ORIGINAL ARTICLE

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

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ABSTRACT

Background: Vascular Endothelial Growth Factor (VEGF), originally known as vascular permeability factor (VPF), is a signal protein produced by cells that stimulates the formation of blood vessels. To be specific, it's a sub-family of growth factors. They are important signaling proteins involved in both vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus.
Aim: To explore the relevance of VEGF level with complication of diabetes mellitus (diabetic retinopathy (DR) and diabetic nephropathy (DN)).
Result we have confirmed previous observations of increased plasma VEGF Levels in patients with diabetic retinopathy and nephropathy. A total 103 quality control subjects divided to 4 groups (diabetics with DR, diabetics with DN diabetics without DR or DN and health individual) were observed to determine the relationship between serum of VEGF level and diabetes complication DR and DN.
Conclusion: High level of VEGF was detected among DM patients with nephropathy and retinopathy, suggesting it may plays a major role in the pathogenesis of these complications.
Keywords; Naphropathy, retinopathy, diabetes type 2, vasculogenesis

INTRODUCTION

Diabetes mellitus (DM) commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time[1].

If left untreated, diabetes can cause many complications[2]. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death[3]. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment[2,4]

Complications of Diabetes mellitus (DM) 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. **One of these complications:**

Diabetic retinopathy (DR)is a common micro vascular complication of diabetes and is the leading cause of blindness in adults aged 20–74 years in developed countries [5][6]. It occurs in more than 60% of patients with type 2 diabetes mellitus (T2DM)it's any damage to the retina of the eyes, which may cause vision impairment [7]. Retinopathy often refers to retinal vascular disease, or damage to the retina caused by abnormal blood flow. . Frequently, it's an ocular manifestation of systemic disease as seen in diabetes or hypertension [8]. The development of retinopathy can be broken down into proliferative and non-proliferative types. Both types cause disease by altering the normal 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[9].

Also known as diabetic kidney disease[10] is the chronic loss of kidney function occurring in those with diabetes mellitus. Protein loss in the urine due to damage to the glomeruli may become massive, and cause a low serum albumin with resulting generalized body swelling (edema) and result in the nephrotic syndrome. The estimated glomerular filtration rate (eGFR) may progressively fall from a normal of over 90 ml/min/1.73m2 to less than 15, at which point the patient is said to have end-stage kidney disease (ESKD)[11]. It usually is slowly progressive over years[12].

Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis and has been investigated as a candidate gene in a number of conditions, including diabetes and its micro vascular complications (e.g., retinopathy and nephropathy). It's a substance made by cells that stimulates the formation of new blood vessels, a process called angiogenesis. VEGF also acts as a mitogen for vascular endothelial (vessel lining) cells, stimulating these cells to divide and multiply. It can stimulate angiogenesis, enhance collateral vessel formation, and increase the permeability of the microvasculature [13][14]Diabetic micro vascular changes in the retina lead to hypoxia, which stimulates production of VEGF, a multifunctional cytokine that promotes angiogenesis and is a potent mediator of micro vascular permeability[15].VEGF is believed to play a significant role in the development of DR by inducing hyper permeability of retinal vessels, breakdown of the blood–retinal barrier and neovascularization[16][17]Complications can arise as a result

of abnormal barrier function of new vessels, leading to intraregional hemorrhage andexudation. New blood vessels have increased fragility leading to sudden severe loss of vision due to vitreous hemorrhage.

VEGF is implicated in the development of diabetic nephropathy because neutralization with anti-VEGF antibodies in experimental models significantly reduces hyper filtration, albuminuria, and glomerular hypertrophy[18,19]. VEGF is a secreted mitogen highly specific for vascular endothelial cell which have been implicated in endothelial cell proliferation and migration.

At difference from other tissues that cease expressing VEGF-A at the completion 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 2 (VEGFR1 and VEGFR2), and co-receptors neuropilin 1 and 2 (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 abundant in endothelial cells but they are expressed by multiple cells, including podocytes and tubular cells Hypoxia and high glucose up regulate podocyte VEGF-A protein expression[23].

In addition, genetic variations in the VEGF gene might lead to 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 in 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 highest prevalence of DM in the world. Seven countries in this region have a high prevalence of DM and a further seven countries (including Sudan) have a medium prevalence (9–12%) of DM[25]. Type 2 diabetes mellitus (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].

SUBJECTS AND METHODS

This study was carried out in Khartoum-Sudan from August, 2018 to August 2020. This case-control study enrolled 103 subjects divide to 4 groups. The 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 (DM) without (DR) and (DN) which attended from ZENAM hospital. The fourth group health individual

The practical side of the study was performed at the laboratory of biochemistry department in College of medical laboratory in ALNILEEIN, ALRIBAT, national university and Selma center of kidney disease, each one submit to Full medical examination which include

- 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)
- ELISA KITS which include serum level of VEGF in all groups under certain condition

Methods Laboratory investigations:

Sample collection: 3 milliliters of blood were collected in 1 tubes, sterile plain vacationer tube (3 ml) was centrifuged, and the serum was stored at–20 °C to measure VEGF level by using ELISA kits E-EL-H0111 96T

ELISA KITS:

A hundred micro preferred or sample changed into introduced to each properly. Incubate for 90 minutes at 37c

-eliminated the liquid, one hundred micro of biotinylated detection AB/AG become delivered, and incubated for 1 hour at 37c

-aspirated and washed for three instances

- 100 micro of HRPconjugate become delivered. Incubated for 30 minutes at 37c

-aspirated and washed for 5 times

- Ninety micro of substrate reagent turned into delivered; incubate for 15 mints at 37 c

- 50 micro of prevent answer decide turned into introduced to the OD value at 450nm right away-the end result became calculated.

Statistical Analysis Methodology: Data was examined using statistical package of social science (IBM SPSS version 20.0) for windows software package. A P value of ≤ 0.05 was interpreted as statistically significant. Comparison of groups was done by Welch test and the post hoc was done using Games-Howell Test. Correlation between quantitative variables was done by Spearman's rank correlation test.

RESULTS

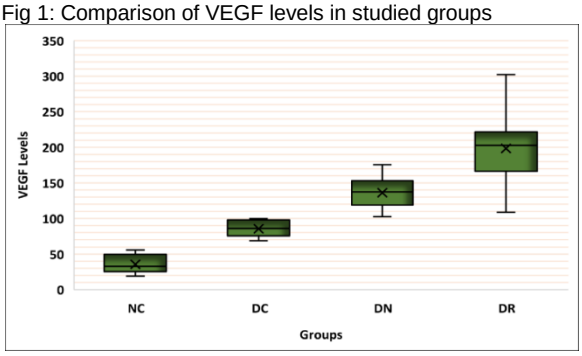
Descriptive: The Present of study including a total of 103 subjects categorized into four groups 26 each Subject NC, DC, DR and DN. Regarding the gender in the present study there was 46.2%, 42.30%, 57.7% and 53.8% males, and 53.8%, 57.7%, 42.3% and 46.2% females in NC, DC, DR and DN groups respectively.

The age mean for the NC group was 65.88±2.61, the DC group was 64.32±2.50, the 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.

VEGF Level between four groups : There was a significant difference within all four groups (Welch test, P Value <0.001). Likewise, a significant difference was discovered between groups (Games-Howell post-hoc Test), (NC-DC. P value <0.001) (NC-DR P value <0.001), (NC-DN P value <0.001), (DC-DR P value <0.001), (DC-DN P value <0.001) (DR-DN P value = 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

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 ± Std	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

Fig 1: Comparison of VEGF levels in studied groups



DISCUSSION

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

In this study, we have confirmed previous observations of increased plasma VEGF levels in diabetic patients, as well as previous observations of higher plasma VEGF levels in patients with retinopathy and nephropathy. In DR may progress through several stages, including early non-proliferative diabetic retinopathy (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 lead to fibro vascular changes, vitreoretinal traction and retinal detachment, eventually resulting in blindness [30, 31, 32]. In the retina VEGF is produced by multiple cell types, Including 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 micro albuminuria and eventually proteinuria. Synergistic effects of hyperglycemia and increased VEGF-A in diabetic glomerulopathy may be explained by the unique hypothesis of "uncoupling of VEGF-A with nitric oxide (NO)"[36,37]. Normally, VEGF-A stimulates endothelial NO release, and NO is required for the actions 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, leading to diabetic glomerulopathy.

The results of our study are consistence with the study of Faten, et al. (2010)[38] and Mahdy et al[39] 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. [40].Suggested that a "normal" level of VEGF-A is essential for maintaining the glomerular capillary structure, including the glomerular filtration barrier in the adult kidneys, and both too much and too little VEGF-A in glomeruli can lead to significant renal pathology. VEGF is 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 is sufficient evidence for the treatment of DR with anti-VEGF treatment (American Academy of ophthalmology[41]. further, the American Academy of Ophthalmology (AAO) recognize exercise method committee now stated that there is sufficient guide for the treatment of DR with anti-VEGF treatment (American Academy of ophthalmology 2017).

The reality that the excess expression of VEGF-A in podocytes associated with hyperglycemia command to special glomerular alterations provides the rationale for anti-VEGF therapy against diabetic nephropathy. The trial detect that the management of neutralizing monoclonal anti-VEGF antibodies to type 1 and type 2 diabetic relief the albuminuria and glomerular hypertrophy[42,43], mentioning the activity of anti-VEGF therapy against diabetic nephropathy. Then, SU5416, a pan-VEGF receptor tyrosine kinase inhibitor, was also conveying to minimize albuminuria in type 2 diabetic[44].

CONCLUSIONS

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

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