INTRODUCTION

Diabetes Mellitus (DM) and its systemic and ophthalmic complications represent an enormous public health threat in the 21st century. The ophthalmic complications of diabetes are the leading cause of legal blindness in adults ***(Mahmood, 2008)***.

The World Health Organization (WHO) has estimated that there will be 370 million people affected with DM in the world by the year 2030, and each of them will be at risk of developing diabetic retinopathy (DR) ***(Wild et al., 2004).***

The prevalence of DR was 34.6 % of diabetic patients. Globally, there are approximately 93 million people with diabetic retinopathy of which 17 million with proliferative diabetic retinopathy (PDR), 21 million with diabetic macular edema (DME) and 28 million with vision-threatening retinopathy as severe non-proliferative DR and proliferative diabetic retinopathy (PDR) ***(Yau et al., 2012).***

DME is one of the most common causes of visual impairment in patients with DM ***(Klien et al., 2009).***

Diabetic macular edema is defined as thickening of the retina or/and hard exudates within 1 disc diameter of the center of the macula ***(Yang et al., 2009).***

The pathophysiology of DME involves retinal microaneurysms, dilated capillaries, and loss of pericytes, with impairment of the blood-retinal barrier (BRB) ***(Ciulla et al., 2003).***

Breakdown of the BRB results in leakage of fluid into the extracellular space, which disrupts macular structure and function on a cellular level ***(Knudsen et al., 2002; Rotsos and Moschos, 2008).***

Diabetic retinopathy is due to breakdown of the retinal vasculature integrity and hemodynamic abnormalities ***(Ciulla et al., 2002).***

Studies of Doppler flowmetry indicated that there is a significant decrease of the choroidal blood flow in diabetic patients especially with the presence of macular edema ***(Nagaoka et al., 2004)***.

Similarly, histological studies suggested that Choriocapillaris degeneration is more prominent in patients with diabetes ***(Cao et al., 1998).***

Vascular Changes in Diabetic Retinopathy and choriodopathy:

Retinal microvascular dysfunction in diabetes:

Alterations in vascular structure under diabetic conditions results in breakdown of the blood retinal barrier and diabetic edema ***(Bandello et al., 2013).***

The vascular unit of the retina is composed of endothelial cells, astrocytes and pericytes. Diabetes affects the integrity of BRB by altering the structure of neurovascular unit of the retina. In the diabetic retina, there is an increase in the pericyte loss and VEGF level contributing to vascular leakage ***(Kim et al., 2012).***

Pericytes play a key role in maintaining vascular stability and early depletion of pericytes is a hallmark of DR leading to the formation of pericyte ghosts. The pericyte ghosts are pockets in basement membranes marking the space formed after pericytes have disappeared ***(Mizutani et al., 1996).***

Degeneration of the pericytes leads to pericyte ghosts; however the number of endothelial cells in retinal vessels is not affected. Pericyte loss also increases proliferation of endothelial cells contributing to microaneurysm formation in retinal vessels. The loss of pericytes in diabetic retinal vasculature is detected by the formation of acellular capillaries. Acellular capillaries are basement membrane tubes without cell nuclei that have at least one-fourth of the normal capillary diameter ***(Barber et al., 2005).***

There is a strong inverse correlation between pericyte density and the formation of a range of retinal microvascular abnormalities similar to those seen in diabetic patients. pericyte loss also may be a causal pathogenic event in diabetic retinopathy ***(Enge et al., 2002).***

In the diabetic retina, non perfused and obliterated microvessels are observed in the early stages of retinopathy. Vessel closure promotes the proliferative retinal neovascularization and can be histologically observed as the prevalence of acellular capillaries increases ***(Engerman, 1989)***.

Adhesion of leukocyte to the retinal blood vessel induces endothelial cell death, pericyte loss and vascular closure leading to retinal non perfusion with subsequent proliferative neovascularization ***(Bandello et al., 2013).***

Leukocyte adhesion to retinal vessels is mediated by ICAM-1 and vascular cellular adhesion molecule ***(Bai et al., 2003).***

The activation of neovascularization is mediated by hypoxia-inducible factor 1-alpha (HIF-1α) which is a transcription factor and regulates the expression of various pro-angiogenic genes including vascular endothelial growth factor (VEGF), VEGF receptor-1, and angiopoietin-1 ***(Costa and Soares, 2013).***

Under diabetic conditions, aberrant activation of these pro-angiogenic factors results in pathological neovascularization. Thus, vascular changes in the diabetic retina promote the breakdown of BRB, macular edema, and proliferative neovascularization leading to impairment of vision ***(Shin et al., 2014).***

Effects on vascular regulation:

While vascular endothelial growth factor plays a major role in the development of proliferative retinopathy, it may also have a role in the auto regulation of the retinal perfusion as it increases retinal blood flow in rats ***(Clermont and Aiello, 1997; Grunwald, 1998).***

In a porcine coronary arterial model, vascular endothelial growth factor produces a dramatic dilatory Norepinephrine-dependent response with development of tachyphylaxis ***(Lopez et al., 1997).***

Macrovascular pathophysiology in diabetic retinopathy:

Retinal capillary perfusion in diabetes:

Studies of capillary perfusion in diabetes primarily measure the capillary density and blood velocity in the perifoveal region. Both fluorescein angiography and blue light entopy have been utilized in such studies. While the more objective fluorescein studies are more reliable, the reduction of entopically determined macular capillary blood flow in diabetic patients correlates with the severity of changes measurable by fluorescein angiography and aqueous flare intensity ***(Applegate et al., 1997; Nakahashi et al., 1992).***

The perifoveal intercapillary area and foveal avascular zone are significantly larger in diabetic patients than in healthy

control. In addition, The visual acuity correlated significantly with foveal avascular zone and perifoveal intercapillary area, indicating an association between enlargement and declined visual acuity) ***(Oliver et al., 1995b).***

In addition, the visual function as measured by contrast sensitivity is affected from the very early stages of diabetic retinopathy. This affection is related to the presence of perifoveal ischemia even with normal visual acuity and no clinically significant macular edema ***(Oliver et al., 1997).***

Indeed, in diabetic cats, retinal hypoxia is demonstrated in early diabetic retinopathy before capillary dropout is evident clinically ***(Linsenmeier et al., 1998).***

Moreover, in diabetic patients, enlargement of the foveal avascular zone correlates with the degree of diabetic ischaemic maculopathy.The previous findings together suggest that enlargement of the foveal avascular zone and intercapillary area produce localized photoreceptor ischemia, hypoxia and reduced cellular function ***(Ciulla et al., 2002).***

This hypothesis is supported by a study that demonstrated the reversibility of contrast sensitivity deficits in diabetic patients by hyperoxia ***(Harris et al., 1996).***

There is consensus that perifoveal capillaries are destroyed by diabetes in proportion to disease severity, however. It remains unclear whether capillary blood velocity

within the remaining capillaries remains normal ***(Oliver et al., 1995a).***

For example, perifoveal capillary blood velocity is lower in diabetic patients with retinopathy than non diabetic subjects, but within the retinopathy group, decreasing visual acuity is not associated with a decrease in capillary blood velocity ***(Oliver et al., 1995b).***

Clearly, however, even maintenance of normal capillary blood velocity in the remaining perifoveal capillaries is insufficient for adequate macular nutrient delivery in diabetes, when capillary numbers steadily decline as the disease advances. This conclusion is supported by the finding that perifoveal capillary density correlates with the degree of visual impairment while the capillary blood velocity do not ***(Oliver et al., 1995a; Oliver et al., 1995b).***

In a related issue, no difference was found between diabetic subjects with and without cystoid macular oedema as related to perifoveal capillary density, the extent of the foveal avascular zone and retinal capillary blood velocity and both groups were found to have lower capillary blood velocity than control subjects ***(Oliver et al., 1995a).***

Effects of diabetes on retrobulbar and choroidal vascular regulation:

Although diabetic patients clearly suffer from dysfunctional retinal perfusion, deficiencies in the retrobulbar and choroidal circulation are also apparent in this disease. While these deficiencies may in part reflect responses to a primary event occurring in the retinal microvasculature, they may independently contribute to development and progression of this disease. Because the retina is supplied via the central retinal artery, defects in central retinal arterial haemodynamics are manifest in diabetes. A study to assess the retrobulbar circulation using Color Doppler imaging reported a lower mean peak systolic velocity in the central retinal artery in patients with nonproliferative diabetic retinopathy, as compared to preretinopathy patients ***(Guven et al., 1996).***

Similarly, the severity of diabetic retinopathy has been found to correlate strongly with decreased flow velocities in the retrobulbar vessels, most prominent in the central retinal artery ***(Goebel et al., 1995).***

These changes in central retinal arterial flow velocity do not, however, necessarily reflect changes in bulk flow, and it is presumed as well that changes in the central retinal vessel result from, rather than cause, changes in vasoregulation within the retina itself. Similarly, autoregulatory defects emergent in the diseased retina have their central retinal arterial correlates:

hyperoxia significantly reduces central retinal artery end diastolic velocity and significantly increases the resistance index in healthy but not in diabetic subjects, a result that parallels findings obtained from direct retinal flow measurements ***(Evans et al., 1997).***

Little is known about choroidal blood flow in diabetes. While the bulk choroidal blood flow can be roughly estimated from measurements of corneal pulsations, contact tonometer studies provide conflicting results, with some finding increased and others finding decreased choroidal perfusion in diabetic subjects ***(Langham et al., 1991).***

No association is found between fundus pulsation magnitude and retinopathy severity by studies utilizing the more reproducible method of noncontact laser interferometry ***(Schmetterer et al., 1997).***

These results, which suggest that total choroidal perfusion is not altered by diabetes, are supported by evidence that flow velocities in the posterior ciliary arteries feeding the choroid are also unchanged by the disease ***(Guven et al., 1996).***

Although the choroidal vessels are innervated by the autonomic system, the effect of diabetic autonomic dysfunction on choroidal blood flow has been examined in only a few studies. While neuropathy has been demonstrated by electron microscopy in the choroidal autonomic nerve plexus in

streptozotocin-induced diabetic rats, the significance of this change for regulation of the choroidal perfusion remains unclear ***(Gartner and Fischer, 1989).***

Additionally, while current models of choroidal vascular regulation emphasize myogenic regulatory mechanisms modulated by neurohumoral factors, little is known of how acute or chronic hyperglycaemia may impact this homeostatic system ***(Kiel and Lovell, 1996).***

Choroidal anatomy

The choroidal vasculature, especially the choriocapillaris, provides nutrients and oxygen to the outer half of sensory retina and retinal pigment epithelium and is responsible for maintaining the highly metabolically active photoreceptor cells ***(Alm, 1992).***

The choroid is the posterior part of the middle tunic of the eye, called uvea (Fig. 1). The uvea develops from the mesenchyme surrounding the two vesicles that bud off the embryonic forebrain at the end of the first month, eventually becoming the eyes. By that time, melanocyte precursors migrate into the uvea from the neural crest. However, differentiation of melanocyte precursors into pigmented melanocytes does not occur until 7-8 months of gestation ***(Nickla and Wallman, 2010).***

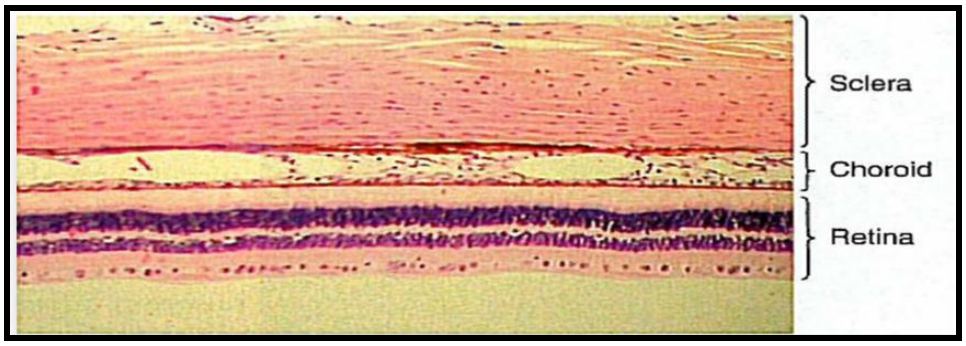

Fig. (1): Photomicrograph of the three tunics at the back of the primate eye) ***(Debora et al., 2011).***

By the second month of gestation, the mesenchyme forms the choriocapillaris but it must be in contact with the developing retinal pigment epithelium (RPE) in order to differentiate. Therefore, the choroid derives from different cell lines than the retina and RPE do, which both derive from the neural ectoderm. The choroid is comprised of blood vessels, fibroblasts, melanocytes, resident immunocompetent cells and supporting elastic and collagenous connective tissue. As one of the most highly vascularized tissues of the body, its main function has been traditionally viewed as supplying oxygen and nutrients to the outer retina ***(Nickla and Wallman, 2010).***

Other likely functions include thermoregulation via heat dissipation, and modulation of intraocular pressure (IOP) by vasomotor control of blood flow. The choroid also has an important role in the drainage of the aqueous humor from the anterior chamber through uveoscleral pathway ***(Alm and Nilsson, 2009).***

Histology of the choroid:

The choroid extends from the margins of the optic nerve to the pars plana, where it continues anteriorly, becoming the ciliary body. Its innermost layer is the complex 5-laminar structure of Bruch's membrane, and its outermost layer is the suprachoroid outside of which is the suprachoroidal space between choroid and sclera ***(Nickla and Wallman, 2010).***

Histologically, the choroid has variously been divided into 4 to 6 layers, depending on whether the vascular region is considered as 1 or 2 layers (Sattler's and Haller's), and whether the lamina fusca is considered to be of scleral or choroidal origin. It is most commonly described as five layers: starting from the retinal (inner) side, these are Bruch's membrane, the choroiocapillaris, the two vascular layers (Haller's and Sattler's), and the suprachoroidea **(Fig. 2A and B)** ***(Hogan et al., 1971).***

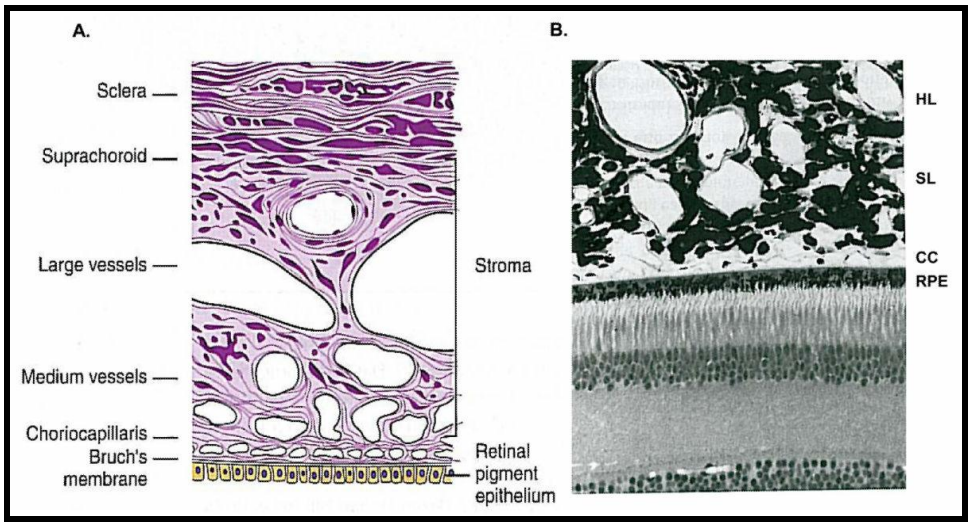

Fig. (2): Histology of the choroid. A. Schematic of the layers of the choroid. B. Semithin resin section of the outer retina and choroid in the primate eye. RPE: retinal pigment epithelium; CC, choriocapillaris; SL: Sattler's layer; HL: Haller's layer ***(Debora et al., 2011).***

In humans, the choroidal thickness is approximately 200 μm at birth and decreases to about 80 μm by age 90 ***(Ramrattan et al., 1994).***

Choriocapillaris: the choriocapillaris is a highly anastomosed network of Capillaries that form a thin sheet opposed to Bruch's membrane (Fig. 3A). The fibrous basement membrane of the capillary endothelial cells forms the outermost layer of Bruch's membrane in humans. The choriocapillaris thickness is about 10 μm at the fovea, where there is the greatest density of capillaries while thinning to about 7 μm in the periphery. The capillaries arise from the arterioles in Sattler's layer where each arteriole gives rise to a hexagonal (or lobular)-shaped domain of a single layer of capillaries, giving a

patch-like structure to the choriocapillaris (Fig. 3B) ***(Nickla and Wallman, 2010).***

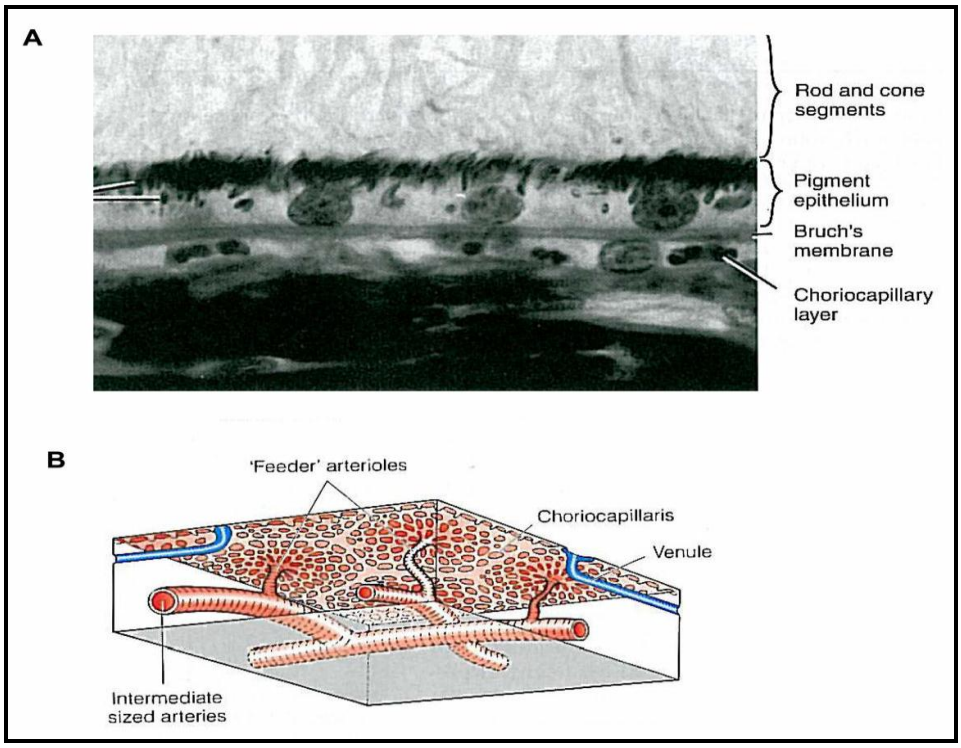

Fig. (3): Histology of the choriocapillaris. A. The choriocapillaris is located adjacent to Bruch's membrane. B. Each feeder arteriole in Sattler's layer supplies a hexagonally-arrayed area of capillaries ***(Debora et al., 2011).***

Finally, the innermost choroidal layer is Bruch's membrane that is a 5-layered structure consisting of (from outer to inner): basement membrane of the choroiocapillaris, outer collagenous zone, elastic layer, inner collagenous zone, and basement membrane of the retinal pigment epithelium ***(Nickla and Wallman, 2010).***

Choroidal vascular layers and suprachoroid: The vascular region of the choroid consists of the outer Haller's layer

of large blood vessels and the inner Sattler's layer of medium and small arteries and arterioles that feed the capillary network, and veins **(Figure 2).** The stroma (extravascular tissue) contains collagen and elastic fibers, non-vascular smooth muscle cells fibroblasts, and numerous very large melanocytes that are closely apposed to the blood vessels. As in other types of connective tissue, there are numerous macrophages, lymphocytes and mast cells ***(Nickla and Wallman, 2010).***

The suprachoroid is a transitional zone between choroid and sclera containing elements of collagen fibers, fibroblasts and melanocytes ***(Krebs and Krebs, 1991).***

The outermost layer of the suprachoroid is the lamina fusca that is approximately 30 μm thick. It is consisted of several layers of closely-apposed, flattened fusiform melanocytes and fibroblast-like cells with interspersed bundles of myelinated axons ***(De Stefano and Mugnaini, 1997b).***

The retinal vasculature is not present in the foveal region so that impairment of the choriocapillaris may cause severe functional damage to the retinal tissue in the fovea. Therefore, in vivo evaluation of the structural changes in the choroid may be very insightful to determine the pathogenesis of progression of the macular changes in diabetic eyes ***(Querques et al., 2012).***

There are few clinical studies on choroidal angiopathy in diabetes due to difficulty of imaging the choroid in vivo ***(Regatieri et al., 2012).***