Asma'a H. Mohamed et al /J. Pharm. Sci. & Res. Vol. 11(1), 2019, 247-250

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of Vascular Endothelial Growth Factor level of diabetic peripheral neuropathy patients in Babylon Province

Asma'a H. Mohamed[1], Haider K. Zaidan[2]

[2]Departement of scholarships and Cultural Relations, Ministry of Higher Education and Scientific Research, Baghdad-Iraq,[1] Department of Biology-College of Science-University of Babylon/Iraq

Abstract

Diabetic peripheral neuropathy (DPN) or nerve damage evolve from chronically hyperglycemia can be one of the most complicated and debilitating complexity of diabetes because of the severe consequences such as pain, discomfort, and disability. Vascular endothelial growth factor (VEGF) is a growth factor responsible for angiogenesis and play an important role in neurodegeneration and neuroregeneration in diabetic peripheral neuropathy. The aim of this study was to point out the role of VEGF in diabetic peripheral neuropathy and study the correlation with other factors. A significant increases ($P \leq 0.01$) has been shown in VEGF levels in type2 patients with DPN compared with type2 patients without DPN and healthy control subjects with significant positive correlation have been shown between VEGF with fasting blood glucose (FBG) and HbA_{1c} in both type2 patients with and without DPN. Conclusions: Increased vascular endothelial growth factor mean levels in diabetic peripheral neuropathy patients were significantly correlated with hypoxia and nerve supply blood vessels damage.

Keywords: Diabetic neuropathy, Neurotrophic factor, VEGF, VEGF receptors

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is a term which set out the damage of peripheral nerve. There are numerous different conditions can cause diabetic peripheral neuropathy and the most common one is diabetes. The symptoms of DPN rely on which of nerves are damaged (sensory, motor, or autonomic nerves) (1). DPN in diabetics is a nerve injury caused by chronically hyperglycemia it leads to numbness, loss of sensation, and pain in feet, legs, and hands(2) Nearly 60-70% of all diabetic patients ultimately develop peripheral neuropathy, but not all be in pain. Because the nerve damage is reversible, patients with diabetes can minimize their risk of developing nerve damage by holding their blood sugar levels near to normal (3).

The neurotrophic gene family constitute four structurally linked basic proteins, nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and brain-derived neurotrophic factor (BDNF). Although they have high structural homology, these neural proteins operate through different high affinity membrane receptors, differ in target specificity, patterns of spatial and temporal distribution, and responses to injury of the neural tissue(4).

Vascular endothelial growth factor (VEGF) is a family of many types of growth factors with high specificity for endothelial cells . VEGF has recently been the factor of interest as a result of its effects on neurons and glia . In 1983, VEGF was discovered as a promoter of angiogenesis (5), but other studies has implicated that VEGF as a potential therapeutic in nervous system disorders such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, strocke, andperipheral diabetic neuropathy(6) .

Two types of receptors mediate the actions of VEGF these include VEGER-1 (Flt-1) and VEGFR-2 (Flk-2) both are tyrosine kinases receptors. Despite VEGFR-2 transduces intracellular signals in response to VEGF binding, but VEGFR-2 receptors don't seem to function in signal transduction but act to modulate free VEGF levels(7). The expression of these receptors is reduced under normal conditions but when there is tissue hypoxia causes over-expression of these receptors and increased VEGF levels. The proliferation and migration of endothelial cells result in angiogenesis and development of collateral circulation in hypoxic tissues(8) .

Recent studies indicated that during the development of the peripheral nervous system, VEGF induce Schwann cell proliferation and axonal growth, promotes glial cells growth, neurogenesis and has a neuroprotective effect, also there is evidence that VEGF guide neuronal migration in the embryonic brain and stimulates the growth of vascular and axonal epidermal network(9), but these actions are blocked by inhibition of VEGFR-2 which is expressed in dorsal root ganglion and Schwann cells(10). It has been suggested that the survival and migratory effects of VEGF may counteracts Schwann cell loss due to nerve injury and may enable newly formed Schwann cells to migrate to, and ensheathe, nerve axons thereby promoting nerve regeneration(11).

The major risk factors that causes alteration in VEGF expression in heart and peripheral nervous system are diabetes mellitus, high cholesterol (12), and age(13). VEGF gene therapy or VEGF injections are able to stimulates angiogenesis processes and prevention of ischemia-induced tissue necrosis(14). In case of Schwann cells hypoxia, the transfer of DNA encoding VEGF in animal experiments had promising results on peripheral nerves(15, 16). In order to know the ischemic nature of diabetic peripheral neuropathy and the need to restore circulation at microcirculation level, VEGF is the subject of resent studies regarding microvascular complications of diabetes especially diabetic peripheral neuropathy(17, 18).

MATERIALS AND METHODS

The current study comprises study of profile of diabetic peripheral neuropathy in 30 patients of DPN compared with 30 patients without DPN and with 30 healthy control subjects. The study was conducted between May 2017-September 2017 in Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq, some of studies were done in Babylon University-College of Science laboratories. These cases include patients of both sex and different age group ranging from (35-65 year) and they had varying duration of diabetes mellitus. Diagnosis of peripheral neuropathy was made by symptoms, signs and the detailed history was taken in each case. The patients were specially questioned to rule out factors which may cause peripheral neuropathy such as peripheral vascular disease, spinal injury or trauma, increased or decreased thyroid stimulating hormone TSH, anemia, tumor, and alcoholism. Venous blood samples were collected from fasting patients and control subjects after a period of fasting 8-10 hours(19) by vein puncture using 5ml disposable syringes, one ml was placed into EDTA tubes and the remaining 4ml pushed slowly into disposable tubes containing separating gel .

Blood in EDTA tubes was mixed gently for 3 minutes and then being used in hematological tests and especially for HbA_{1c} assay, while blood in the gel containing tubes was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 2000rpm for approximately 10-15 minutes (20) and then the sera were obtained and stores at -20 ºC until analysis for hormonal assay .The levels of human serum FBGand VEGF were calculated by using manual ELISA kits.

RESULTS AND DISCUSSION

The statistical analysis of this study shows (Table:1) that the mean VEGF level in type2 patients with DPN (311.78±1.47) was significantly higher than that of type2 patients without DPN (290.28±1.65) and also higher than healthy control (303.01±1.80)(P≤0.05), and the mean VEGF levels in type2 patients without DPN (290.28±1.65) was significantly lower than that in healthy controls (303.01±1.80)(P≤0.05). No significant differences was found for age in all three groups, whereas there was significant differences in mean duration among the three groups also significant differences have been found in FBG and HbA_{1c} among the type2 patients with and without DPN and healthy controls.

The correlation analysis showed significant positive correlation (P≤0.01) between VEGF with FBG and HbA_{1c} in type2 diabetic patients with DPN as shown in (figures 1,2). Similarly, the correlation results revealed a significant positive correlation (P≤0.05) with FBG and (P≤0.01) with HbA_{1c} in type2 diabetic patients without DPN as shown in (figures 3,4).

Table (1): Basic parameters in type2 patients with and without diabetic peripheral neuropathy, and control subjects.

	Patients with DPN(n = 30)	Patients without DPN(n = 30)	Healthy control(n = 30)	P Value
Age (Years)	51.73±8.04	50.76±9.64	49.06±10.06	--------
FBG (mmol/l)	12.37±1.57	10.63±0.26	5.81±0.29	*
$HbA_{1c\%}$	9.92±0.96	8.45±0.03	4.89±0.26	**
BMI (kg/m^2)	27.44±2.57	27.48±0.69	27.58±2.22	--------
VEGF (Pg/ml)	311.78±1.47	290.28±1.65	303.01±1.80	**
Duration of diabetes	12.33±7.64	7.76±6.95	---------	*

FBG: Fasting blood glucose. HbA_{1c}: Glycated hemoglobin, BMI: Body mass index, VEGF: Vascular endothelial growth factor.
The data expressed as the Mean ± Standard Deviation
*P significant at P≤0.05
**P significant at P≤0.01

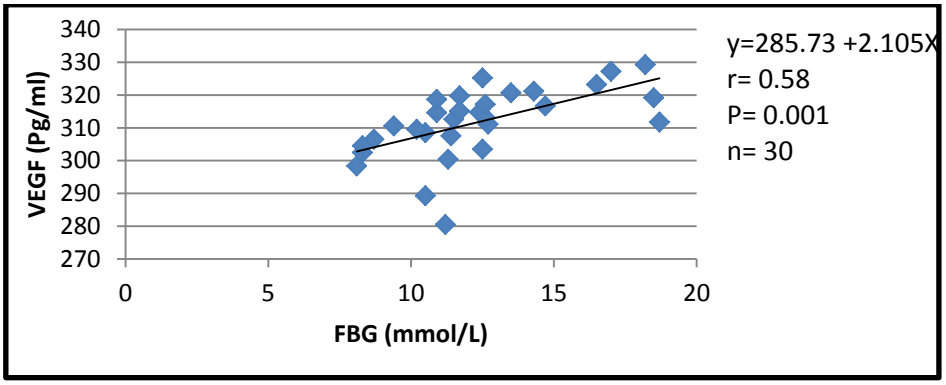


Figure (1): Relationship between VEGF (Pg/ml) and FBG (mmol/L) in type2 diabetic patients with peripheral neuropathy.

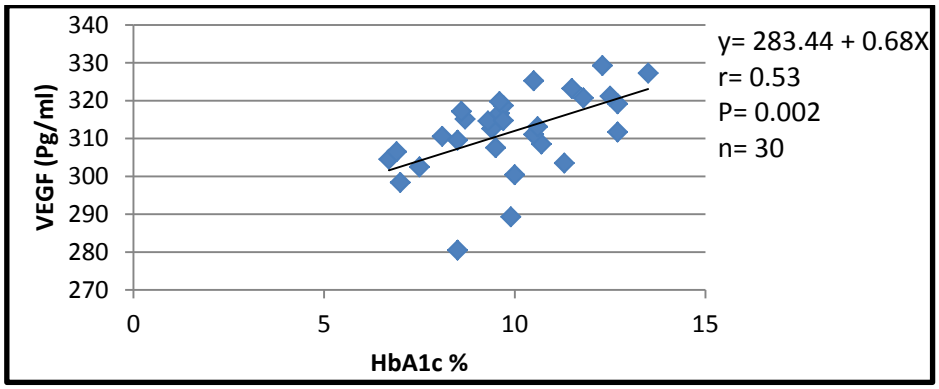
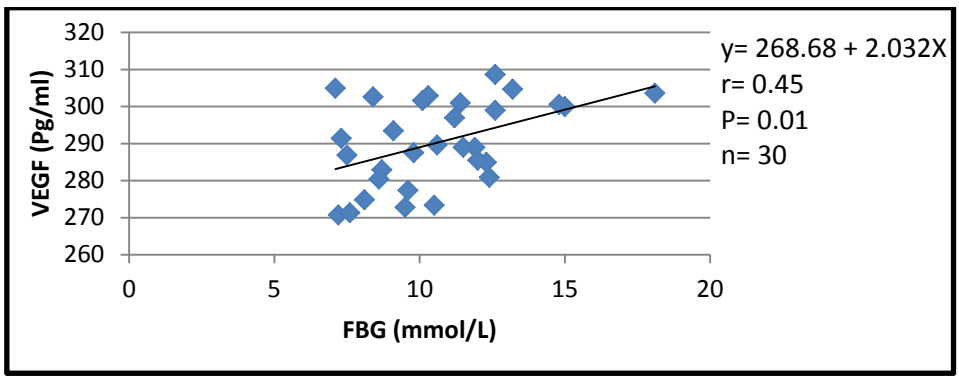


Figure (2): Relationship between VEGF (Pg/ml) and HbA_{1c} % in type2 diabetic patients with peripheral neuropathy.



. Figure (3): Relationship between VEGF (Pg/ml) and FBG (mmol/L) in type2 diabetic patients without peripheral neuropathy.

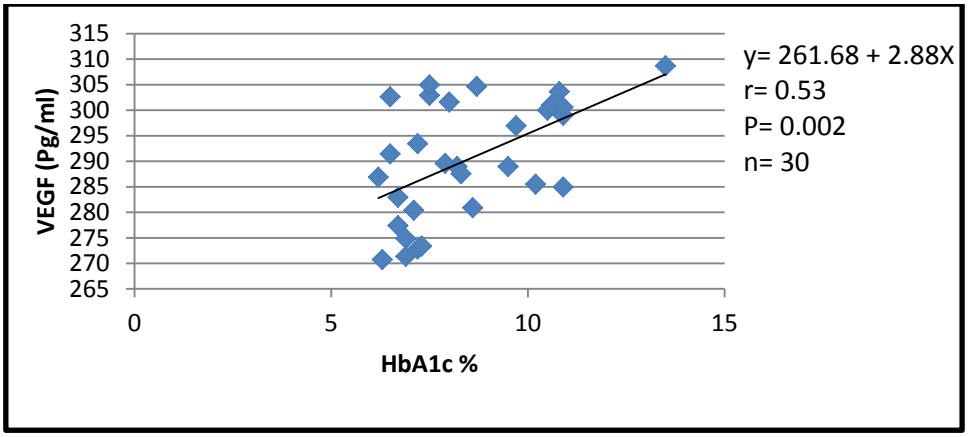


Figure (4): Relationship between VEGF (Pg/ml) and HbA_{1c} % in type2 diabetic patients without peripheral neuropathy.

The results of this study showed that the levels of FBG and HbA_{1c} were higher in type2 patients with DPN than in control groups, this study agrees with numerous previous studies (21,22, 23,24) as the hyperglycemia be the main risk factor that participate in the progression of DPN by the accumulation of intracellular sugar and then induction the generation of glycating sugars and advanced glycation end products, enhanced oxidative damage, and activation of protein kinase C(25, 26,27).

The serum VEGF levels shown to be higher in type2 patients with DPN compared with control groups, this findings agree with Dorle*et al*., 2002 where they found that the acute hyperglycemia is greatly associated with an elevation in serum VEGF in human. In humans the concentration of VEGF is tightly associated with the levels of hypoglycemia.Moreover,the studies suggested that VEGF release during hypoglycemia is positively correlated to keeping of neurocognitive function(28).

According to the duration of diabetes, the DPN patients have longer duration of diabetes than patients without DPN, this study agree with (29, 30) where they demonstrated that the longer duration of diabetes is the major risk factor for the pathogenesis of DPN. A positive correlation has been shown between the prevalence of diabetic peripheral neuropathy and duration of diabetes (31,32). Edward's *et al*., 2008 they proposed that these positive correlation is associated with poorer glycemic control, accumulation of injurious effects of metabolic control on peripheral nerves, acute atherosclerosis which eventually results in Microvascular insufficiency and enhanced neuropathy (33,34,35).

In conclusion, The serum VEGF level was higher in type2 patients with DPN than in control groups, this findings suggested that diabetes and the symptoms of DPN both affected by the serum VEGF level. A positive significant correlation has been shown between duration of diabetes and progression of type2 DPN. An inverse correlation has been shown between duration of diabetes and the serum levels of VEGF, where a higher serum levels of VEGF showed in newly diagnosed patients with DPN, these findings suggest that the duration of diabetes may influence the VEGF levels, indicating that the duration of diabetes may increase the prevalence of DPN in patients with type2 diabetes mellitus.

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