	0.545

VEGF: Vascular endothelial growth factor, PEDF: Pigment epithelium-derived factor

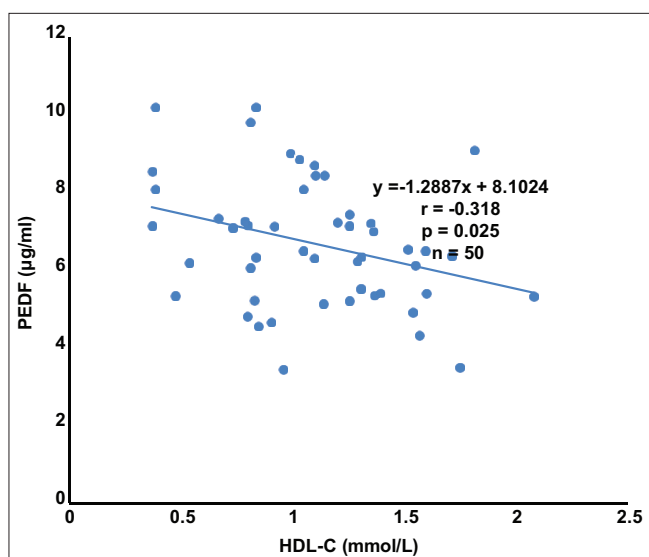


Figure 1: Correlation plot of PEDF against HDL-cholesterol in diabetics without retinopathy

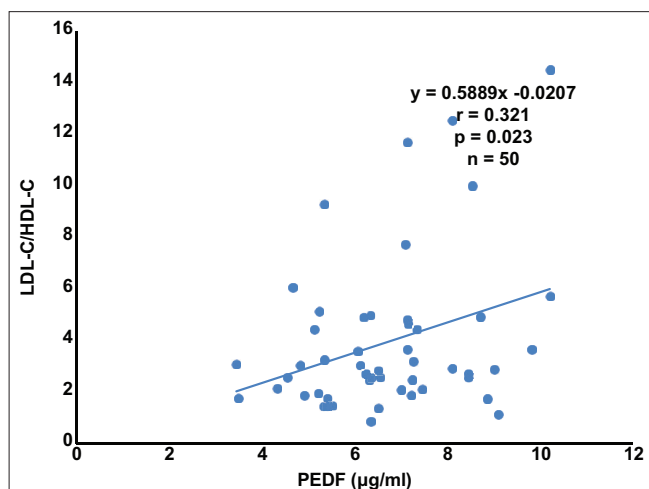


Figure 2: Correlation plot of PEDF against LDL/HDL-cholesterol in diabetics without retinopathy

cytokines, growth factors, and other molecules are released, in response to vascular damage. This stimulates angiogenesis through VEGF, which is crucial for the repair process.^[25] Another probable mechanism is that elevation of VEGF may be a reflection of endothelial cell damage which occurs in diabetics.^[26] The high levels of VEGF in these subjects may be due to the overexpression of VEGF gene in diabetic patients which induces angiogenesis so as to compensate for oxygen need induced by tissue hypoxia in this environment (the retina). A variety of conditions, including diabetes, can cause hypoxia within human tissues leading to damage in those cells.^[27] However, hypoxia can also be beneficial in human physiology and development. Changes in oxygen levels can activate or result in the expression of homeostatic regulating genes, which allows cells and tissues to survive fluctuations in environmental conditions. One of such genes is hypoxia-inducible factor 1- α (HIF1- α). The function of HIF1- α is the promotion of

angiogenesis. It directs the migration of mature endothelial cells toward hypoxic environments through HIF-1 α regulation of VEGF transcription.^[28] Pathological conditions, including diabetes, increases VEGF mRNA expression in response to tissue hypoxia. HIF-1 α is a high regulator of these hypoxia responses. HIF-1 α dimerizes with HIF-1 β and creates a stable complex that binds to hypoxia receptor elements in the promoter region of the VEGF gene.^[29] This agrees with the findings of Mahdy *et al.*^[30] who reported that VEGF was significantly increased in patients with diabetes mellitus particularly within those who had developed micro- and macrovascular complications.

The level of VEGF was significantly higher in diabetics with retinopathy than diabetics without retinopathy. Several studies have reported an increase in VEGF expression in patients with retinopathy.^[30-32] The synthesis of more VEGF observed here may be due to defective endothelial cell replication seen in diabetes mellitus which accelerates the process of retinal ischemia by exhausting the cells' replicative capacity. As the vascular cells degenerate, late structural consequences set in, including the leaking of fluid by capillaries into the adjacent retinal tissue resulting in microaneurysms and intraretinal hemorrhages.^[33] Although the endothelial cells attempt repair of the damage, by proliferating on the inner membrane, this only results in occlusion of the affected capillaries. Thus, the release of VEGF is signaled by the ischemic retina, causing neovascularization.^[34] Besides hyperglycemia, many other metabolic regulators, including reactive oxygen species, which is a common finding in diabetics, causes an increase of VEGF and its receptors. Synovial fibroblasts release prostaglandin – E2 and interleukin-1 α (IL-1 α) which upregulate the synthesis of VEGF, thus bringing about inflammation-mediated angiogenesis.^[34] Numerous growth factors, including tumor necrosis factor (TNF)- α , epidermal growth factor, TNF- β , IL-6, basic fibroblast growth factor, keratinocyte growth factor, platelet-derived growth factor, and insulin-like growth factor – I upregulate VEGF which suggests that these growth factors work in conjunction with tissue hypoxia. Mechanical forces such as shear stress and stretch, which is common in vitreomacular traction also promote VEGF production.^[35] The action of VEGF is inhibited by a factor known as PEDF. This inhibition regulates the level of VEGF which in good health is maintained at homeostatic level. PEDF is a widely expressed multifunctional glycoprotein. It displays a cytoprotective activity in several cell types, including photoreceptor cells in the eye.^[36]

In this study, the plasma PEDF levels were also significantly higher in diabetics with or without retinopathy than in the controls. The *post hoc* analysis shows that the levels of plasma PEDF in diabetics with retinopathy were significantly higher than those of diabetics without retinopathy. The high level of PEDF is produced in response to high plasma levels of VEGF – angiogenic stimulation. The high level of PEDF in patients with retinopathy performs a counter-regulatory function and is protective against damage to the vasculature by hyperglycemia

and chronic inflammation induced by VEGF due to tissue hypoxia.^[24] Zhou *et al.*^[37] reported that the levels of plasma VEGF are particularly high in patients with proliferative DR and with other microvascular complications. Some researchers have speculated that PEDF levels might be elevated to counter the increased production of VEGF by atherosclerotic environment triggered by vascular injuries. This is due to PEDF's anti-oxidative, anti-inflammatory, anti-angiogenic, and anti-thrombotic properties on vascular tissues.^[38]

The diabetics with or without retinopathy had a significantly lower ratio of VEGF/PEDF than the control group. It is reported that in normal condition, there is a balance of activity between these two systems and this balance is essential to maintain the quiescence of retinal vessels and the integrity of blood-retinal barrier.^[39] Other studies have associated higher VEGF/PEDF with development and unfavorable prognosis in patients with DR.^[40,41] This is in contrast with our findings and the reasons for this are unclear except to mention that the ratios may have decreased due to the increase in PEDF levels in both groups of diabetics.

Dyslipidemia is one of the major risk factors in diabetes mellitus and DR. Its characteristic features, namely a high plasma triglyceride, low HDL-C, and high LDL-C concentration are a commonly reported pattern in diabetics with or without retinopathy.^[42] This pattern was observed in the diabetics without retinopathy in our study as they had high LDL-C/HDL-C ratio. The cause of these three fundamental features of dyslipidemia in diabetics is reported to be the increased release of free fatty acid from insulin-resistant fat cells.^[43] The increase in free fatty acids influx into the liver with adequate glycogen store stimulates the production of triglycerides, which in turn causes the stimulation of apolipoprotein B secretion and VLDL-C. Insulin's impaired ability to inhibit the release of free fatty acid leads to enhanced VLDL-C production by the liver.^[44] However, the HDL-C level in diabetics with retinopathy was significantly higher than those without retinopathy and controls whereas LDL-C level was significantly lower than diabetics without retinopathy and an LDL-C/HDL-C comparable to that of the non-diabetics. The patients with retinopathy, in our study, were on strict dietary regimens and exercises besides various pharmacologic interventions in the management of diabetes and DR. Lifestyle changes such as dietary modifications and increased physical activity could have contributed to these favorable changes in the lipid pattern observed; as it has been shown that exercise can improve insulin sensitivity and increase levels of HDL-C, especially in people with a high baseline HDL-C level.^[45]

The LDL-C/HDL-C ratio is regarded as highly predictive in evaluating atherosclerotic risk. From our study, it was observed that the diabetics without retinopathy had higher LDL-C/HDL-C ratio and therefore higher atherosclerotic risk. About 46% of diabetics without retinopathy were dyslipidemic compared to 16% of diabetics with retinopathy who had

dyslipidemia. However, there were no significant differences in the levels of VEGF and PEDF or the VEGF/PEDF ratio between the patients who were dyslipidemic and those who were not in the diabetics without retinopathy. However, the significant negative correlation between PEDF and HDL-C and a significant positive correlation between PEDF and LDL-C/HDL-C suggests that increasing PEDF levels are associated with higher atherogenic risk.

The highest priority for diabetic individuals is to achieve good glycemic control with the expectation that this approach will reduce the progression of diabetic complications.^[11] In our study, more than 75% of both diabetic groups had poor control. This may be attributed to poor compliance to drug or dietary regimen, most likely due to poverty, forgetfulness, side effects, perceived non-effectiveness, and pill burden.^[46] Abdulazeez *et al.*^[46] reported in their study that 73.6% of the diabetic patients were non-compliant to their drug regimen. It is higher than 46% reported in Benin City and 62% in Umuahia which are other parts of Nigeria.^[47,48] Despite this, there were no significant differences in levels of VEGF and PEDF or the VEGF/PEDF ratio as the levels of these parameters were comparable in those with poor and good glycemic control in both diabetic groups. Hence, despite good glycemic control, levels of PEDF and VEGF were still high in both diabetics with retinopathy and those without retinopathy. These findings disagree with those of Cavusoglu *et al.*,^[31] who reported that poor glycemic control caused an increase in plasma VEGF levels. This observation suggests that once ischemia sets in as a result of hyperglycemia, there are interplays of other metabolic pathways that result in the activation of VEGF and PEDF even with good glycemic control. More studies have shown that continuous oxidative damage is experienced by the retina even in tight glycemic control and oxidative stress is important in the pathogenesis of DR.^[49] As such, adjunct therapy targeting these molecules should also be employed besides the treatment of dyslipidemia and hyperglycemia. This may explain why some diabetics may still develop DR even with good glycemic control. A limitation of this study is the small number of subjects used.

Conclusion

DR is associated with higher levels of VEGF and PEDF which suggests that the two contributing systems, angiogenic stimulator (VEGF) and angiogenic inhibitor (PEDF) play important role in DR possibly to maintain the quiescence of retinal vasculature and integrity of the blood-retinal barrier. Good glycemic control and dyslipidemia seem not to have a profound effect on VEGF and PEDF levels in diabetics with or without DR. Higher PEDF levels are associated with higher atherogenic risk in the diabetics with retinopathy. Hence, early screening of patients to detect diabetes and prevents its complications remain the best course of action. These patients may also benefit from adjunct therapy with anti-VEGF drugs and PEDF.

Authors' Declaration Statements

Ethics approval and consent to participate

Ethical permission was obtained from the National Eye Centre ethics and Research Committee (NECEC/PATH/20170015). Written informed consent was also obtained from each subject before being enrolled in the study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, IEB, upon reasonable request.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding statement

Nil.

Authors' Contributions

PJU: Conceptualization, data curation, investigation, methodology, project administration, writing – original draft preparation. IEB: Formal analysis, methodology, writing – original draft preparation, writing – review and editing. MHE: Conceptualization, supervision, methodology, validation, writing – review and editing. AEU: Conceptualization, supervision, methodology, validation, writing – review and editing. MBA: Conceptualization, writing – review and editing. UOA: Writing – original draft preparation, writing – review and editing.

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