INTRODUCTION

Type 2 diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90% of cases. It has become one of the world's most important public health problems and it is now well established that the 21st century will be characterized by a global epidemic of it ***(Wild et al., 2004).***

Diabetes is a disease that is strongly associated with both microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (ischemic heart disease, peripheral vascular disease and cerebrovascular disease) complications, resulting in organ and tissue damage in approximately one third to one half of people with diabetes ***(Cade, 2008).***

Diabetic retinopathy (DR) is a microvascular complication of diabetes that can affect the peripheral retina, the macula or both and is a leading cause of visual disability and blindness in people with diabetes ***(Ciulla, 2004 and Pandit & Sultana, 2012).*** DR is the main cause of blindness in individuals aged 20-64 years. The risk of blindness is approximately 30-fold higher in people with diabetes mellitus compared to the population in general ***(Esteves et al., 2009).***

The cause of complications in the diabetic state has been a subject of intense research for over half of a century. However, two major clinical trials established the relationship of poor glycemic control to DR ***(Diabetes Control and***

Complications Trial, 1993 and United Kingdom Prospective Diabetes Study, 1998). The precise relationship of other factors to diabetic complications is still not clear. In particular, the biochemical pathways involved in the development of these complications, the relative importance of these pathways in specific tissues and the role of endocrine agents remain topics requiring further research.

Vitamin D is now recognized as an important prohormone in health and disease ***(Johal & Levin, 2009)***. The major and most well-known function of vitamin D is to maintain calcium and phosphorus homeostasis and promote bone mineralization. Moreover, ***Holick (2006)*** suggested that vitamin D and calcium homeostasis may also be important for a variety of non-skeletal outcomes including neuromuscular function and falls, psoriasis, multiple sclerosis, colorectal cancer and prostate cancer.

Vitamin D deficiencies are associated with numerous problems including osteoporosis, fractures, falls, cancer, autoimmune diseases, diabetes and cardiovascular disease ***(Holick & Chen, 2008).***

A factor that may impact the development of diabetic nephropathy is vitamin D. The role of the kidney in the hydroxylation of the vitamin D metabolite 25-hydroxycholecalciferol from the liver to the biologically active form of 1, 25- dihydroxycholecalciferol was well established ***(Holick, 2007).***

Diaz et al. (2009) demonstrated an association between both vitamin D deficiency and vitamin D insufficiency with nephropathy in individuals with diabetes even after controlling for factors such as race/ethnicity, presence of hypertension and use of ACE inhibitors or ARBs. The high prevalence of vitamin D deficiency and vitamin D insufficiency in individuals with diabetes suggest that further study of this relationship may lead to new interventions to delay the progression of diabetic nephropathy. ***Taverna et al. (2005)*** demonstrated that the vitamin D receptor (VDR) was extensively expressed in retina.

AIM OF THE WORK

This is a pilot (an exploratory) study. The aim is to study the relation between 25-hydroxy (25-OH) vitamin D_3 level and diabetic retinopathy in patients with type 2 diabetes mellitus and to evaluate for any relation between 25 (OH) vitamin D_3 level and different stages of diabetic retinopathy.

Diabetic Retinopathy

Diabetic retinopathy (DR) is a prototypical microvascular disorder associated with microaneurysms, intraretinal hemorrhages, capillary nonperfusion, intraretinal microvascular abnormalities and neovascularization ***(Gardner et al., 2011).*** These morphological abnormalities in the retinal microvasculature can remain relatively stable, that is non-proliferative DR (NPDR) or progress to diabetic macular edema (DME) and/or proliferative DR (PDR), which are leading causes of both moderate and severe vision loss in working-age adults ***(Feener, 2009 and Kumar et al., 2012).***

Classification of Diabetic Retinopathy (DR):

Diabetic retinopathy can be classified into four types: ***(Rohilla et al., 2012).***

- Mild non proliferative retinopathy: in which the retina swells like balloon and is recognized as the earliest stage of DR.
- Moderate non proliferative retinopathy: in which the blood vessels nourishing the retina get blocked.
- Severe non proliferative retinopathy: in which the retinopathy spreads and blood vessels get blocked in several areas in retina.
- Proliferative retinopathy: in which the retina sends signals to trigger the growth of new blood vessels.

Risk Factors Associated With Diabetic Retinopathy:

(A) Type of diabetes:

Henricsson et al. (2003) conducted a cohort study for 10 years including newly diagnosed patients with DM. After 5 years duration, there was no significant difference in DR risk between type 1 and type 2 participants, while after 10 years, 37% of type 1 and 41% of type 2 cases had developed retinopathy. The authors suggested that this trend could indicate that improvements in metabolic control had brought the DR risks in people with T1DM and T2DM closer together.

(B) Duration of diabetes:

Duration of diabetes is the most important risk factor for the development of DR ***(Dizdarevic et al., 2012).***

A cross sectional study conducted on 470 type 2 diabetic patients revealed that the duration of diabetes was the strongest predictor for any DR as well as its severity ***(Niazi et al., 2010).*** Patients with duration 5-10 years had 5 times more chances to have NPDR and 2×10^6 times more chances for advanced retinopathy than patients with duration less than 5 years. Similarly patients with duration more than 10 years had 32 times more chances to have NPDR and 2×10^8 times more chance to have PDR than patients with duration less than 5 years ***(Niazi et al., 2010).***

Moreover, a large cross-sectional study by ***Pradeepa et al. (2008)*** revealed that duration of diabetes was an independent risk factor for severity of DR.

The influence of increased duration on the occurrence of DR and its severity was probably related to the magnitude or prolonged exposure to hyperglycemia, or both, coupled with other risk factors ***(Pradeepa et al., 2008 and Rani et al., 2009).***

(C) Systemic risk factors include:

- **Glycemic control**

Two important trials, ***Diabetes Control and Complications Trial (DCCT) (2000)*** and ***United Kingdom Prospective Diabetes Study (UKPDS) (1998)***, were designed to compare the effects of conventional with intensive diabetes therapy in the development and progression of early microvascular complications. These studies conclusively demonstrated that intensive glycemic control significantly reduces the risk of DR development and progression in both type 1 and type 2 diabetes though not preventing retinopathy completely. Mean glycated hemoglobin (HbA1c %) was the dominant predictor of DR progression ***(UKPDS, 1998)***.

- **Blood pressure control**

Arterial hypertension had been shown to be an independent risk factor for the development and progression of DR ***(Falcao et al., 2010).***

Hypertension control in patients with type 2 diabetes had been shown to help prevent retinopathy and other microvascular complications ***(Matthews et al., 2004).*** Moreover, ***Zavrelova et al. (2011)*** suggested that systolic blood pressure (SBP) control appears to play a more important role than diastolic blood pressure (DBP) control on the developmental patterns of DR.

- **Blood lipid control**

Hyperlipidaemia was well established as a risk factor for DR particularly for macular hard exudates deposition and clinically significant macular edema (CSME) ***(Ucgun et al., 2007).*** Hard exudates, in turn, were associated with visual impairment and subretinal fibrosis from macular oedema ***(Yam & Kwok, 2007).***

Other factors include:

- **Genetic risk factors**

Variations in several genes had been found to be associated with risk for developing DR in different population worldwide ***(Uthra et al., 2008 and Daniel, 2010).***

One potential candidate gene was the aldose reductase gene2 (ALR2). Different ALR2 polymorphisms had been associated with a higher likelihood of DR in Asian Indians with T2DM ***(Kumaramanickavel et al., 2003).***

Katakami et al. (2011) reported that the C allele of the polymorphism at position −106 in the promoter of aldose reductase gene, which codes a rate-limiting enzyme of the polyol pathway, was a susceptibility allele for DR in Japanese type 2 diabetic patients.

Gene polymorphisms in the receptor for advanced glycation end products (RAGE) gene in type 2 diabetic patients with DR was investigated by ***Yuan et al. (2012).*** There research confirmed an association between the RAGE -374T/A polymorphism and retinopathy in subjects with type 2 diabetes but they found no significant difference between DR patients and those without retinopathy for the -429T/C variant. The authors suggested that the RAGE Gly82Ser polymorphism might be considered a significant risk for DR in Asian populations ***(Yuan et al., 2012).***

Other genes polymorphisms associated with risk for developing DR include the VEGF gene ***(Errera et al., 2007 and Yang et al., 2011)***, the endothelial nitric oxide synthase (eNOS) gene ***(Suganthalakshmi et al., 2006 and Bazzaz et al., 2010)*** and vitamin D receptor (VDR) ***(Taverna et al., 2002 and Taverna et al., 2005).***

A meta-analysis that examined the association between DR in T2DM and polymorphisms in the gene for hyperhomocystinaemia methylenetetrahydrofolate reductase (MTHFR) found only a marginal association with large heterogeneity between different studies ***(Zintzaras et al., 2005).***

Pathogenesis of Diabetic Retinopathy:

The retina is the most metabolically active tissue of the human body needing high oxygen concentrations. It is very sensible to hypoxia responding to this noxious stimulus by producing cytokines and growth factors ***(Falcao et al., 2010).***

Physiologically, retinal capillaries are composed of non-fenestrated endothelial cells (ECs) with tight-junctions and low permeability. There is a high proportion of pericytes surrounding endothelial cells. Pericytes regulate blood flow through the retinal capillaries due to their contractile structure. These cell types, along with other retinal cells (Muller cells and Astrocytes) form the inner blood-retinal barrier ***(Kaur et al., 2008).*** This barrier is fundamental for the maintenance of retinal homeostasis as it prevents the leakage of macromolecules into retinal tissues ***(Falcao et al., 2010).***

The outer blood-retinal barrier occurs between the tight junctions of the cells of the retinal pigment epithelium. It is also fundamental for ocular homeostasis but has no role in the pathogenesis of DR ***(Falcao et al., 2010).***

The pathogenesis of DR is multifactorial and a range of hyperglycemia-linked pathways have been implicated in the initiation and progression of this condition ***(Stitt, 2010).*** *Figure (1)* summarizes the pathogenesis of DR and represents a schematic overview of the effects of chronic hyperglycemia and its implications on cellular damage.

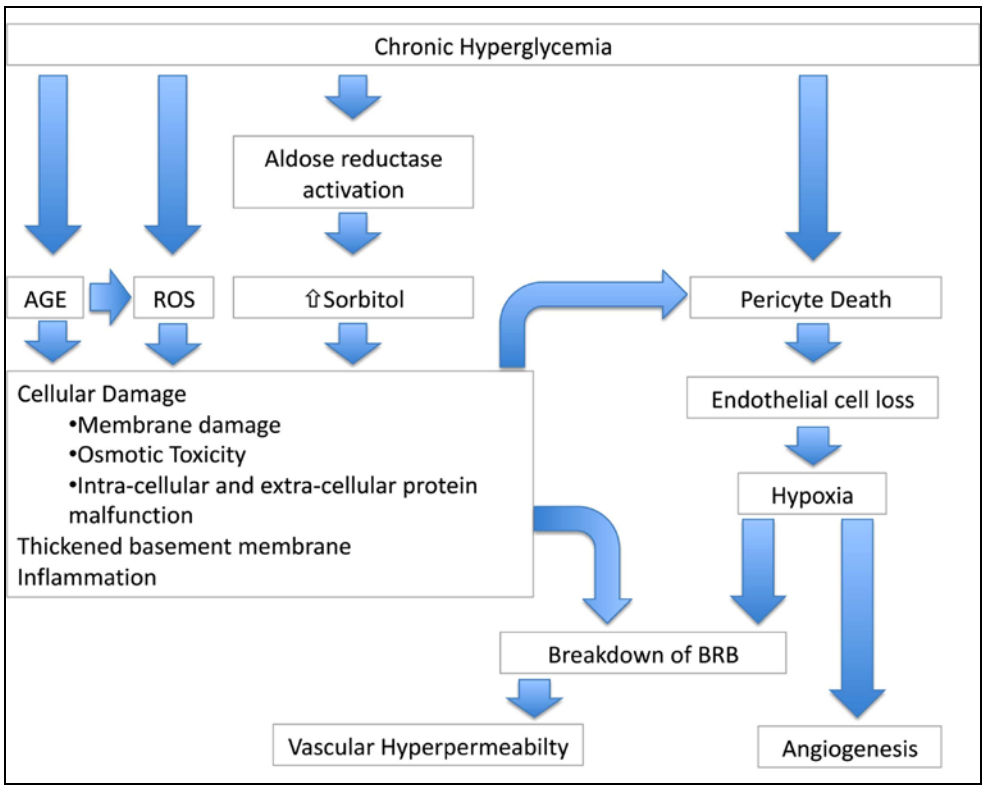

Figure (1): *Pathogenesis of diabetic retinopathy* ***(Falcao et al., 2010).***

Most retinal cells are affected by the metabolic abnormalities of diabetes ***(Gardner et al., 2011).*** However, clinicians attribute visual impairment of DR to macular edema due to abnormal permeability of barrier capillaries, ischemia due to capillary closure, epiretinal membranes, retinal neovascularization caused by angiogenesis, vitreous hemorrhages and/or traction detachments ***(Curtis et al., 2009 and Gardner et al., 2011).*** The existence of neovascularization marks the beginning of the proliferative disease ***(Falcao et al., 2010).***

Macular edema is the most frequent cause of moderate visual loss in DR ***(Falcao et al., 2010).*** It can exist with or without PDR and their severities are not directly related. Severe cases of DME can occur in patients with moderate NPDR and severe proliferative disease can occur without macular edema ***(Silva et al., 2009).***

Biochemical Mechanisms Involved In DR:

The increased circulating levels of glucose accumulate in the retinal ECs and result in the activation of various biochemical pathways ***(Rohilla et al., 2012)*** that include:

1. Oxidative Stress:

It has been noted that retina has high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue ***(Rohilla et al., 2012)***

Diabetes activates a small molecular weight G-protein, H-Ras, in the retina and its capillary cells. H-Ras activation is implicated in the apoptosis of retinal capillary cells. Matrix metalloproteinase (MMP)-9 is regulated by H-Ras and its activation in diabetes is associated with increased vascular permeability ***(Kowluru, 2010).***

Diabetes and hyperglycemia are associated with increase in oxidative stress and overproduction of reactive oxygen species (ROS)**.** Production of ROS may result from various mechanisms, including glucose auto-oxidation, protein

glycation, increased flux through the polyol pathway and prostanoid production**.** These high ROS levels are thought to determine structural and functional changes in all cellular components leading to DNA and protein modification and lipid peroxidation ***(Giusti & Gargiulo, 2007)***.

Moreover, in diabetes, the activities of antioxidant defense enzymes responsible for scavenging free radicals such as superoxide dismutase, glutathione reductase, glutathione peroxidase and catalase are diminished in the retina ***(Rohilla et al., 2012)***.

Pericytes are highly sensitive to the oxidative stress. As damage progresses, the blood vessel wall becomes more porous letting proteins and other materials leak out abnormally, thus determining the typical features of NPDR e.g. hard exudates and CSME ***(Giusti & Gargiulo, 2007).***

2. Aldose reductase and polyol pathway:

Aldose reductase (AR) is an NADPH-dependent oxidoreductase which is primarily known for catalyzing the reduction of glucose to sorbitol, the first step in polyol pathway of glucose metabolism. The polyol pathway may contribute to advanced glycation end product formation and development of secondary diabetic complications ***(Rohilla et al., 2012)***.

The polyol (sorbitol) pathway of glucose metabolism can generate cellular oxidative stress through a variety of biochemical abnormalities including myo-inositol depletion, down regulation of Na/K ATP-ase activity, NAD^+/NADH and

$NADP^+$/NADPH redox imbalances, changes in fatty acid metabolism, impaired neurotrophic support and up regulation of vascular endothelial growth factor (VEGF) ***(Giusti & Gargiulo, 2007)***.

3. Advanced glycation end products (AGEs):

Among the several pathogenic mechanisms that may contribute to DR are the formation and accumulation of AGEs. These are a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. AGEs are found in retinal vessel of diabetic patients and their levels correlate with severity of retinopathy ***(Tarr et al., 2013)***.

Numerous AGE adducts have been identified in vivo, including; *N*-carboxymethyl lysine (CML), crossline, pentosidine, furoyl-furanyl imidazole (FFI), hydroimidazolone, argpyrimidine, glyoxal lysine dimer (GOLD) and methylglyoxal lysine dimer (MOLD) ***(Goldin et al., 2006).***

Both AGEs and receptors for AGEs (RAGEs) have been localized to the retinal vasculature and vascular ECs. Moreover, it has been demonstrated that AGEs lead to altered protein function, interfere with the extracellular matrix (ECM) function and cause elaboration of cytokines ***(Rohilla et al., 2012)***.

4. Protein kinase C activation:

Protein kinases play modulatory role in transducing the adverse effects of hyperglycemia in the retinal vasculature which was proved by the fact that the exposure of cultured ECs to high levels of glucose leads to the rapid induction of protein kinase family members ***(Rohilla et al., 2012)***.

In vascular cells, hyperglycemia induces synthesis of diacylglycerol (DAG). This promotes the activation of protein kinase C (PKC) which, in turn, is associated with a number of biochemical and metabolic abnormalities including increased expression of extracellular matrix proteins such as collagen and fibronectin as well as increased expression of vasoactive mediators such as endothelin. The net effect of these changes may be manifested as basement membrane thickening and changes in vessel permeability and/or blood flow which in turn further contribute to the pathogenesis and progression of DR ***(Giusti & Gargiulo, 2007 and Tarr et al., 2013)***.

The PKC family is a family of enzymes with several isoforms ***(Steinberg, 2008).*** Although the activity of multiple PKC isoforms (α, β*1*, β*2*, δ and ε) is increased in vascular diabetic tissues, studies suggest that the PKC-β*2* isoform is preferentially activated. PKC-β has been shown to be an integral component of cellular signaling by reducing VEGF induced hyperpermeability, thus stimulating retinal pericyte proliferation ***(Giusti & Gargiulo, 2007)***.