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Diabetic Retinopathy and the Role of VEGF

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Literature Review

Abstract

Diabetic retinopathy (DR) is the leading cause of blindness in the working-age population. It works by disrupting the neural and vascular components in the retina and leads to loss of neural interaction/function, vascular permeability, and angiogenesis. It can be classified into two general stages: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). DR can also lead to vascular leakage and cause diabetic macular edema (DME), the most common cause of vision loss in DR (Duh, Sun et al. 2017). There are currently a wide range of therapies, but they are limited in their efficacy and side effects. None of these therapies are as effective as early identification of the disease. Therefore, new studies are being done that focus on this aspect of DR prevention. As of now, the most promising therapy is anti-VEGF intravitreal injections. These are shown to prevent irreversible vision loss in the population of diabetics affected with proliferative diabetic retinopathy. Due to the increasing number of individuals diagnosed with diabetes worldwide, it can be predicted that DR will continue to be a leading cause of vision loss and therefore there is a significant need for new developments in the field. This literature review aims to consolidate the current knowledge on diabetic retinopathy and delve into the role of VEGF in the pathogenesis and treatment of the disease.

Introduction

Diabetic retinopathy (DR) is a microangiopathy caused by the thickening, inflammation, and leakage of the blood vessels within the retina of the eye. Microangiopathies are more commonly related to diabetes but are also seen in various other diseases, such as leukemia, glaucoma, hypertensive retinopathy, and retinoblastoma. Recently diabetic retinopathy has also been classified as a neuropathy due to neurodegenerative alterations preceding the vascular changes, such as the apoptosis of Muller cells and retinal ganglion due to a glutamate accumulation (Martins 2020). The retina has very high metabolically active tissue with a high mitochondrial population that consists of complex interactions between photoreceptors, neurons, and retinal cells. The dysfunction in this relationship causes the two main components of diabetic retinopathy: the breakdown of blood-retina barrier and neuronal impairment (Antonetti, Silva et al. 2021). DR is the most common complication of diabetes as one third of the population with diabetes mellitus presents with signs of the disease. Even though the affected population is so numerous and wide-spread, the is no reliable gold-standard treatment protocol for DR. With anti-VEGF injections gaining momentum in proliferative diabetic retinopathy therapy, more research is being done to spread that treatment efficacy into other stages of DR. Early detection of the disease is still the most effective method to preventing diabetes-related blindness. According to Nagi, by 2030 the population of patients affected with diabetic retinopathy is projected to rise from 127 million to 191 million, yet there are not nearly enough reliable methods to detect the early signs of diabetic retinopathy (Nagi 2021). The issue of delayed detection is now being targeted in various ways to save diabetic patients losing vision due to this disease. There are different technologies being investigated for accurate early detection, such as spectral-domain optical coherence tomography (SD-OCT) (Tang, Chan et al. 2021) or frequency domain optical coherence tomography (FD-OCT) (Simo, Hernandez et al. 2014) and the two-class algorithm (Nagi 2021) along with other studies to determine the

earliest signs and risk factors for DR. The role of VEGF and the therapeutic intravitreal anti-VEGF injections can be monumental in the future of DR. The toll that diabetic retinopathy has in a global public health standpoint highlights the importance of discovering better approaches for DR diagnosis and treatment than the current standards.

Results and Discussion:

Diabetic Retinopathy

In America, diabetic retinopathy is the leading cause of blindness in the 20-70 age range adults (Lee, Wong et al. 2015). In order of severity, the stages of DR are NPDR- mild, moderate, and severe- followed by PDR and diabetic macular edema (DME). DME is accumulation of fluid in the macula and is core reason for vision loss in the diabetic population. Each stage has different identifying features and management techniques. The pathophysiology of each stage, and hyperglycemia in general, can develop using multiple different molecular pathways such as, the polyol pathway, hexosamine pathway, advanced glycation end products (AGEs), and inflammation. The retina is more vulnerable to hyperglycemic states because of the regulation of glucose uptake. The sodium-independent glucose transporters (GLUTs) regulate the glucose transport. While there are fourteen GLUT receptors, GLUT1 and GLUT3 are the most expressed in the retina. GLUT1 is insulin dependent and therefore is always open to transport glucose in. This receptor is expressed in the retinal pigment epithelium, a layer of cells that maintain the photoreceptors in the retina, independent of the presence of diabetes. GLUT1 changes have been directly correlated to the pathogenesis of DR (Aragones, Rowan et al. 2020). Since retinal endothelial cells are not effective in regulating glucose uptake, it makes them highly sensitive in hyperglycemic states. As discussed above, retinal neurodegeneration is a major factor in the progression of DR and has been a key player in recent studies and developments.[7] Studying each of these pathways and their roles in each stage of DR is vital for understanding the disease and each pathway has plenty potential for future therapeutic intervention. For a high-level overview, the evolution of DR starts with neurodegeneration and the dysfunction of glial and Muller cells. It then proceeds to affect the endothelial membrane of the thin-walled retina vessels by breaking down the blood-retina barrier (BRB) irreversibly. Then we start seeing endothelial proliferation and capillary cell changes on the venous side followed by pericyte damage due to increased blood flow from the capillary dilation. The endothelial proliferation also brings on basement membrane thickening and causes capillary closure and arterio-venous shunt formation. The SV shunts and capillary close coupled with arterial autoregulation causes an increase in blood flow as well.

Technology and Imaging

The mainstream imagine methods for DR are optical coherence tomography (OCT), fundus photography, and fundus fluorescein angiography. In recent years, vitreous fluorophotometry and fluorometric determination have gained popularity as well. SD-OCT/FD-OCT and multifocal electro-retinography (mfERG) are the most sensitive technologies that are used because of their ability to sense the loss of ganglion cells which precedes the vascular changes which are sensed by most of the other technology. Fundus photography in the posterior pole relies on markers for macular thickening.[8] To clinically stage DR, fluorometric determination of

segmental retinal blood is most used. This is only useful when there is at least a minimal change in background retinopathy and the disease isn't limited to BRB disruption. The readings increase with the severity and increase in blood flow. This could be due to an increase in AV shunts, because of capillary closure, in addition to the loss of arterial autoregulation.[10]

Table 1 *Evolution of retinal vascular lesions in diabetes*

Stages	*Vitreous fluorophotometry*	*Blood flow*	*Ophthalmoscopy*	*Pathology*		
				Small vessels		*Large vessels*
				Venous side	*Arterial side*	
0.	5·7	4·3	——	?		
1. Initial	12·8	4·8	Rare aneurysm	Endoth. prol. + +	Endoth. deg. +	Vein +
				Aneurysm + +		
2. Intermediate			Numerous aneurysms haemorrhages exudate	Endoth. prol. + +	Endoth. deg. + +	Vein +
				Aneurysm + +	Focal cap. clos.	
3. Advanced	36·7	6·4	Same lesions as in stage 2	Endoth. deg. + + +		Vein +
				Aneurysm + + +		Artery +
				A-V shunts		
				Large area cap. clos.		
4. Final	300	4·2	Same lesions plus retinitis proliferans	Endoth. deg. + + + +		Vein + +
				Aneurysm + + +		Artery + + +
				Generalised cap. clos.		

Table 1: Evolution of Retinal Vascular Lesions in Diabetes
This table consolidates the different technologies used and pathologies seen in various stages of DR.

Risk Factors

Before discussing the stages and pathways for pathogenesis, it is vital to investigate identified risk factors for diabetic retinopathy. Some well understood factors include diabetes mellitus type 1 (compared to type 2), poor glycemic control, hyperlipidemia, hypertension, and pregnancy. The duration of type 1 diabetes along with the age of the patient also are directly correlated to the relative risk of the disease. Nearly all of type 1 diabetes patients that have had the disease for at least 15 years have diabetic retinopathy, compared to the 75% of type 2 patients at that same duration. [8] Glycosylated hemoglobin levels are also directly correlated with increasing severity of proliferative diabetic retinopathy specifically. Serum cholesterol levels greater than 240 mg/dL are also correlated with higher incidence of DR. Studies have seen a 34% decrease in DR with blood pressure control in hypertension patients using ACE inhibitors. There are other factors- sleep apnea, genetic mutations, fatty liver disease, smoking, and serum prolactin levels- that have a more complicated connection to DR whose relationship is still unclear. [8]

Stages

Diabetic Retinopathy is staged using fundus photography and the grading guidelines developed in 1977 by the Early Treatment Diabetic Retinopathy Study group (ETDRS) (1991). There are a total of 5 stages of diabetic retinopathy. The first three can be classified under NPDR- mild, moderate, severe. The next two are more clinically severe- PDR and DME. As a brief summary, in the NPDR phase the disease can progress from mild, moderate, to severe. During the early NPDR stage, the key features in the retinal vasculature are vascular permeability and capillary occlusion. The key pathologies that can be detected in this stage are microaneurysms, hemorrhages, and hard exudates (Wang and Lo 2018). In PDR, the key feature is neovascularization, the development on new, weak, and potentially leaky blood vessels (Kusuhara, Fukushima et al. 2018). DME can occur during any stage due to the BRB breakdown and consequent swelling and thickening of the macula which causes fluid accumulation in the sub or intra retinal space. It can cause severe vision loss in the eye and maybe even blindness. A summary of the key features and the identification methods of these features are shown in Table 1 (Cunha-Vaz 1978).

Non-Proliferative Diabetic Retinopathy

Non-Proliferative Diabetic Retinopathy (NPDR) was found in 25% of diabetic patients within 5 years of diagnosis (Koetting 2019). The hallmark of this stage is that neovascularization has not developed. The sub0stages help to identify the severity level of disease progression.

Mild

The mild stage is characterized by at least one microaneurysm, caused by loss of pericyte function, in the retinal blood vessels. In this stage, this is the only abnormality present (Koetting, 2019). DR in this stage is very difficult due to the microscopic and subtle changes. At this stage, advocating for dietary modifications and hyperglycemia management is key to prevent further progression. Educating the patient on complications and potential ramifications, yearly follow-ups, and concurrent interdisciplinary communication with the PCP and other members of the healthcare team is the best treatment plan available.

Moderate

The moderate stage is characterized by interference of retinal blood flow due to the increased swelling of blood vessels. The findings in this stage are microaneurysms in three qudrants of the retina along with other signs such as dot and blot hemorrhages, hard exudates, cotton wool spots, and venous beading (Koetting, 2019). Patients with moderate NPDR have a 12-27% risk of progressing to proliferative diabetic retinopathy within 1 year of diagnosis (Koetting 2019). In addition to the plan for the mild stage, patients should be seen every 6 months on average to assure early identification of potential progression and referred to the PCP for hyperglycemia treatment modification.

Severe

The severe stage is characterized by a large blockage of retinal blood flow and signals for new blood vessel growth. The findings in this stage are ones in the moderate stage PLUS 20 or more intraretinal hemorrhages in each quadrant of the retina, definite venous beading in 2 quadrants, intraretinal microvascular abnormalities in at least 1 quadrant, without signs of proliferative retinopathy (Koetting, 2019). Since there is a 52% risk of PDR development in a

year from the DR diagnosis, follow-up vists ever 3-4 months are recommended along with continuation of hyperglycemia monitoring and treatment (Koetting 2019).

Proliferative Diabetic Retinopathy

Proliferative Diabetic retinopathy (PDR) was found in 2% of diabetic patients within 5 years of diagnosis. For patients who have had the diagnosis for 15 or more years, the number jumps up to 15.5 (Koetting 2019). This stage is characterized by neovascularization, new and hyperpermeable blood vessel formation in the retina, due to ischemia. Findings in this stage are the severe NPDR stage and one or more of the following: neovascularization or vitreous/preretinal hemorrhage. During proliferative diabetic retinopathy, the blood vessels that are formed are not regularly oriented and can invade the vitreous cavity which can lead to hemorrhage and potentially retinal detachments (Wang and Lo 2018). This is usually when symptoms start affecting the patient causing them to come in for a check-up. These symptoms can be floaters, blurry vision, and a decreased visual field. At this point, damage to the tissues cannot be revered but only managed. Potential treatment is hyperglycemia management, laser photocoagulation, vitrectomy, and the most commonly used, anti-VEGF intravitreal injections.

Diabetic Macular Edema

Diabetic Macular Edema (DME) is assessed separately from the stages and can occur in any stage of diabetic retinopathy. DME is the most common complication seen in DR patients. It should be caught and managed quickly due to the irreversible blindness it can cause. There are two classifications of DME: non-central and central involved DME. Findings that have to be present for a noncentral-involved DME diagnosis is retinal thickening in the macula without central subfield involvement. For a central-involved DME diagnosis, the thickening would have to involve the central subfield zone (Koetting, 2019). It is caused by diabetes induced blood-retina-barrier breakdown and subsequent protein/fluid leakage into the neural layer of the retina (Duh, Sun et al. 2017). This can cause retinal layer thickening, cysts, subretinal fluid, and swelling of the neuropil and therefore precipitate blindness.

	NPDR Mild	*NPDR Moderate*	*NPDR Severe*	*PDR*	*DME*
Findings	Microaneurysms (MA) only	MA + dot or blot hemorrhages or venous beading or cotton wool spots	Moderate findings + >20 hemorrhages in each quadrant or venous beading in more than 2 quadrants or IRMA in at least one quadrant	Neovascularization or vitreous hemorrhage (early would only have presence of vessels and none of the above findings)	Hard exudates or retinal thickening within one disk diameter of the fovea
Management	6-12 months	3-6 months	Referral to ophthalmologist in 2-4 months	Referral to ophthalmologist immediately in less than a week	Referral to retina specialist within two weeks

Pathways of Pathophysiology

There are multiple pathophysiological pathways for DR development, such as, oxidative stress, AGE synthesis, protein kinase C-β activation, and increased VEGF production (Ciulla, Amador et al. 2003). In the next few paragraphs, I expand on a few of these pathways.

Neurodegeneration

The neurodegeneration aspect of DR is defined as a reduction in the nerve layer caused by glutamate accumulation and consequent apoptosis of ganglion and muller cells in the retina. This glutamate accumulation can be due to the dysfunction of glutamine-synthetase enzyme in Muller cells. This dysfunction disables the glial cells and they can no longer remove the extra glutamate. In addition to this, the Muller cells can no longer oxidize glutamate as well. This glutamate accumulation causes an increase in intracellular calcium and therefore neural cell death. Through studying areas without vascularization, such as the cornea, with confocal microscopy, it was discovered that this neurodegeneration pathway of pathogenesis is independent from the microvascular pathway. (Martins 2020) Although microvascular changes still can cause the neurovascular changes because the endothelial changes can cause the apoptosis of pericytes and capillary occlusion of capillaries supplying the optic nerve. The pericyte lesion absence defined the earliest stage of DR before neurodegeneration was discovered. Now there is a need for the discovery of new technology that can test for these nervous system changes in the absence of pericyte lesions. Further research in this area can also shine light on the addition risk factors of diabetic retinopathy that need to be controlled in addition to hyperglycemia.

Treatment for the neurodegeneration aspect of DR is brimonidine tartrate and somatostatin eye drops. These drugs cause vasodilation and increased blood flow in the retina. Somatostatin, an antioxidant, also reduces glutamate cell accumulation and prevents neovascularization by inhibiting VEGF production (Grauslund, Frydkjaer-Olsen et al. 2019). PEDG, insulin, neuroprotectin D1, glial cell line neurotrophic factor, nerve growth factor also neuroprotective agents that need to be further studied to provide additional insight into the neurodegenerative aspects of diabetic retinopathy (Grauslund, Frydkjaer-Olsen et al. 2019). Further investigation from this perspective could offer new and less invasive novel therapies.

Microvascular Complications

Hyperglycemia is the start of different microvascular cascades, such as polyol pathway, AGE pathway, RAAS pathway, PKC pathway, and inflammation. These all eventually lead to oxidative stress and cause the release of cytokines that cause dysfunction in the retinal vasculature. This leads to blood flow changes, pericyte apoptosis, and basement membrane thickening which can cause capillary exclusion and microaneurysms. Once this happens, hypoxia and ischemia occur with subsequent neovascularization. The retinal vasculature dysfunction can also cause increase in vascular permeability and separately lead to diabetic macular edema which is one of the most dangerous complications of DR. Hyperglycemia can increase ECM protein and collagen expression in the retinal endothelial cells (REC). The ROS production also induces the expression and cross-linking of collagen and induction causes the structural rigidity that leads to pericyte

loss. In addition, it promotes endothelin-1 transcription which is a vasoconstrictor that causes basement membrane thickening. The blood-retina barrier, which regulates the exchange of substances between the blood and neural retina, is also highly affected by oxidative stress. This can also be caused by the increase in VEGF production that happens in diabetic retinopathy as it results in the loss of Caludin-1, an important protein that maintains the tight junctions in the BRB (Deissler, Deissler et al. 2013). Hyperglycemic induction of the PKC pathway causes pericyte loss and oxidative stress related induction of the NF-kB pathway leads to inflammation, which both lead to further incapacitation of the BRB. Angiogenesis and inflammation processes are closely linked due to the angiogenic factors released through inflammatory cascades, such as prostaglandin E2 (PGE2) and Cyclooxygenase-2 (COX-2).

AGE pathway

Advanced glycation end (AGE) products are one of the many pathways in the pathogenesis of diabetic retinopathy. In general, there is a positive feedback loop where the AGE products promote ROS production (hydrogen peroxide, superoxide, nitric oxide) which then further promote AGE formation. AGE development is a normal physiological process, but diabetes accelerates the formation of end products and adduct accumulation due to the increased stores of glucose. The process starts with sugars and the nonenzymatic reduction with amino groups. The first product in this reduction is from the Maillard reaction, the Schiff base. The Schiff base is a subclass of imines and is formed between glucose and ε-amino groups. This base is relatively un-stable, so it freely rearranges into the more stable Amadori adducts. Amadori products can form α-dicarobonyls, such as methylglyoxal. From there various other reactions result in AGE adducts such as pentosidine and Nε-carboxy-methyl-lysine (CML), which are the main adducts in diabetic patients. AGEs affect the cells in three ways: as adducts- either on serum proteins or endogenously formed through glucose metabolism, or as ECM modifications on structural proteins (Zong, Ward et al. 2011). Specifically in diabetic retinopathy, the involvement of AGEs is mainly in relation to the direct glycation of the extracellular matrix, and the resulting decrease in elasticity of the vasculature, and indirectly inducing intracellular pathways such as Ras-MAPK and RhoA/ROCK that activate NF-kB (Okamoto, Yamagishi et al. 2002).The intracellular cascade changes release inflammatory cytokines and angiogenic factors, generates ROS, increases vasopermeability, and compromised blood-retina barrier (Okamoto, Yamagishi et al. 2002). The main receptor involved in this pathway, which also has increased expression in diabetic patients, is the receptor for advanced glycation end products (RAGE) which is expressed in microglia, muller cells, RPE cells, and pericytes (Chen, Curtis et al. 2013). The interaction of AGEs and this receptor induces inflammation, apoptotic, and proliferation processes

VEGF

Vascular endothelial growth factor (VEGF), a type of angiogenic growth factor, is the most potent stimulator of endothelial cells, causing extracellular matrix degradation and subsequent blood vessel proliferation. The interaction of VEGF with its receptors activates the Gq pathway. In this pathway, phospholipase Cγ gets phosphorylated and converts inositol phosphate into diacylglycerol which activates protein kinase C. VEGF synthesis is upregulated by hypoxia

inducible factor 1 (HIF1) activation due to oxidative stress caused by retinal blood vessel obstruction. A mechanism involving signal transducer and activator of transcription factor 3 (STAT3) was also identified. The process starts with angiogenic growth factors activating the endothelial cell receptors. They then release protease enzymes that breakdown the basement membrane. This allows the endothelial cells to branch out and leave the parent vessel wall to use integrins and invade in the surrounding matrix (Caldwell, Bartoli et al. 2003). This hypoxic state also increases VEGF sensitivity by inducing it's receptor expression (Cai and Boulton 2002). VEGF works through a number of different mechanisms. Mainly, it effects junctional proteins such as VE cadherin and occludin causing vascular leakage (Cai and Boulton 2002) and promotes proliferation though activation of mitogen activation protein (MAP) (Wang and Lo 2018). VEGF is also a survival factor for vascular endothelial cells by causing dephosphorylation of p38, a key proapoptotic modulator (Caldwell, Bartoli et al. 2003). VEGF is one of the main predictive factors for progression to DME as well.

Therapy wise, anti-VEGF intravitreal injections are the mainstay for PDR and DME. Compared to surgical interventions, there are less side effects, such as peripheral visual field loss and DME onset, due to the highly specific nature of the receptors and their key role in the pathogenesis of the later stages of DR (Duh, Sun et al. 2017). In fact, anti-VEGF treatment showed a higher average visual acuity compared to PRP over a 2 year period in a study done by the Diabetic Retinopathy Clinical Research Network (Writing Committee for the Diabetic Retinopathy Clinical Research, Gross et al. 2015). A major setback in anti-VEGF therapy is the short life of the injection. Patients need repeat injections ever 1-2 months in order to prevent disease progression, and non-adherent patients would still be at risk for further irreversible damage. Some of the other adverse events with this therapeutic approach are potential geographic atrophy and inhibition of the neuroprotective effects of VEGF on retinal neurodegeneration from long-term exposure to anti-VEGF injections (Simo, Sundstrom et al. 2014). Another limitation is the fact that it's therapeutic effect is specific for late stages of diabetic retinopathy. Due to these reasons, laser photocoagulation and corticosteroid injections still play a role in late stage DR treatment. Regardless, prevention of vision loss is still difficult with the current therapeutic approaches.

Conclusion

Diabetic retinopathy is one of the most highly pervasive illnesses in our population and the number of people affected is only growing more every year. It has multiple pathways of pathogenesis which makes it much harder to create a general fix and treatment. While the current treatment regimens are effective in stopping progression during the late stages of the disease, there is no treatment for earlier stages or general cure for DR. Anti-VEGF injections are the current gold standard treatment for PDR and DME due to the lower level of invasiveness and side effects compared to other surgical therapies used in the past. Targeting the earlier stages of VEGF production looks to be promising in potential novel therapies. By targeting the earlier stages of VEGF production, instead of the current antagonist injections, the downstream effects could be stopped before they even begin. As the VEGF production correlates to the severity of the disease, this treatment strategy could decrease the probability of DR causing blindness. Now that there is an understanding of the role of neurodegeneration in early stage

DR, exploring technology that can identify it could help stop the disease in the earlier stages. In general, treatment for the early stages of DR is what is needed to overcome its wide-spread effects. In order to do this, more patients need to be screened and found in earlier stages. The first step would be to increase the awareness for diabetic retinopathy and perform at least yearly screenings on those with Type 1 Diabetes and/or other risk factors. By identifying gaps of preventative measures and starting from the baseline, the medical field can progress to finding more effective treatments and maybe even a cure to diabetic retinopathy.

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