INTRODUCTION

Diabetes mellitus is a chronic disorder characterized by the impaired metabolism of glucose due to insulin deficiency or its resistance, leading to hyperglycemia and late development of vascular and neuropathic complications. It is of two types: type 1 primarily caused by autoimmune pancreatic b-cell destruction and characterized by absolute insulin deficiency, and type 2 characterized by insulin resistance and relative insulin deficiency ***(Alghadyan, 2011).***

The prevalence of diabetes in Egypt was estimated to be 7.3 million in 2011 and is estimated to be 12.4 million by the year 2030 ***(IDF Diabetes Atlas 5th edition, 2012).***

Diabetic retinopathy (DR) is one of the commonest and easily demonstrable examples of microvascular damage that diabetes inflicts throughout the body. DR is among the leading causes of blindness in people of working age, affecting both the genders equally**.** Patients with type 1diabetes may show evidence of retinopathy as early as 5 years after the onset of diabetes, and almost all patients will show varying degrees of retinopathy 20 years after the onset of diabetes ***(Shah, 2008).***

Risk factors of diabetic retinopathy include duration of diabetes (most important risk factor in patients diagnosed before 30), poor glycemic control, pregnancy, hypertension, nephropathy, hyperlipid-aemia, smoking, cataract surgery, obesity and anaemia ***(Kanski et al., 2011).***

Diabetic Retinopathy Disease severity scale ranges from no apparent retinopathy, non-proliferative retinopathy and proliferative diabetic retinopathy. Non-proliferative diabetic retinopathy is further subdivided into mild (microaneurysms only), moderate and severe (presence of >20 intraretinal haemorrhages in each quadrant, two quadrants of venous beading, or one quadrant of prominent intraretinal microvascular abnormalities (IRMA). Proliferative diabetic retinopathy (PDR): one or more of: neovascularization (i.e. of the iris, angle, optic disc, or elsewhere), or vitreous/preretinal haemorrhage ***(Ehler et al., 2008).***

Maculopathy is a disease of macula and can accompany any stage of DR including background retinopathy. Maculopathy is a serious condition and may affect central vision. It is characterized by macular edema and ischemic maculopathy. Macular edema is due to extravasation of plasma proteins due to damage of blood-retinal barrier ***(Shah, 2008).***

Proliferative diabetic retinopathy (PDR) is a leading cause of blindness and visual impairment among adults aged <40 years in the developed world. Diabetic macular oedema (DME), another important event that occurs in the setting of diabetic retinopathy (DR), is more frequent in type 2 than type 1 diabetes. Although DME does not cause total blindness, it frequently leads to a severe loss of central vision. Because of the high prevalence of type 2 diabetes, DME is the main cause of visual impairment in diabetic patients. In addition, DME is

almost invariably present when PDR is detected in type 2 diabetes ***(Simó & Hernández, 2008).***

Our understanding of the pathophysiology of both proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) is increasing as new biochemical pathways are identified. Hypoxia-driven angiogenesis is a crucial pathway in the development of PDR, whereas the leakage of plasma from the small blood vessels in the macula following the disruption of the tight junctions of the blood–retinal barrier is the main factor responsible for DME. Vascular endothelial growth factor (VEGF) plays an essential role in the development of both PDR and DMO ***(Caldwell et al., 2003).***

Vascular Endothelial Growth Factor (VEGF) is a pluripotent growth factor that functions as an endothelial cell-specific mitogen and vasopermeability factor and through these mechanisms the VEGF plays a critical role in promoting angiogenesis and vascular leakage ***(Grant et al., 2004).*** Three decades of intense research has uncovered the detailed biochemistry of VEGF and its receptors. More than just a single molecule, VEGF is actually several isomers that segregate into 5 distinct subgroups— VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor—with VEGFA emerging as the key regulator of both physiologic and pathologic angiogenesis and with VEGF165, the most common isoform (molecular weight of 30 kD), being the most important for angiogenesis ***(Stewart, 2012).***

In diabetic retinopathy, the impairment of the blood retinal barrier and the increased permeability are responsible for the diabetic macular edema and several investigations underline the active role of vascular endothelial growth factor. By disrupting the intercellular tight junctions between the retinal endothelial cells, vascular endothelial growth factor increases the extracellular accumulation of fluid from the intravascular compartment ***(Gardner et al., 2002)***. Moreover, vascular endothelial growth factor shows a role in mediating active intraocular neovascularization. In essence, vascular endothelial growth factor is an attractive candidate as therapeutic target of pharmacological treatment in the management of DR ***(Iacono et al., 2010).***

There are 4 major anti-VEGF agents that have been evaluated in treating DR: pegaptanib sodium (Macugen), ranibizumab (Lucentis), bevacizumab intravitreal injection (Avastin), and VEGF Trap-Eye (VTE; aflibercept), Ranibizumab is the only FDA approved drug for diabetic macular edema ***(Allen et al., 2012).***

Pegaptanib *(Macugen, OSI/Eyetech, Melville, NY, USA)* is a pegylated aptamer that targets the VEGF165 isoform. It has been shown to inhibit VEGF's endothelial mitogen activity and its vascular permeability effects ***(Ishida et al., 2003 and Bell et al., 1999).***

The US Food and Drug Administration (FDA) has approved Macugen for the treatment of neovascular AMD. The VEGF Inhibition Study in Ocular Neovascularization (VISION)

trial established its safety and efficacy in neovascular AMD ***(Gragoudas et al., 2004).***

Ranibizumab *(Lucentis, Genentech, Inc., South San Francisco, CA, USA)* is a recombinant, humanized antibody fragment that binds all isoforms of VEGF. Ranibizumab was approved by the FDA in June 2006 for the treatment of all subtypes of neovascular AMD and was approved in 2010 for the treatment of macular edema associated with retinal vein occlusion and in 2012 for diabetic macular edema ***(Allen et al., 2012).***

Bevacizumab *(Avastin, Genentech, Inc.)* is a recombinant, full-length, humanized antibody that also binds all VEGF isoforms.

Avastin is used on an off-label basis for a variety of ophthalmic conditions. Large clinical trials of Avastin are currently underway for AMD, DME, and vein occlusions, but the safety and efficacy of Avastin for intraocular use remains to be demonstrated ***(Nicholson and Schachat, 2010).***

VEGF Trap-Eye, also known as aflibercept, is the most recent anti-VEGF agent approved by the FDA, in 2011, for the treatment of neovascular AMD.11 VEGF Trap-Eye is a 115-kDa recombinant fusion protein consisting of the VEGF binding domains of the human VEGF receptors 1 and 2 fused to the Fc domain of human immunoglobulin G1. VEGF Trap-Eye

competitively inhibits VEGF and binds placental growth factors 1 and 2 ***(Allen et al., 2012).***

The safety up to date data for these four medications is reassuring, although a large prospective trial with methodical surveillance of adverse events is still lacking for bevacizumab ***(Nicholson and Schachat, 2010).***

AIM OF THE WORK

This review of literature aims to elicit the effect and drawbacks of anti VEGF in diabetic retinopathy.

Chapter 1

VASCULAR ENDOTHELIAL GROWTH FACTOR

Angiogenesis and the Eye:

Concerning the eye, the angiogenic process has to be considered as a pathologic phenomenon. There are actually several conditions leading to the formation of abnormal neovascularization. Age-related macular degeneration is one of the most important diseases characterized by the formation of choroidal new vessel in the macular region finally leading; if untreated, to vision loss. The other major disease characterized by abnormal formation of retinal vessels is diabetic retinopathy, in particular the so-called proliferative stage of this disease ***(Tremolada et al., 2012).***

Vascular endothelial growth factor is involved not only in angiogenesis but also in vasculogenesis ***(Folkman and D'more, 1996)***. It interacts with specific tyrosine kinase receptors, receptor 1/fms like tyrosine liver kinase (VEGF-RI/flt1) and receptor 2/fetal liver kinase (VEGFRII/flk), stimulating receptor autophosphorylation, and Endothelial Cell (EC) replication and migration. Mice deficient in VEGF have impaired angiogenesis and vasculogenesis, and die by day 9 of gestation ***(Ferrara et al., 1996)***.

Regarding both retina and choroid, vascular endothelial growth factor (VEGF) was shown to be a major contributor to angiogenesis by increasing the number of new capillaries ***(Tremolada et al., 2012).***

VEGF concentration levels, in particular, were found to be significantly increased in ocular tissues from patients with diabetes ***(Lutty et al., 1996)***.

Intravitreal injection of VEGF into normal primate eyes induced the same pathologic processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability. Levels of VEGF in the vitreous highly correlated with growth of new vessels and blockage of VEGF has been associated with the inhibition of iris neovascularization and suppression of retinal new vessel formation in primates ***(Shoeir et al., 2009).***

These findings raised the question of the potential role of VEGF in the pathogenesis of DR ***(Tremolada et al., 2012).***

Vascular Endothelial Growth Factor (VEGF)

History and Identification of VEGF:

In ***1948, Michaelson*** proposed that a diffusible angiogenic "factor X" produced by the retina is responsible for retinal and iris neovascularization that occurs in proliferative diabetic retinopathy and other retinal disorders, such as central retinal vein occlusion ***(Michaelson, 1948; Ferrara, 2004).***

In ***1983, Senger et al.*** described the partial purification from the conditioned medium of a guinea-pig tumor cell line of a protein able to induce vascular leakage in the skin, which was named "tumor vascular permeability factor" (VPF) ***(Senger et al., 1983; Ferrara, 2004).***

In June ***1989, Ferrara and Henzel*** reported the isolation of a diffusible endothelial cell-specific mitogen from a conditioned medium of bovine pituitary folliculostellate cells, which they named VEGF, to reflect the restricted target cell specificity of this molecule. NH2-terminal amino acid sequencing of purified VEGF proved that this protein was distinct from the known endothelial cell mitogens such as Fibroblast Growth Factor -2(FGF-2) and indeed did not match any known protein in the available databases ***(Ferrara and Henzel, 1989).*** Subsequently, ***Connolly et al. (1989)*** reported the isolation and sequencing of human VPF from a hepatocarcinoma cell line ***(Connolly et al., 1989).***

In December 1989, both studies described the complete complementary DNA sequences encoding VEGF and VPF ***(Kech et al., 1989; Leung et al., 1989)*** and these turned out to be identical ***(Ribatti, 2004).***

The finding that VEGF is potent, diffusible, and specific for vascular endothelial cells led to the hypothesis that this molecule might play a role in the regulation of physiological and pathological growth of blood vessels ***(Ferrara and Henzel, 1989; Leung et al., 1989; Ferrara et al., 1991).***

VEGF Isoforms

Three decades of intense research has uncovered the detailed biochemistry of VEGF and its receptors.

More than just a single molecule, VEGF is actually several isomers that segregate into 5 distinct subgroups: VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor ***(Ferrara et al., 2003).***

VEGFA is the emerging key regulator of both physiologic and pathologic angiogenesis. Variable splicing of the 8 exons of the VEGFA gene results in the synthesis of 6 different human isoforms: VEGF121, VEGF145, VEGF165, VEGF183, VEGF189, and VEGF20614—with VEGF165, the most common isoform (molecular weight of 30 KD), being the most important for angiogenesis ***(Stewart, 2012).***

It is a potent angiogenic stimulator, promoting proliferation, migration, proteolytic activity and capillary tube formation of endothelial cells, thus playing a crucial role in both normal and pathological angiogenesis ***(Aiello and Wong, 2000; Dvorak et al., 1995; Ferrara and Henzel, 1989).***

On the basis of these isoforms and their relative importance, distinct therapeutic strategies have developed: specific blockade of VEGF165, pan-VEGFA blockade, and pan-VEGF blockade ***(Stewart, 2012).***

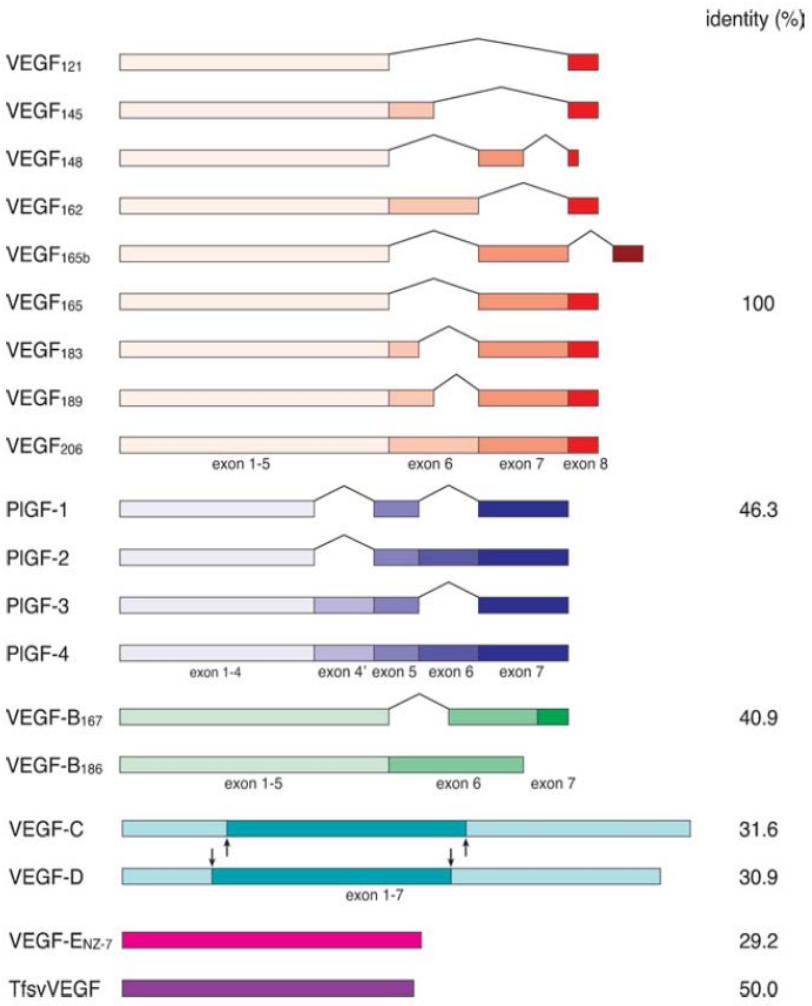

Figure (1): Comparison of structures of the VEGF family. Numbers on the right side of structures indicate identities with VEGF165 at the amino acid level. Arrows denote positions of proteolytic cleavage that give rise to mature VEGF-C or VEGF-D ***(Takahashi and Shibuya, 2005).***

VEGF Receptors:

Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1)

- **Molecular weight:**180 kDa
- **High-affinity receptor for:** VEGFA, VEGF-B, placental growth factor (PlGF) and Trimeresus flavoviridis snake venom VEGF (*Tf*svVEGF) ***(Takahashi and Shibuya,2005).***
- **Site of expression:** It is expressed in vascular endothelial cells and a range of non-endothelial cells, including ***macrophages*** and monocytes ***(Sawano et al., 2001)***, and haematopoietic stem cells ***(Hattori et al., 2002).***
- **Binding site:** The second Ig domain of VEGFR-1 is the major binding site for VEGF-A and PlGF ***(Christinger et al., 2004)*** VEGFR-1 binds VEGF-A with at least 10-fold higher affinity than VEGFR-2; however, ligand binding results in a maximal 2-fold increase in kinase activity ***(Takahashi and Shibuya, 2005).***
- **Function:**

By activating VEGFR-1, VEGF promotes assembly of endothelial cells (ECs) into tubes. The role of VEGFR-1 is context dependent. In embryos and some adult tissues, it acts as a decoy receptor that modulates angiogenesis and in some adult tissues it mediates VEGF signaling and is proangiogenic ***(Luttun et al., 2002).***

In the eye, VEGFR-1 is proangiogenic, and its inhibition can suppress retinal or choroideal neovascularization ***(Shen et al., 2006)***.

Vascular Endothelial Growth Factor Receptor-2(VEGFR-2)

- **High-affinity receptor for:** VEGFR-2 binds VEGF and proteolytically modified VEGF-C and –D ***(Kinnunen, 2009).***
- **Site of expression:** VEGFR-2 is expressed in vascular and lymphatic endothelial cells, and other cell types such as megakaryocytes and haematopoietic stem cells ***(Takahashi and Shibuya, 2005)***.
- **Function:**

VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGFA. Furthermore, recent studies have indicated that the activation of VEGFR-2 also promotes lymphangiogenesis ***(Nagy et al., 2002; Hong et al., 2004)***.

Vascular Endothelial Growth Factor Receptor-3(VEGFR-3)

- **Molecular Weight:** 195 kDa
- **High-affinity receptor for:** VEGFC and VEGF-D ***(Lohela et al., 2003).***

- **Function:**
- Stimulation of VEGFR-3 alone is sufficient to induce lymphangiogenesis.
- The stimulation of VEGFR-3 also protects the lymphatic endothelial cells from serum deprivation-induced apoptosis ***(Makinen et al., 2001).***
- A recent study has demonstrated that blockade of VEGFR-3 signalling significantly suppresses corneal dendritic cell trafficking to draining lymph nodes as well as the induction of delayed-type hypersensitivity and rejection of corneal transplants, suggesting a role for VEGFR-3 in adaptive immunity ***(Chen et al., 2004).***

Neuropilin-1 (NRP-1) and Neuropilin-2(NRP-2)

- **Molecular Weight:** 130–140 kDa cell-surface glycoprotein ***(Neufeld et al., 2002).***
- **High-affinity receptor for:**
- NRP-1 is able to bind VEGF165, VEGF-B, PlGF-2 and some VEGF-E variants.
- NRP-2 can bind VEGF145, VEGF165, PlGF-2 and VEGF-C ***(Takahashi and Shibuya, 2005).***
- **Site of expression:**
- In early development, Nrp-1 is expressed in arteries and Nrp-2 is expressed in veins ***(Herzog et al., 2001)***.