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Immunological Risk Factors for the Development and Progression of Diabetic Retinopathy

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1. Epidemiology

International epidemiological studies indicate that over the last 50 years there has been progressive raise in diabetes incidence. According to International Diabetes Federation (IDF) currently there are 284 millions of people affected by diabetes worldwide and IDF prognosis predicts that in 2030 this number for all countries and human races will reach 438 million which will account for 7.7% of global population (IDF Atlas, 2010). The highest prevalence of diabetes is in North America where it reached the level of 10.2% of adult population whereas in Europe the prevalence is 6.9% of population aged 20 to 79 years. Particularly worrying is constant increase in diabetes incidence of both type 1 (Patterson et al., 2009; Jarosz-Chobot et al., 2011) as well as type 2 (D'Adamo & Caprio, 2011) in developmental age population. As a consequence of increase in diabetes prevalence there is higher number of patients with microangiopathic complications including diabetic retinopathy (DR) in children and youth (Cho et al., 2011) and in adults (Rosenson et al., 2011). Chronic complications reduce the quality of life and are a main cause of disability. Diabetic retinopathy has become a leading reason for blindness and visual impairment in developed countries and is constantly increasing (Fong et al ., 2004). Nearly all patients with type 1 diabetes will develop some manifestation of DR, whereas in type 2 diabetic patients 80% of insulin- dependent patients and 50% of patients not requiring insulin therapy will have DR within 20 to 25 years following disease onset (Lamoureux & Wong, 2011). Among younger-onset patients with diabetes, the prevalence of any retinopathy was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years. The prevalence of proliferative diabetic retinopathy (PDR) was 0% at 3 years and increased to 25% at 15 years (National Health and Nutrition Examination Survey, 2006).

2. Pathomechanism of diabetic retinopathy

Although there is a high incidence of diabetic retinopathy, its pathogenesis still remains enigmatic. Before changes in the eye fundus become visible in ophtalmoscopic examination or fluorescein angiography, in immunohistopathological study there are already visible morphological changes in precapillary arterioles, capillaries and venules of diameter less than 100 μm. In initial stage of development of diabetic retinopathy there is thickening of basal membrane of small vessels, its narrowing and closure, disappearance of pericytes, weakening

and distension of small vessel walls and, in consequence, formation of microaneurisms and endothelial cell proliferation. Additionally, the following take place: functional changes in capillaries, increase in their permeability and disruption of blood-retina barrier. As a result of enhanced vascular permeability, oedemas and haemorrhages appear in the retina. Moreover, the closure of retinal vessels leads to areas with loss of blood flow within retina, which causes its chronic ischaemia and hypoxia and subsequent increase in the production of growth factors that, in turn, induce angiogenesis, formation of arterio-venous anastomoses and proliferation of fibrous tissue within retina and optic nerve disc (Yoshida et al., 2004; Curtis et al., 2009; Roy et al., 2010; Lange et al. 2011). It is commonly accepted that hyperglycaemia plays crucial role in pathogenesis of diabetic angiopathy (Roy et al., 2010; Kowluru et al., 2010). Hyperglycaemia leads to the formation of advanced glycation end products (AGEs) which are durable, irreversible and their characteristic feature is to create cross-links between proteins which in turn affects flexibility of vessels (Wa et al., 2007; Roy et al., 2010; Yamagishi et al., 2011). It was proved that AGEs interaction with receptor for advanced glycation end products (RAGEs) plays a key role in development and progression of late diabetic complications (Thomas et al., 2011; Yamagishi et al., 2011; Zong et al., 2011). In multiple studies investigating diabetic retinopathy pathogenesis more and more attention is paid to inflammatory and angiogenic factors (Naldini et al., 2005; Maier et al., 2006; Campa et al, 2010; Praidou et al., 2010).

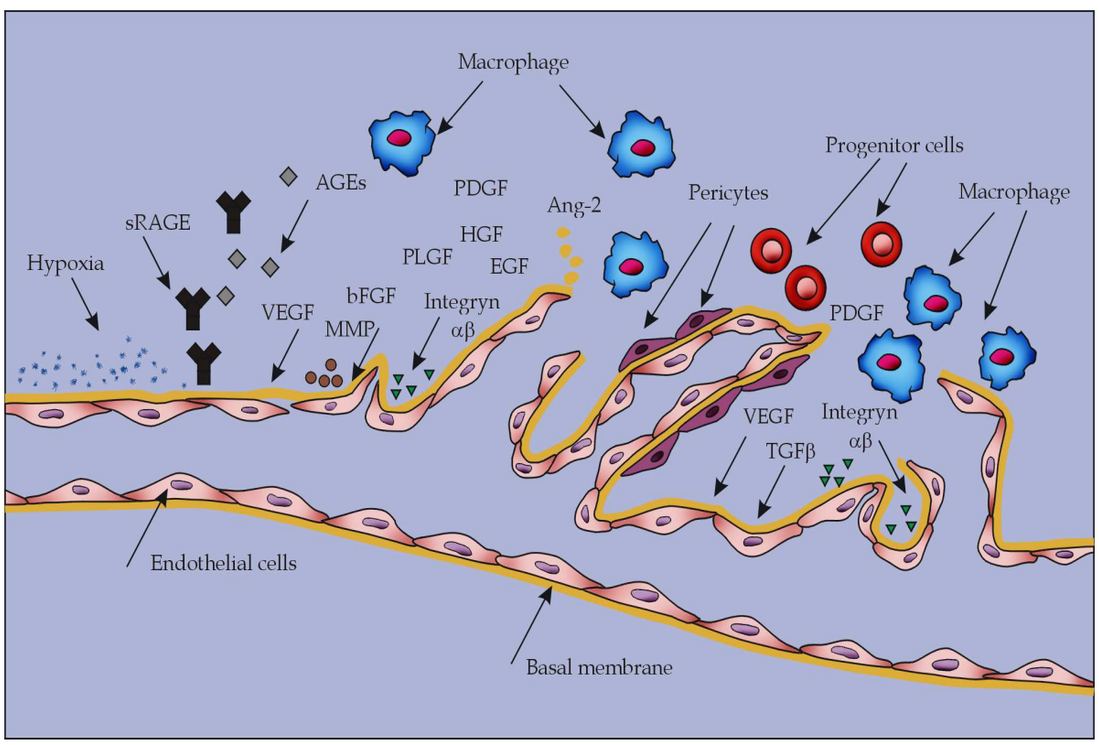


Fig. 1. The selected factors involved in the development and progression of diabetic retinopathy. AGEs - advanced glycation end products, RAGEs - receptor for advanced glycation end products, VEGF- vascular endothelial growth factor, IGF-I - insulin like growth factor, PLGF - placental growth factor, HGF – hepatocyte growth factor, PEDF - pigment epithelium derived factor, bFGF - basic fibroblast growth factor, TGF-β transforming growth factor beta, MMPs – metalloproteinases PDGF-platelet-derived growth factor , EGF-Epidermal growth factor, Ang-2 - Angiopoietin-2

In the eye of a healthy person endothelial cells are mitotically inactive thanks to pro-angiogenic and anti-angiogenic factors remaining in balance. Healthy organism maintains perfect equilibrium between angiogenesis modulators (Carmeliet, 2003; Kvanta, 2006). However in the case of hypoxia or inflammation this balance may be shifted towards neoangiogenesis (Kvanta, 2006; Campa et al., 2010). Eye angiogenesis is a complicated multi-stage process in which new vessels are created from existing ones (Kvanta, 2006; Curtis et al., 2009). This usually leads to significant loss of vision in patients with type 1 as well as type 2 diabetes (Rosenson et al., 2011; Durham & Herman, 2011). Results of studies conducted within last ten years proved that eye angiogenesis may involve choroid, cornea as well as retina (Kvanta, 2006; Caporali & Emanueli, 2011). During retinal angiogenesis newly formed vessels grow into vitreous where they may break and cause haemorrhage or retinal detachment. Fig. 1-3 shows three examples of a fundus of the eyes from normal and diabetic individuals.

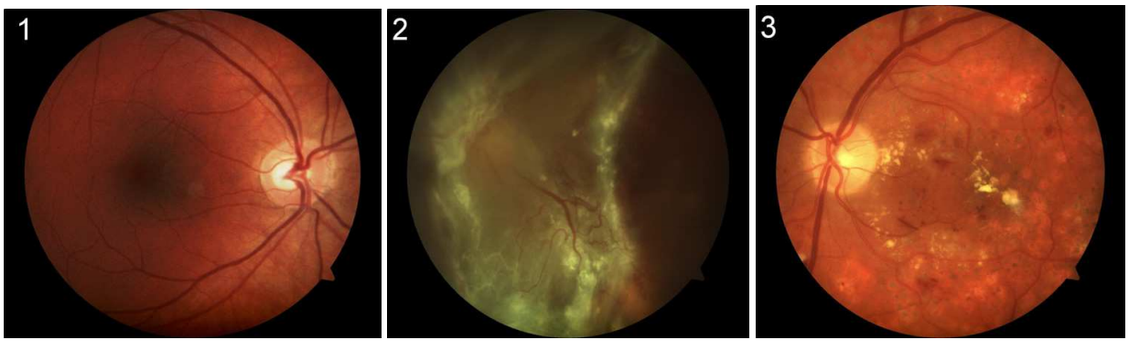


Fig. 2. Fundus photos of eyes from normal and diabetic individuals. 1. Normal fundus of the eye. 2. Proliferative diabetic retinopathy with tractional retinal detachment. 3. Diabetic retinopathy accompanied by macular oedema, status after argon laser therapy.

3. Inflammatory and angiogenic factors of diabetic retinopathy

3.1 Growth factors

3.1.1 Vascular Endothelial Growth Factor (VEGF)

VEGF is the most potent factor stimulating physiological and pathological angiogenesis. It is a 46-48 kD of molecular weight glycosylated homo-dimer produced by endothelial cells, macrophages, CD4 lymphocytes, plasmatic cells, myocytes, megakaryocytes as well as neoplasm cells (Ferrara et al., 2003, Ferrara, 2004). VEGF induces new blood vessel formation through binding to receptors. The VEGF family encompasses 6 proteins: VEGF-A,-B,-C,-D,-E and PLGF. Best known and most frequently used clinically is VEGF-A. There are also other VEGF isoforms known: VEGF121, VEGF145, VEGF148, VEGF162, VEGF165, VEGF183, VEGF189, VEGF206, with different amino acid chain length, ability to bind heparin, mitogenic activity and VEGF receptor affinity (Ferrara, 2004; Simó et al., 2006; Wirostko et al., 2008). VEGF stimulates proliferation and migration of endothelial cells and increases blood vessel permeability (Aiello et al, 1994; Wirostko et al., 2008). Furthermore it induces production of tissue collagenase and increases macrophage and monocytes chemotaxis. The authors (Matsumoto et al., 2002; Oh et al., 2010; Suzuki et al., 2011)

demonstrated that VEGF increases monocyte chemoattractant protein-1 (MCP-1) mRNA expression. VEGF induces MCP-1 most likely through activation of transcription factors such as NFκB and AP-1 and signalling pathways dependent and independent from ERKs (Mohammad & Kowluru, 2010). It is known that pro-inflammatory protein MCP-1 is a potent attractant for monocytes and has been detected in the majority of patients with proliferative retinopathy. As it causes monocyte/ macrophage infiltration, it leads to vascular abnormalities (Czepluch et al., 2009; Zhang et al., 2009).The hypothesis that monocytes / macrophages participate in the pathogenesis of retinopathy is further supported by the results (Kataoka et al.,2011). The vitreous macrophages are attracted to the pathological vessels induced by retinal ischemia. In children and adolescents with T1DM there was a significant increase of VEGF levels in serum, not only in patients with type 1 diabetes and non-proliferative retinopathy, but also in patients in whom the ophthalmic examination showed no changes in the organ of sight (Chiarelli et al., 2000; Santilli et al., 2002; Zorena et al., 2007; Myśliwiec et al., 2008). Those findings suggest that VEGF may play a role in the development of vascular changes within the eye in children and adolescents already in first few years of disease when available diagnostic methods can't detect retinopathic changes. Furthermore, in other studies (Zorena et al., 2010) it has been noted that VEGF level has been higher in patients with hypertension, retinopathy and nephropathy compared to diabetic patients without hypertension although with retinopathy and nephropathy.In addition, there were no significant differences in VEGF levels between patients with T1DM group without hypertension, but with retinopathy and nephropathy compared to healthy controls group. Furthermore, how does hypertension lead to increased production of VEGF in children and adolescents with retinopathy is not fully known. It is believed that it is a multidirectional process. On one hand it is known that persistent hyperglycaemia leading to the elevation of HbA1c levels may lead to the production and accumulation of advanced glycation end products. Formation of AGEs promotes production of pro-inflammatory cytokines which may further initiate increase of VEGF levels and thus indirectly lead to the development of hypertension in T1DM (Gallego et al., 2008; Roy et al., 2010). It was shown that VEGF binds to VEGFR-2 receptor at the endothelial cell surface which leads to phosphorylation of transcription factors via MAPK (mitogen activated protein kinase) (Suzuma et al., 2001; Wirostko et al., 2008). As a consequence, there is increase in expression of adhesive molecules, cytokines, chemokines which in turn increase proliferation of endothelial cells and production of extracellular matrix with simultaneous impairment of its degradation. This leads to progressive fibrosis and closure of vessels' lumen and subsequently increases blood flow resistance. Higher resistance in vascular system results in further raise in VEGF production which in turn causes changes in structure and ratio of collagen to elastin which makes blood vessels stiffer and leads to increase in blood pressure (Iglesias-de la Cruz et al., 2002; Kvanta et al., 2006; Wirostko et al., 2008). Increased levels of VEGF has been detected in vitreous of adult patients with proliferative diabetic retinopathy (Maier et al., 2008; Marek et al., 2010; Lange et al., 2011). Moreover, it has been demonstrated that high level of VEGF in vitreous of patients with proliferative diabetic retinopathy has been associated with increased VEGF level in serum (Maier et al., 2008). The emerging new therapies based on application of anti VEGF gave promising results in treatment of proliferative diabetic retinopathy (Adamis et al,. 2006; Starita et al., 2007;. Hernández-DaMota et al., 2010; Engelbert et al., 2011). The first drug in this group was sodium pegaptanib (Macugen) with anti VEGF165 properties and has been

administered intravitreally. Pegaptanib has been used as a treatment of both diabetic macular edema (DME) as well as PDR (Adamis et al., 2006; Querques et al., 2009; Hornan et al., 2010). After intravitreal injection of another VEGF-A inhibitor – ranibizumab (registered by FDA in June 2006 as a Lucentis) is good antiangiogenic with vision improvement in 95% of patients (Jorge et al., 2011; Rosenfeld et al., 2011).

3.1.2 Insulin like Growth Factor (IGF-I)

IGF-I is a polypeptide showing high similarity to insulin. Two different forms are distinguished: IGF-I and IGF-II. IGF-I circulates in blood in the form of IGF-binding protein (IGF-BP), probably inhibiting activity of free IGF. IGF-I is a pivotal growth factor secreted as a result of stimulation by human growth hormone. Both in vivo and in vitro studies indicate its anti-apoptotic and anti-inflammatory properties (Goes et al., 1996; Sukhanov et al., 2007; Sun et al., 2010). There are reports that IGF-I has protective actions in ischaemic rat kidney due to inhibition of inflammatory cytokine production (Goes et al., 1996) and anti-apoptotic in Parkinson disease via inhibition of GSK-3β signalling pathway (Sun et al., 2010). IGF-I exerts its protective actions also in central nervous system and cardiomyocytes (Sun et al., 2010). In premature babies a small concentration of IGF-I is a risk factor of retinopathy of prematurity (Pérez-Muñuzuri et al., 2010). IGF-I deficiency after birth may play a role in development and deterioration of neurological deficiencies in premature babies (Lofqvist et al., 2006). On the other hand in children and youth with T1DM and microangiopathy IGF-I levels have been found to be lower compared to group of patients without microangiopathy (Peczyńska et al., 2004). Furthermore, IGF-I levels were lowest in children with T1DM for over 10 years. Interestingly, the same group of children had raised VEGF levels in serum and the longer the duration of the disease, the higher were the levels (with maximum levels in patients with diabetes for over 10 years) (Chiarelli et al., 2000; Santilli et al., 2001; Peczyńska et al. 2004; Zorena et al., 2009). Also much lower IGF-I concentrations were found in adolescents with microangiopathy compared to diabetic patients without complications and healthy children (Wedrychowicz et al., 2005). However, IGFBP-1 levels in serum were much higher whereas IGFBP-3 were lower in patients with microangiopathy compared to those without complications. Thus circulating IGFBP-1 may play a role in development of diabetic complications while IGFBP-3 may be protective (Kielczewski et al., 2011). In adults with PDR, levels of IGF-I and VEGF in vitreous were significantly higher than in control group (Simo et al., 2002; Poulaki et al., 2004; Hartnett et al., 2009). Surprisingly there were no differences in levels of both factors in serum in each group. This effect could be explained by two mechanisms: higher concentration of IGF-I binding protein (IGFBP's) in vitreous may neutralize the increased IGF-I production or may lower the production of free IGF-I in tissues of diabetic patients (Simo et al., 2002). Additional evidence supporting IGF-I in PDR result from use of IGF-I inhibitors. Somatostatin and octreotide, a somatostatin analogue, inhibited IGF-I receptor (IGF-1R) phosphorylation and decreased VEGF production (Sall et al. 2004). Systemic inhibition of IGF-I signalling in a relevant animal model with a receptor-neutralizing antibody, or with inhibitors of PI-3 kinase (PI-3K), c-Jun kinase (JNK), or Akt, suppressed downstream signalling pathways, VEGF expression, ICAM-1 levels, leukostasis, and BRB breakdown. Intravitreal administration of IGF-I increased retinal factors AKT, JNK, HIF-1alpha, NF-κB, AP-1 activity, and VEGF levels. Haurigot et al., 2009 demonstrated that high intra-ocular

concentration of IGF-I in retina is sufficient to activate processes leading to disruption of blood-retina barrier and increase in vascular permeability as a consequence of high expression of IGF-I in retina.

3.1.3 Placental Growth Factor (PLGF)

PLGF is a homodimer protein belonging to VEGF family, showing structural similarity to VEGF-A. Three PLGF isoforms have been described (PLGF-1,-2,-3). Those isoforms do not interact with VEGFR-2 but they bind to VEGFR-1 (Christinger et. al., 2004). PLGF has been recently isolated as a factor, stimulating neovascularization. Over the last decade, the direct or indirect pro angiogenic effect of PLGF was demonstrated during ischaemia, inflammation and wound healing. There are controversies regarding the pro-angiogenic activity of placental growth factor in diabetic retinopathy. In the eye, loss of PLGF does not hamper retinal development (Feeney et al., 2003) but impairs choroidal neovascularization (Rakic et al., 2003). Intravitreal injections of PLGF prevent oxygen-induced retinal ischaemia, without inducing neovascularization (Campochiaro et al., 2006). In animals′ retinal cells only small amounts of PLGF have been detected. However, increased levels have been detected in eyes affected by advanced neovascularization in retina (Khaliq et al., 1998; Miyamoto et al., 2007).

3.1.4 Hepatocyte Growth Factor (HGF)

HGF is a pleiotropic factor derived from mesenchymal tissue regulating growth and migration of various cells. HGF is synthesized mainly in liver but also in lungs, kidney, smooth muscles and corneal endothelial cells (Stoker et al., 1987). It is secreted as a single chain precursor which is activated by proteolytic cleavage. Simó et al., 2006 have detected increased concentrations of both HGF as well as VEGF in vitreous of patients with PDR compared to patients with diabetes but without complications and healthy control group. However, they have not demonstrated relevant correlation between HGF and VEGF. Yoshida et al., 2010 investigating levels of angiopoietin-2 , HGF, bFGF, PDGF, TIMP-1 and TIMP-2 in the vitreous body of PDR patients before and after vitrectomy has shown a marked decrease of HGF as well as angiopoietin-2 levels in the vitreous body of PDR patients after vitrectomy.

3.1.5 Pigment Epithelium Derived Factor (PEDF)

PEDF also known as serpin F1 (SERPINF1), is a multifunctional secreted protein that has anti-angiogenic and neurotrophic functions. Found in vertebrates, this 50 kD protein holds promise in the treatment of such conditions as heart disease, cancer and choroidal neovascularization (Filleur et al., 2009). PEDF is secreted by many retinal cells including Müller cells, endothelial cells, pericytes, and pigment epithelium cells of retina (Doll et al., 2003; Barnstable et al., 2004; Tombran-Tink et al., 2010). Studies conducted on PEDF depleted mice, showed that lack of this gene results in serious abnormalities in both, cell differentiation as well as in retinal morphology (Doll et. al., 2003). In 2003 the first hypothesis emerged that PEDF may hamper angiogenesis by direct reduction of VEGF gene expression (Yamagishi et al., 2003). Most recent data confirm also that PEDF has direct effect on vascular endothelial growth factor receptor 1 (VEGFR-1) by increasing g-secretase

complex activity (Cai et al., 2011). Activation of g-secretase leads directly to proteolytic cleavage within VEGFR-1 and C-end domain of receptor is removed which subsequently migrates from cell membrane into cytosol (Cai et al., 2006; Cai et al., 2011). Additionally, PEDF inhibits production of reactive oxygen species (ROS) and MCP-1 and furthermore, it neutralizes negative effects of AGE (Inagaki et al., 2003). Other studies suggest that transcription factor, NF-κB and Fas ligand and its receptor play a role in PEDF mechanism of action. Volpert et. al. 2002, proved that anti-FasL antibodies and application of inhibitors hampers PEDF action. PEDF may equally prevent cell apoptosis by activation of transcription factor NF-κB, as well as lead to programmed cell deaths via increasing Fas ligand expression (Volpert et al., 2002). Studies (Cai et al., 2006) have demonstrated that PEDF used in bovine retinal microvascular endothelial cells culture (BRMECs) did not affect their migration and formation of primary vessel canals. However, when an addition of PEDF had been preceded by application of VEGF then this factor reduced significantly proliferation and endothelial cells migration induced by VEGF. Studies evaluating the antiangiogenic properties of PEDF have also shown that exerts a modulating effect on the formation of new retinal blood vessels and promote angiogenesis in hypoxia (Barnstable et al., 2004; Elayappan et al., 2009; Subramanian et al., 2011).

3.1.6 Basic fibroblast Growth Factor (bFGF)

Fibroblast growth factors, or FGFs, are a family of growth factors involved in wound healing, embryonic development, and angiogenesis. There are two FGF distinguished: acidic (aFGF) and basic (bFGF), the latter being attributed to play the most significant role in angiogenesis. This factor has an ability to directly activate endothelial cells, it also affects proliferation, migration of endothelial cells and fibroblasts, induces proteolytic enzymes and synthesis of fibronectin, collagen, proteoglycans and hyaluronic acid (Shi et al., 2011). Synthesis of bFGF takes place in retina cells as well as in cornea and is activated by inflammation or a local injury (Polykandriotis et al., 2011). It has been suggested that bFGF exerts its paracrine effects on the eye by inhibition of apoptosis. Other studies have demonstrated that beta-FGF works via two pathways a calcium independent FGFR1 through PI 3-K, P70(S6K) and Akt to increased VEGF from the RPE and a calcium dependant FGFR2. The inhibition of those two pathways suppresses bFGF-induced choroidal endothelial cells proliferation (Rosenthal et al., 2005). Deissler et al., 2011, have discovered that VEGF165 but not bFGF is mainly responsible for changes in cell permeability observed in retinal endothelium.

3.1.7 Transforming Growth Factor beta (TGF-β)

TGF-β is a member of transforming growth factor family which has immunomodulatory function. It is secreted primarily by monocytes, macrophages, lymphocytes, and dendritic cells. This cytokine takes part in angiogenesis, stimulates synthesis and degeneration of extracellular matrix proteins, regulates induction of apoptosis and stimulates proliferation of mesenchymal cells.

TGF-β exists in three isoforms coded by different genes β1, β2, β3; best known is TGF- β1 (Bertolino et al., 2005; Orlova et al., 2011) . TGF-β is believed to be the most important ligand in the pathogenesis of fibrotic diseases in the eye. Such ocular fibrotic diseases include

scarring in the cornea and conjunctiva, fibrosis in the corneal endothelium, post-cataract surgery fibrosis of the lens capsule, excess scarring of tissue around the extraocular muscles in the strabismus surgery and proliferative vitreoretinopathy (Saika et al., 2009; Sumioka et al., 2011; Hills et al., 2011). Those properties of TGF-β are confirmed in animal models (Yingchuan et al., 2010; Kowluru et al., 2010) as well as in patients with diabetic retinopathy (George et al., 2009; Abu El-Asrar et al., 2010). It is believed that TGF-β plays a role in pathogenesis of diabetic retinopathy via hyperglycaemia and inflammation. Kowluru et al., 2010 have reported that both the duration of the initial exposure to high glucose, and normal glucose that follows high glucose, are critical in determining the outcome of the alterations in the inflammatory mediators such as IL-1 beta, NF-kB, VEGF, TNF-α including with TGF-β in retinal.

3.1.8 Angiogenin

Angiogenin is a small protein that is implicated in angiogenesis. Angiogenin mRNA expression is detectable in epithelial cells, fibroblasts and blood cells (Tello-Montoliu et al., 2006). Higher level of angiogenin was observed in serum in children and adolescents with non-proliferative retinopathy as compared to the group of children and adolescents without DR (Chiarelli et al., 2002; Raczyńska K et al., 2008). Maier et al., 2006 have revealed that angiogenin level has been increasing in the vitreous in diabetic patients. Nevertheless it is suggested that the increased level was an effect of blood-retina barrier disruption and leakage of growth factors from blood vessels into eye (Maier et al., 2006). Lower levels of serum angiogenin was demostrated in patients with type 2 diabetes (Siebert et al., 2007; Siebert et al., 2010). Lower level of angiogenin but higher VEGF were found also in the vitreous of patients T1DM with PDR (Marek et al., 2011).

4. Cytokines

4.1 Interleukin 6 (IL-6)

Interleukin 6 has been well known as a pro-inflammatory cytokine. However, there is an increasing number of reports about its anti-inflammatory character (Sanchez et al., 2003; Sappington et al., 2006; Nandi et al., 2010). *In vitro* in presence of increased pressure and injury resulting from ischaemia, IL-6 inhibited apoptosis of retinal ganglion cells (Sanchez et al., 2003; Sappington et al.,2006). On the other hand (Dace et al., 2008) M1 macrophages prevented neovascularisation within retina due to high secretion of IL-6, IL-12 and IL-23. Although, majority of reports confirm its negative role in the onset and progression of diabetic retinopathy (Noma et al., 2009; Funk M et al., 2010; Koleva-Georgieva et al., 2011). IL-6 is produced mainly by macrophages, monocytes, lymphocytes T and B, while in the eye IL-6 is produced by keratocytes, Müller cells, pigmented epithelium, corneal epithelium, iris and ciliary body (Yoshida et al., 2001). IL-6 secretion is activated by hypoxia, AGEs, and PKC (protein kinase C) (Giacco & Brownlee, 2010; Adamiec-Mroczek et al., 2010; Lange et al., 20011). In children and adolescents with diabetic retinopathy, higher levels of IL-6 were demonstrated in serum (Lo Hui-Chen et al., 2004; Zorena et al., 2007; Myśliwiec et al., 2008 , Bradshaw et al., 2009). A significant increase in the level of IL-6 was found in PDR patients compared to NPDR and healthy children. Authors

recorded significant gradation in the IL-6 increase when comparing healthy children, children with T1DM without abnormalities in the eyes, and diabetic children with non proliferative diabetic retinopathy. Higher IL-6 and TNF levels in diabetic children are attributed to worse metabolic balance and chronic inflammation (Zorena et al., 2007; Myśliwiec et al., 2008). Apart from IL-6 influence on the set and progression of diabetic retinopathy in young patients with diabetes, higher levels of VEGF and C-reactive protein were also found (Coulon at al., 2005; Zorena et al., 2007). Coulon et al., discovered in their studies that children with long standing diabetes and retinopathy as well as nephropathy had five times higher levels of CRP than patients with diabetes but without complications (Coulon et al., 2005). Authors concluded that high level of pro-inflammatory cytokines in long standing diabetes is a result of ongoing inflammatory process. In inflammatory conditions IL-6 levels in serum may increase even 100 times, therefore IL-6 is regarded as an early and sensitive but non-specific indicator of inflammatory process affecting the organism (Abrahamsson et al, 1997). High IL-6 levels have been observed also in patients with type 2 diabetes and retinopathy as compared to patients without retinopathy (JH Lee et al., 2008; Goldberg., 2009). Also in aqueous from eyes with diabetic macular edema (Funk et al. 2010) found to be significantly increased IL-6 and VEGF. When patients were given bevacizumab, it was noted that VEGF levels dropped below physiological levels. Oh et al., 2010, demonstrated positive correlation between the aqueous levels of IL-6 and macular thickness indicating that IL-6 may play a central role in the development of diabetic macular edema. Other studies concerned the increased levels of IL-6, TNF, ET-1 vWF, sE-selectin in vitreous detected in patients with type 2 diabetes and PDR (Adamiec-Mroczek et al., 2010). Furthermore, authors noted correlation between TNF-α, ET-1 and HbA1c suggesting that there is close relation between metabolic equilibrium and inflammatory factors in T2DM patients. Thus, those studies support pro-inflammatory and proangiogenic role of IL-6.

4.2 Interleukin 10 (IL- 10)

Several recent studies reported that local IL -10 production may lead to angiogenesis in retina. Studies demonstrated that IL-10 may polarize macrophages in proangiogenic direction (Apte RS., 2006, Kelly et al., 2007, Dance et al., 2008). In macrophages of C57BL/6 mice with induced retinopathy, there has been significantly higher proangiogenic cytokine gene expression. This hasn't been found in macrophages of mice with IL-10 -/- phenotype (Dace et al., 2008). In children and adolescents with long-standing type 1 diabetes more than 60% of patients with symptoms of diabetic retinopathy showed no activity of this cytokine in serum. IL-10 levels analysis in group of children with T1DM and various grades of diabetic complications in the eye suggests that higher secretion of IL-10 may protect from late complications (Myśliwiec et al., 2006). Also, there were no significant differences in the IL-10 levels in the vitreous of patients with proliferative retinopathy as compared to those without PDR (Hernandez et al., 2005). However in T2DM patients, IL-10 was progressively lower with more advanced retinopathy (JH Lee et al., 2008). Suzuki et al., 2011 demonstrated higher levels of IL-10 as well as positive correlation between IL-10 and VEGF in patients with PDR compared to those with central retinal vein occlusion (CRVO).

4.3 Interleukin 12

Interleukin 12 (IL 12) has been described initially as a factor stimulating natural cytotoxic cells and causing maturation of cytotoxic lymphocytes. Under physiological circumstances it is produced mainly by macrophage, dendritic cells, keratinocytes, granulocytes, and mast cells (Trinchieri, 1998). In vivo, it was demonstrated that IL-12 is a potent antiangiogenic cytokine and this effect is mediated through interferon-γ (Voest et al., 1995). Few studies conducted in recent years indicate that IL-12 does not affect *per se* endothelial cells, but just by IFN-γ (Voest et al., 1995). This in turn regulates production of second line chemokines via induction of protein (IP)-10 being recognized as the most important mediator for IL-12 in angiogenesis activation (Sgadari et al., 1996). Furthermore, another report suggests that inhibition of IL-12 production may be mediated by natural killer cells (NK) (Ghiringhelli et al., 2006). A significantly lower IL-12 level but higher TNF and VEGF levels were found in a group of children and adolescents with diabetes and NPDR as compared to a group of patients without DR (Zorena et al., 2007). Another study (Zorena et al., 2008) reported imbalance between pro and antiangiogenic factors in serum of children and adolescents with long term T1DM. This imbalance was demonstrated between TNF-α and IL-12. It has been observed that patients who had both low TNF-α and IL-12 levels did not develop diabetic complications like retinopathy or nephropathy. However, when patients had high level of TNF-α and absent IL-12 they developed microangiopathic complications. Shift in balance towards TNF-α promotes late diabetic complications. Loss of equilibrium between pro and antiangiogenic actions of TNF-α and IL-12 underpins late diabetic complications. Applied monoclonal antibody against p40 subunit of IL-12 – Uteskinumab gave positive results in a patient with Crohn's disease (Sansó Sureda et al., 2011).

4.4 Tumor Necrosis Factor alpha (TNF-α)

TNF-α is one of most important inflammatory cytokines. It is produced primarily by monocytes and macrophages on which it exerts its endo-, para- and autocrine actions. It stimulates cytotoxic properties of monocytes and macrophages and simultaneously is a mediator of cytotoxicity. Its biological effects depends strongly on quantity and intensity of TNF-α secretion. Apart from taking part in inflammatory processes it also plays important role in neovascularisation (Wilson &Balkwill, 2002). TNF-α exerts versatile effects due to its ability to induce synthesis of other cytokines functionally related to TNF-α, extracellular matrix proteins, monocyte and fibroblast chemotaxis modulation and also influences the expression of adhesive molecules in retinal vessels (Doganay et al.,2002; Naldini & Carraro, 2005). Teflon implants soaked in 3.5 ng TNF-α and implanted into rat cornea caused a visible growth of new blood vessels after 7 days (Fajardo et al., 1992). A similar effect was achieved on chicken embryo membranes (Hooper et al., 2005). However, (Patterson et al., 1996) showed an antiangiogenic action of TNF-α using human endothelial cells. The authors showed that incubation of those cells with known proangiogenic factor VEGF for 24h augmented their proliferative activity more than two fold, whereas 12h pre-incubation abolished this effect. However, TNF-α alone revealed a weak cytotoxic effect towards endothelial cells. Inhibition of endothelial cell proliferation by TNF-α was associated with reduction in VEGFR-2 (KDR/Flt-1) receptor mRNA transcription level which depends on dose and the duration of cytokines. Low concentrations of TNF-α can trigger signalling pathways through p55 and p75 receptor, but in high concentrations only through p55 (Bigda

et al., 1994). In young patients with newly diagnosed diabetes increased activity of TNF-α has been demonstrated (Myśliwiec et al, 2006). High levels of TNF-α have been detected also in type 1 diabetic children and adolescents with non-proliferative retinopathy (Myśliwiec et al, 2006, Zorena et al. 2007). TNF-α may become a relevant indicator of development and risk of diabetic retinopathy. Similar observations were made in adult patients with PDR (Gustavsson et al., 2008; Koleva–Georgieva et al., 2011). Levels of TNF-α in vitreous of Type 2 diabetic patients with PDR were higher than those found in control group. Furthermore a correlation between TNF-α and HbA1c is observed, suggesting that there is a close relation between glycaemic control and inflammatory factors in T2DM patients (Adamiec-Mroczek et al., 2008; Lee JH, 2008). TNFRI and TNFRII receptors' levels in vitreous of patients with PDR and proliferative vitreoretinopathy were much higher than in patients with perforation in macula (Limb et al., 2001). Attempts are being made to block TNF-α actions with monoclonal antibodies (Sfikkakis et al., 2010; Giganti et al., 2010; Biswas et al., 2010). In randomized studies, the intravenous use of infliximab has improved visual acuity in patients with diabetic macular edema (DME) (Sfikakis et al., 2010).

Etanercept is a soluble TNF-α receptor that acts as competitive inhibitor blocking effects of TNF-α binding to cells. Etanercept reduced leukocyte adherence in retinal blood vessels of diabetic rats for 1 week as compared to control. Etanercept did not reduce retinal VEGF levels, but it inhibited blood-retinal barrier breakdown and NF-κB activation in the diabetic retina (Joussen et al., 2002; Zheng et al., 2004).

5. Adipocytokines

5.1 Leptin

Leptin was discovered as a first adipocytokine in 1994 (Zhang Y et al., 1994). Team of researchers (Ates et al., 2008) have found relationship between leptin and retinal vein occlusion. The authors suggest that leptin may play a role in pathogenesis of retinal vein occlusion probably by influencing vessel wall homeostasis. In diabetes, leptin may affect angiogenesis process by induction of vascular endothelial growth factor (VEGF) and suppression of pigment epithelium derived factor (PEDF) and also production of intracellular reactive oxygen species (ROS) in retinal vascular endothelial cells. (Gariano et al. 2000, Yamagishi et al., 2003). However, in other studies the authors reported no association between leptin and the development and progression of retinopathy (Sari et al., 2010).

5.2 Adiponectin

Adiponectin is a 28 kD protein and its structure is similar to TNF-α, collagen VIII and IX and C1q molecule of complement. As demonstrated in vitro adiponectin exerts antyaterogenne, by inhibiting the adhesion of monocytes to endothelial cells and the transformation of macrophages into foam cells (Diez & Iglesias, 2003; Tan et al., 2004; Abi Khalil et al., 2011). Zhou et al., 2011 demonstrated that adiponectin hampers monocytes adhesion to endothelial cells in blood vessel walls by reduction in adhesive protein expression. The study also reported that adiponectin inhibits macrophages transformation into foam cells, reduces smooth muscle proliferation, increases NO synthesis and stimulates angiogenesis. Several studies present adiponectin as an anti-inflammatory cytokine (Matzuzawa et al., 2005; Goldberg et al., 2009; Zhou et al., 2011). In vitro studies revealed that TNF-α inhibits

adiponectin gene expression through inhibition of nuclear factor NFκB, which is activated by adiponectin. Increasing insulin resistance and growth of adipose tissue increases TNF-alpha expression leading to a decrease of adiponectin concentration (Savino et al., 2008). Additional studies have shown that adiponectin directly stimulates the production of IL10 by macrophages and decreases the production of proinflammatory cytokines TNF-α and IL6 (Wulster-Radcliffe et al., 2004, Kumada et al., 2004, Kollias et al., 2011, Zhou et al., 2011). Adiponectin inhibits adhesive molecules expression in endothelial cells and also production of cytokines in macrophages thus reduces the inflammatory process which is present in early stages of atherosclerosis and microangiopathy (Diez & Iglesias, 2003; Goldberg et al., 2009). Increase in adiponectin level in serum is thought to be a response to endothelium damage (Schalwijk et al., 2006; Goldberg et al, 2009).Correlation was found between severity of diabetic retinopathy and adiponectin in patients with T1DM and T2DM (Frystyk et al., 2005; Zietz et al., 2008; Kato et al., 2008).

6. Chemokines

6.1 Stromal Cell-Derived Factor (SDF-1)

SDF-1 is a small cytokine of the chemokines family (C-X-C motif) or a ligand 12 (CXCL12). SDF-1 plays an important role in the angiogenesis by recruiting endothelial progenitor cells from bone marrow (Unoki et al, 2010). In the animal model it has been shown that VEGF, SDF-1 alpha and CXCR-4 all play part in the development of diabetic retinopathy. An increase in SDF-1 alpha expression has been observed, and it correlated with the duration of the disease(Lin et al., 2009) as well as with the level of pro-inflammatory cytokines IL-6 and IL-8 (Otsuka et al, 2010). An increase in SDF-1 concentration in the vitreous has been observed also in patients with proliferative retinopathy, the more pronounced, the more acute had been the disease course (Meleth et al., 2005; Chen et al., 2008).

6.2 Monocyte Chemotactic Protein-1 (CCL2/MCP-1)

Chemokine (C-C motif) ligand 2 (CCL2) is a small cytokine belonging to the CC chemokine family that is also known as monocyte chemotactic protein-1 (MCP-1) and small inducible cytokine A2. CCL2 is a monomeric polypeptide, with a molecular weight of approximately 13kD (Yoshimura & Leonard., 1992). The cell surface receptors that bind CCL2 are CCR2 and CCR4 (Xia & Sui.,2009). MCP-1, as well as its interaction with CCR2, plays a pivotal role in mediating persistent mononuclear phagocyte infiltration that leads to chronic inflammatory (Romagnani et al., 2004). Inhibition of CCL2-CCR2 signalling blocks the recruitment of inflammatory monocytes, inhibits metastasis in vivo and prolongs the survival of tumour-bearing mice (Qian et al., 2011). Also, MCP-1 has been recognized as an angiogenic chemokine (Strieter et al.,2005). In vivo angiogenesis assays showed that MCP-1-induced angiogenesis was as potent as that induced by vascular endothelial growth factor (VEGF). The angiogenic effect of MCP-1 was completely inhibited by a VEGF inhibitor, suggesting that MCP-1-induced angiogenesis is mediated through pathways involving VEGF (Kim et al., 2005). In young patients with long term diabetes and microangiopathy levels of CCL2/MCP-1 were higher than in those without microangiopathy (Zorena et al., 2010). The authors suggest active role of this chemokine in onset and development of diabetic retinopathy. Increased level of CCL2/MCP-1 was higher also in the vitreous of

adult patients in PDR. (Hernandez et al., 2005; Abu El-Asrar et al., 2006). The aqueous levels of MCP-1, IP-10, IL-8, and VEGF were higher in the eyes of diabetic patients than in the eyes of non-diabetic subjects. The aqueous levels of MCP-1 and IP-10 were elevated in the eyes with severe NPDR and PDR compared to eyes with less severe DR and eyes of non-diabetic subjects (Oh et al., 2010).

7. Matrix metalloproteinases (MMPs) and metalloproteinase inhibitors (TIMP)

7.1 Metalloproteinases (MMPs)

Increased levels of metalloproteinases such as MMP-2, MMP-9 and MMP-14 have been demonstrated in early stages of retinopathy (Giebel et al., 2005). It has been demonstrated that high glucose increases the production of MMP-9 in retina cells (Kovluru et al., 2010; Mohammad & Kowluru, 2011). Increased level of MMPs leads to faster degradation of basement membrane proteins thereby increasing permeability. Pericyte apoptosis and basement membrane changes result in dilatation of capillaries and formation of microaneurisms. These conditions impair blood flow and in consequence lead to ischaemia in the retina. Disruption of physiological blood-retina barrier leads to formation of hard exudates. Retinal hypoxia worsens abnormalities in MMP/TIMP system caused by hyperglycaemia leading to excessive production of basement membrane material and proliferation of pathological capillaries. During retinal hypoxia the production of growth factors including VEGF increases, which stimulates metalloproteinases expression in extracellular matrix. It has been noted that in proliferative retinopathy the MMP-2 and MMP-9 activity increases (Jacqueminet et al., 2006, Kowluru et al., 2010).

7.2 Tissue inhibitor of matrix metalloproteinase 3 (TIMP-3)

Tissue inhibitor of matrix metalloproteinase 3 (TIMP-3) belongs to zinc (Zn) binding endopeptidases group which takes part in remodelling and degradation of extracellular matrix. In the eye TIMP-3 is closely related to Bruch's membrane and regulates angiogenesis (Janssen et al., 2008). Analysis of micromatrix demonstrated that out of investigated metalloproteinases and its inhibitors: MMP1, MMP2, MMP11, MMP14, MMP25, TIMP1, TIMP2, TIMP3 and TIMP4 which were present in pericytes, only TIMIP3 mRNA may play role in impairment of microcirculation in diabetic retinopathy (Barth et al., 2007).

8. Conclusion

In developed countries diabetic retinopathy has become the most prevalent cause of blindness and loss of visual acuity, and its incidence is on the rise. Occurrence and progression of diabetic retinopathy may be a result of activation of immunological cells in the metabolic imbalance of the disease. Late diabetic complications may also reflect the ongoing autoimmunological process that started already in the *prediabetic* period. It has been proved that plasma levels of pro-inflammatory/pro-angiogenic factors was higher in patients with proliferative retinopathy than in those with diabetes but without morphological changes in the eye fundus. Moreover, high concentrations of VEGF, TNF-α, IL-6 has been found not only in plasma but also in the vitreous of PDR patients. High hopes have been placed on the use of monoclonal antibodies anti-VEGF and anti-TNF. Particularly

etanercept, bevacizumab and ranibizumab have been used to prevent ocular neovascularisation. However only concomitant use of several therapeutic methods is regarded as effective in achieving plausible results in treatment of proliferative retinopathy.

9. References

Abrahamsson J, Påhlman M, Mellander L. (1997). Interleukin 6, but not tumour necrosis factor-alpha, is a good predictor of severe infection in febrile neutropenic and non-neutropenic children with malignancy. *Acta Paediatr.*, 86(10):1059-64.

Abi Khalil C, Mohammedi K, Aubert R, Abou Jaoude E, Travert F, Hadjadj S, Fumeron F, Roussel R, Marre M.(2011). Hyperadiponectinemia is independent of kidney function, diabetes duration, and control in type 1 diabetic patients without microangiopathy. *J Clin Endocrinol Metab.*,96(3):E485-7

Abu El-Asrar A.M., Struyf S., Kangave D. (2006). Chemokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. *Eur Cytokine Netw*, 17, 155-65.

Abu El-Asrar AM, Missotten L, Geboes K. (2010). Expression of advanced glycation end products and related molecules in diabetic fibrovascular epiretinal membranes. *Clin Experiment Ophthalmol.*, 38(1):57-64; .

Adamiec-Mroczek J, Oficjalska-Młyńczak J. (2008). Assessment of selected adhesion molecule and proinflammatory cytokine levels in the vitreous body of patients with type 2 diabetes--role of the inflammatory-immune process in the pathogenesis of proliferative diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol,* 246(12):1665-70

Adamiec-Mroczek J, Oficjalska-Młyńczak J, Misiuk-Hojło M. (2010). Roles of endothelin-1 and selected proinflammatory cytokines in the pathogenesis of proliferative diabetic retinopathy: Analysis of vitreous samples. *Cytokine*, (49) 3: 269-74

Adamis AP, Altaweel M, Bressler NM, Cunningham Jr ET, Davis MD, Goldbaum M (2006). Changes in retinal neovascularization after pegaptanib (Macugen) therapy in diabetic individuals. *Ophthalmology.;*113:23–8.

Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. (1994). Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 331: 1480–1487.

Apte RS, Richter J, Herndon J, Ferguson TA. (2006). Macrophages inhibit neovascularization in a murine model of age-related macular degeneration. *PLoS Med.* 3:e310.

Ates O, Keles M, Bilen H, Kiziltunc A, Kocer I, Kulacoglu DN, Türkeli M, Baykal O. (2008). Increased serum levels of leptin in retinal vein occlusion. *Tohoku J Exp Med.*, 215(4):373-6.

Barnstable C, Tombran-Tink J. (2004). Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Prog. Retin Eye Res*., 23: 561–577

Barth JL, Yu Y, Song W, Lu K, Dashti A, Huang Y, Argraves WS, Lyons TJ. (2007). Oxidised, glycated LDL selectively influences tissue inhibitor of metalloproteinase-3 gene expression and protein production in human retinal capillary pericytes. *Diabetologia,* 10: 2200-8.

Bertolino P, Deckers M, Lebrin F, Ten Dijke P. (2005). Transforming growth factor-β signal transduction in angiogenesis and vascular disorders. *Chest*, 128(6 Suppl):585S-590S.

Bigda J, Beletsky C, Brakebusch Y. (1994). Dual role of the p75 tumor necrosis factor (TNF) receptor in TNF cytotoxicity. *J Exp Med*, 180, 2, 445-460

Biswas NR, Das GK, Dubey AK. (2010). Monoclonal antibodies in ophthalmology. *Nepal Med Coll J.*,12(4):264-71.

Bradshaw EM, Raddassi K, Elyaman W, Orban T, Gottlieb PA, Kent SC, Hafler DA. (2009). Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. *J Immunol,* 183(7):4432-9

Campa C, Costagliola C, Incorvaia C, Sheridan C, Semeraro F, De Nadai K, Sebastiani A, Parmeggiani F. (2010). Inflammatory mediators and angiogenic factors in choroidal neovascularization: pathogenetic interactions and therapeutic implications. *Mediators Inflamm.* pii: 546826

Campochiaro PA. (2006). Potential applications for RNAi to probe pathogenesis and develop new treatments for ocular disorders. *Gene Ther.,*13(6):559–562

Caporali A, Emanueli C. (2011). MicroRNA regulation in angiogenesis. *Vascul Pharmacol,* Jul 14. [Epub ahead of print]

Cai J, Jiang WG, Grant MB, Boulton M. (2006). Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1. *J. Biol. Chem,* 281: 3604–3613

Cai J, Wu L, Qi X, Li Calzi S, Caballero S, Shaw L, Ruan Q, Grant MB, Boulton ME. (2011). PEDF Regulates Vascular Permeability by a γ-Secretase-Mediated Pathway. *PLoS One.* 6(6):e21164

Carmeliet P. (2003). Angiogenesis in health and disease. *Nat Med*, 9: 653–660.

Chen L, Lü L, Li Y, Huang X, Zhang J, Li S. (2008). Vitreous levels of stromal cell-derived factor-1 and vascular endothelial growth factor in diabetic retinopathy. *Yan Ke Xue Bao*, 24(1):6-8

Chiarelli F, Spagnoli A, Basciani F, Tumini S, Mezzetti A, Cipollone F, Cuccurullo F, Morgese G, Verrotti A. (2000). Vascular endothelial growth factor (VEGF) in children, adolescents and young adults with type 1 diabetes mellitus: relation to glycaemic control and microvascular complications. *Diabet Med*, 17(9):650-6.

Chiarelli F, Pomilo M, Mohn A, Tumini S, Verrotti A, Mezzetti A, Cipollone F, Wasniewska M, Morgese G, Spagnoli A. (2002). Serum angiogenin concentrations in young patients with diabetes mellitus. *Eur J Clin Invest*, 32(2):110-4.

Cho YH, Craig ME, Hing S, Gallego PH, Poon M, Chan A, Donaghue KC (2011). Microvascular complications assessment in adolescents with 2- to 5-yr duration of type 1 diabetes from 1990 to 2006. *Pediatr Diabetes* [Epub ahead of print] Article by DOI 10.1111/j.1399-5448.2011.00762

Christinger HW, Fuh G, de Vos AM, Wiesmann C. (2004). The crystal structure of placental growth factor in complex with domain 2 of vascular endothelial growth factor receptor-1. *J Biol Chem,* 279:10382–10388

Coulon J, Willems D, Dorchy H. (2005). Increase in C-reactive protein plasma levels during diabetes in infants and young adults. *Presse Med.*,34(2 Pt 1):89-93.

Curtis TM, Gardiner TA, Stitt AW. (2009). Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis? *Eye (Lond)*, 23(7):1496-508

Czepluch FS, Zweigle B, Waltenberger J. (2009). Chemotaxis analysis of circulating monocytes in patients with a recent acute coronary syndrome. *Atherosclerosis.* 204(1):304-8

D'Adamo E, Caprio S. (2011). Type 2 diabetes in youth: epidemiology and pathophysiology. *Diabetes Care*. 34 Suppl 2:S161-5.

Dace DS, Apte RS. (2008). Effect of senescence on macrophage polarization and angiogenesis. *Rejuvenation Res.*11(1):177-85

Dace DS, Khan AA, Kelly J, Apte RS. (2008). Interleukin-10 promotes pathological angiogenesis by regulating macrophage response to hypoxia during development. *pLoS ONE*; 3(10):e3381.

Deissler HL, Deissler H, Lang GE. (2011). Inhibition of vascular endothelial growth factor (VEGF) is sufficient to completely restore barrier malfunction induced by growth factors in microvascular retinal endothelial cells. *Br J Ophthalmol*, 95(8):1151-6

Diez J, Iglesias P. (2003). The role of novel adipocyte –derived hormone adiponectin in human disease. *Eur J Endocrinol*, 148,288-293

Doganay S, Evereklioglu C, Turkoz ERH, Sevinc A, Mehmet N, Savli H. (2002). Comparision of serum NO, TNFα, IL-1β, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. *Eye,*16:163-70.

Doll J.A., Stellmach V.M., Bouck N.P., Bergh A.R., Lee C., Abramson L.P., Cornwell M.L., Pins M.R., Borensztajn J., Crawford S.E. (2003). Pigment epithelium-derived factor regulates the vasculature and mass of the prostate and pancreas. *Nat. Med*., 9: 774-780

Durham JT, Herman IM. (2011). Microvascular modifications in diabetic retinopathy. *Curr Diab Rep*.;11(4):253-64.

Elayappan B, Ravinarayannan H, Pasha SP, Lee KJ, Gurunathan S (2009). PEDF inhibits VEGF- and EPO- induced angiogenesis in retinal endothelial cells through interruption of PI3K/Akt phosphorylation. *Angiogenesis,*12(4):313-24.

Engelbert M, Zweifel SA. and Freund KB. (2010;2011). Long-term follow-up for type 1 (subretinal pigment epithelium) neovascularisation using a modified "treat and extend" dosing regimen of intravitreal antivascular endothelial growth factor therapy, *Retina,* 30(9):1368-75. Erratum in: *Retina.* 2011 Jan;31(1):208.

Fajardo LF Kwan HH, Kowalski J, Prionas SD, Allison AC. (1992). Dual role of tumor necrosis factor-alpha in angiogenesis. *Am J Pathol,*140(3):539.

Feeney SA, Simpson DA, Gardiner TA, Boyle C, Jamison P, et al. (2003). Role of vascular endothelial growth factor and placental growth factors during retinal vascular development and hyaloid regression. *Invest Ophthalmol Vis Sci*.;44(2):839–847

Ferrara N, Gerber HP, LeCouter J. (2003). The biology of VEGF and its receptors. *Nat Med*. 2003;9:669–676

Ferrara N. (2004). Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev*, 25: 581-611.

Filleur S, Nelius T, de Riese W, Kennedy RC (2009). Characterization of PEDF: a multi-functional serpin family protein. *J. Cell. Biochem,* 106: 769–75

Folkman J (1971): Tumour angiogenesis: therapeutic implications. *N Engl J Med*, 285: 1182–1185.

Fong, D.S. Aiello, L.P. Ferris F.L. III and Klein R. (2004). Diabetic retinopathy, *Diabetes Care* 27, pp. 2540–2553

Frystyk J, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A. (2005). Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. *Diabetologia.* 48(9):1911-8

Funk M, Schmidinger G, Maar N, Bolz M, Benesch T, Zlabinger GJ, Schmidt-Erfurth UM. (2010). Angiogenic and inflammatory markers in the intraocular fluid of eyes with diabetic macular edema and influence of therapy with bevacizumab. *Retina*. 30(9):1412-9.

Gallego PH, Craig ME, Hing S, Donaghue KC (2008).. Role of blood pressure in development of early retinopathy in adolescents with type 1 diabetes: prospective cohort study. *BMJ*, 337: a918.

Gariano R, Nath A, D'Amico D. (2000). Elevation of vitreous leptin in diabetic retinopathy and retinal detachment. *Invest Ophthalmol Vis Sci,* 41: 3576-81.

George B, Chen S, Chaudhary V, Gonder J, Chakrabarti S. (2009). Extracellular matrix proteins in epiretinal membranes and in diabetic retinopathy. *Curr Eye Res.* Feb;34(2):134-44.

Gerhardinger C, McClure KD, Romeo G, Podesta F, Lorenzi M. (2001). IGF-I mRNA and signaling in the diabetic retina. *Diabetes.* 50: 175–183.

Ghiringhelli F, Menard C, Martin F, Zitvogel L (2006). The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol Rev*, 214: 229-238.

Giacco F, Brownlee M. (2010). Oxidative stress and diabetic complications. *Circ Res.*,107(9):1058-70

Giebel SJ, Menicucci G, McGuire PG, Das A.(2005). Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. *Lab Invest*, 85(5):597-607.

Giganti M, Beer PM, Lemanski N, Hartman C, Schartman J, Falk N. (2010). Adverse events after intravitreal infliximab (Remicade). *Retina*. 30(1):71-80.

Goes N, Urmson J, Vincent D, Ramassar V, Halloran PF. (1996). Effect of recombinant human insulin- like growth factor-1 on the inflammatory response to acute renal injury. *J. Am. Soc. Nephrol.*, 7, 710-720.

Goldberg RB. (2009). Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. *J Clin Endocrinol Metab*.94(9):3171-82.

Gustavsson C, Agardh E, Bengtsson B, Agardh CD. (2008). TNF-alpha is an independent serum marker for proliferative retinopathy in type 1 diabetic patients. *J Diabetes Complications,* 22(5):309-16.

Hartnett ME, Tinkham N, Paynter L, Geisen P, Rosenberg P, Koch G, Cohen KL. (2009). Aqueous vascular endothelial growth factor as a predictor of macular thickening following cataract surgery in patients with diabetes mellitus. *Am J Ophthalmol*.148(6):895-901.e

Haurigot V, Villacampa P, Ribera A, Llombart C, Bosch A, Nacher V, Ramos D, Ayuso E, Segovia JC, Bueren JA, Ruberte J, Bosch F.(2009). Increased intraocular insulin-like growth factor-I triggers blood-retinal barrier breakdown. *J Biol Chem.* 284(34):22961-9

Hernández-Da Mota and Nuñez-Solorio S.M. (2010). Experience with intravitreal bevacizumab as a preoperative adjunct in 23-G vitrectomy for advanced proliferative diabetic retinopathy, *Eur. J. Ophthalmol.* 20, pp. 1047–1052

Hernández C, Segura RM, Fonollosa A, Carrasco E, Francisco G, Simó R. (2005). Interleukin-8, monocyte chemoattractant protein-1 and IL-10 in the vitreous fluid of patients with proliferative diabetic retinopathy. *Diabet Med*. 22, 719-22.

Hills CE, Squires PE. (2011). The role of TGF-β and epithelial-to mesenchymal transition in diabetic nephropathy. *Cytokine Growth Factor Rev*, 2011 Jul 12. [Epub ahead of print]

Hooper LC, Chin MS, Detrick B, Hooks JJ. (2005). Retinal degeneration in experimental coronavirus retinopathy (ECOR) is associated with increased TNF-alpha, soluble TNFR2 and altered TNF-alpha signaling. *J Neuroimmunol.* 166(1-2):65-74.

Hornan D, Edmeades N, Krishnan R, Khan J, Lochhead J. (2010). Use of pegaptanib for recurrent and non-clearing vitreous haemorrhage in proliferative diabetic retinopathy. *Eye (Lond)*. 24:1315–9.

Iglesias-de la Cruz MC, Ziyadeh FN, Isono M, Kouahou M, Han DC, Kalluri R (2002). Effects of high glucose and TGF-beta1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. *Kidney Int.,* 62: 901–913.

Imai D, Yoneya S, Gehlbach PL, Wei LL, Mori K. (2005). Intraocular gene transfer of pigment epithelium-derived factor rescues photoreceptors from light-induced cell death. *J Cell Physiol*. 202(2):570-8.

Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S. (2003). Pigment epitheliumderived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia;* 46: 284-7.

IDF Atlas . http://www.diabetesatlas.org/

Jacqueminet S, Ben Abdesselam O, Chapman MJ, Nicolay N, Foglietti MJ, Grimaldi A, Beaudeux JL. (2006). Elevated circulating levels of matrix metalloproteinase-9 in type 1 diabetic patients with and without retinopathy. *Clin Chim Acta*. 367(1-2):103-7

Janssen A, Hoellenriegel J, Fogarasi M, Schrewe H, Seeliger M, Tamm E, Ohlmann A, May CA, Weber BH, Stöhr H. (2008). Abnormal vessel formation in the choroid of mice lacking tissue inhibitor of metalloprotease-3. *Invest Ophthalmol Vis Sci*. 7: 2812-2

Jarosz-Chobot P, Polanska J, Szadkowska A, Kretowski A, Bandurska-Stankiewicz E, Ciechanowska M, Deja G, Mysliwiec M, Peczynska J, Rutkowska J, Sobel-Maruniak A, Fichna P, Chobot A, Rewers M.(2011). Rapid increase in the incidence of type 1 diabetes in Polish children from 1989 to 2004, and predictions for 2010 to 2025. *Diabetologia,* 54(3):508-15.

Jorge R, Oliveira RS, Messias A, Almeida FP, Strambe ML, Costa RA, Scott IU. (2011). Ranibizumab for retinal neovascularization. *Ophthalmology*, 118(5):1004-1004.e1.

Joussen AM, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Döhmen S, Adamis AP. (2002),. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. *FASEB J*.16(3):438-40

Kataoka K, Nishiguchi KM, Kaneko H, van Rooijen N, Kachi S, Terasaki H. (2011). The roles of vitreal macrophages and circulating leukocytes in retinal neovascularization. *Invest Ophthalmol Vis Sci*. 52(3):1431-8.

Kato K, Osawa H, Ochi M, Kusunoki Y, Ebisui O, Ohno K, Ohashi J, Shimizu I, Fujii Y, Tanimoto M, Makino H. (2008). Serum total and high molecular weight adiponectin levels are correlated with the severity of diabetic retinopathy and nephropathy. *Clin Endocrinol (Oxf)*. 68(3):442-9.

Kelly J, Ali Khan A, Yin J, Ferguson TA, Apte RS. (2007). Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice. *J Clin Invest.* 117: 3421-3426.

Khaliq A, Foreman D, Ahmed A, Weich H, Gregor Z, McLeod D Boulton M. (1998). Increased expression of placenta growth factor in proliferative diabetic retinopathy. *Lab Invest.* 78, 109-16.

Kielczewski JL, Hu P, Shaw LC, Li Calzi S, Mames RN, Gardiner TA, McFarland E, Chan-Ling T, Grant MB. (2011) Novel protective properties of IGFBP-3 result in enhanced pericyte ensheathment, reduced microglial activation, increased microglial apoptosis, and neuronal protection after ischemic retinal injury. *Am J Pathol*. 178:1517-28.

Kim MY, Byeon CW, Hong KH, Han KH, Jeong S. (2005). Inhibition of angiogenesis by the MCP-1 (monocyte chemoattractant protein-1) binding peptide. *FEBS Lett.* 579: 1597-601.

Koleva-Georgieva DN, Sivkova NP, Terzieva D. (2011). Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. *Folia Med (Plovdiv)*. 53(2):44-50.

Kollias A, Tsiotra PC, Ikonomidis I, Maratou E, Mitrou P, Kyriazi E, Boutati E, Lekakis J, Economopoulos T, Kremastinos DT, Dimitriadis G, Raptis SA. (2011). Adiponectin levels and expression of adiponectin receptors in isolated monocytes from overweight patients with coronary artery disease. *Cardiovasc Diabetol,* 1;10:14.

Kowluru RA, Zhong Q, Kanwar M. (2010). Metabolic memory and diabetic retinopathy: role of inflammatory mediators in retinal pericytes. *Exp Eye Res.*90(5):617-23

Kumada M, S. Kihara, O. Noriyuki, H. Kobayashi, Y. Okamoto, K. Ohashi, K. Maeda, H. Nagarentani, K. Kishida, N. Maeda, A. Nagasawa, T. Funahashi, Matsuzawa Y. (2004). Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages, *Circles 109,* 2046– 2049.

Kvanta A. (2006). Ocular angiogenesis: The role of growth factors. *Acta phthalmol Scand*; 84: 282-288.

Lamoureux EL, Wong TY. (2011). Diabetic retinopathy in 2011: further insights from new epidemiological studies and clinical trials. *Diabetes Care*, 34(4):1066-7.

Lange CA, Stavrakas P, Luhmann UF, de Silva DJ, Ali RR, Gregor ZJ, Bainbridge JW. (2011). Intraocular Oxygen Distribution in Advanced Proliferative Diabetic Retinopathy. Am J Ophthalmol. [Epub ahead of print]

Lee JH, Lee W, Kwon OH, Kim JH, Kwon OW, Kim KH, Lim JB. (2008). Cytokine profile of peripheral blood in type 2 diabetes mellitus patients with diabetic retinopathy. *Ann Clin Lab Sci*.38(4):361-7.

Limb G, Hollifield R, Webster L, Charteris D, Chignell A. (2001). Soluble TNF receptors in vitreoretinal proliferati−ve disease. *Invest Ophthalmol Vis Sci,* 142, 1586–1591.

Lin A, Lei M, Xie X, Xu H. (2009). Expression and meaning of pro-angiogenic factors in retinopathy of diabetic rats. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 34(12):1243-50

Lo Hui-Chen, Lin Su-Chen, Wang Yu-Mei. (2004). The relationship among serum cytokines, chemokine, nitric oxide, and leptin in children with type 1 diabetes mellitus. *Clin Bioch,* 37: 666-72

Löfqvist C, Engström E, Sigurdsson J, Hård AL, Niklasson A, Ewald U, Holmström G, Smith LE, Hellström A. (2006). Postnatal head growth deficit among premature infants parallels retinopathy of prematurity and insulin-like growth factor-1 deficit. *Pediatrics*, 117, 1930-1938.

Losso JN, Truax RE, Richard G. (2010). Trans-resveratrol inhibits hyperglycemia-induced inflammation and connexin downregulation in retinal pigment epithelial cells. *J Agric Food Chem*. 58(14):8246-52.

Maier R., Weger M., Haller-Schober E.M., El-Shabrawi Y., Theisl A., Barth A., Aigner R. (2006). Haas A Application of multiplex cytometric bead array technology for the measurement of angiogenic factors in the vitreous. *Mol Vis.*12:1143-7.

Maier R, Weger M, Haller-Schober EM, El-Shabrawi Y, Wedrich A, Theisl A, Aigner R, Barth A, Haas A. (2008). Multiplex bead analysis of vitreous and serum concentrations of inflammatory and proangiogenic factors in diabetic patients. *Mol Vis*.14:637-43.

Marek N, Myśliwiec M, Raczyńska K, Zorena K, Myśliwska J, Trzonkowski P. (2010) Increased spontaneous production of VEGF by CD4+ T cells in type 1 diabetes. *Clin Immunol*.137:261-70.

Marek N, Raczyńska K, Siebert J, Myśliwiec M, Zorena K, Myśliwska J, Reiwer-Gostomska M, Trzonkowski P. (2011). Decreased angiogenin concentration in vitreous and serum in proliferative diabetic retinopathy. *Microvasc Res*, 82(1):1-5.

Matsumoto Y, Takahashi M, Ogata M. Relationship between glycoxidation and cytokines in the vitreous of eyes with diabetic retinopathy. Jpn J Ophthalmol. 2002 Jul-Aug;46(4):406-12.

Matzuzawa Y. (2005). Adiponectin: identification, physiology and clinical relevance in metabolic and vascular disease. *Atheroscler Suppl* ; 6:7-14.

Meleth AD, Agrón E, Chan CC, Reed GF, Arora K, Byrnes G, Csaky KG, Ferris FL 3rd, Chew EY. (2005). Serum inflammatory markers in diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 46(11):4295-301.

Miyamoto N, de Kozak Y, Jeanny JC, Glotin A, Mascarelli F, Massin P, BenEzra D, Behar-Cohen F. (2007). Placental growth factor-1 and epithelial haemato-retinal barrier breakdown: potential implication in the pathogenesis of diabetic retinopathy. *Diabetologia.* 50(2):461-70

Mohammad G, Kowluru RA. (2010). Matrix metalloproteinase-2 in the development of diabetic retinopathy and mitochondrial dysfunction. *Lab Invest*. 90(9):1365-72.

Mohammad G, Kowluru RA. (2011). Diabetic retinopathy and signaling mechanism for activation of matrix metalloproteinase-9. *J Cell Physiol.* doi: 10.1002/jcp.22822.

Myśliwiec M, Balcerska A, Zorena K, Myśliwska J, Nowacka M, Lipowski P, Raczyńska K. (2006). Selected immunologic and biochemical risk factors of the retinopathy and nephropathy development in children with diabetes mellitus type 1. *Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego.* 4: 269-273.

Myśliwiec M, Zorena K, Balcerska A, Myśliwska J, Lipowski P, Raczyński K. (2006). The activity of N-acetyl-beta-D-glucosaminidase and tumor necrosis factor-alpha at early stage of diabetic retinopathy development in type 1 diabetes mellitus children. *Clinical Biochemistry* 39: 851-856.

Myśliwiec M, Balcerska A, Zorena K, Myśliwska J, Lipowski P, Raczyńska K. (2008). The role of vascular endothelial growth factor, tumor necrosis factor alpha and interleukin-6 in pathogenesis of diabetic retinopathy. Diabetes Research and Clinical Practice; 79:141-146.

Naldini A, Carraro F. (2005). Role of inflammatory mediators in angiogenesis. Curr Drug Targets Inflamm Allergy.

Nandi D, Mishra MK, Basu A, Bishayi B. (2010). Protevtive effects of interleukin-6 in lipopolisacharide (LPS)-induced experimental endotoxemia are linked to alteration in hepatic anti-oxidant enzymes and endogenous cytokines. *Immunobiology*, 215:443-451.

National Health and Nutrition Examination Survey 1999–2002. (2006). Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population. *Diabetes Care*, 29:1263–1268.

Noma H, Funatsu H, Mimura T, Harino S, Hori S. (2009). Vitreous levels of interleukin-6 and vascular endothelial growth factor in macular edema with central retinal vein occlusion. *Ophthalmology*, 116(1):87-93.

Oh IK, Kim SW, Oh J, Lee TS, Huh K. (2010). Inflammatory and angiogenic factors in the aqueous humor and the relationship to diabetic retinopathy. *Curr Eye Res*.35(12):1116-27.

Orlova VV, Liu Z, Goumans MJ, Ten Dijke P. (2011). Controlling angiogenesis by two unique TGF-β type I receptor signaling pathways. *Histol Histopathol*. 26(9):1219-30.

Otsuka H, Arimura N, Sonoda S, Nakamura M, Hashiguchi T, Maruyama I, Nakao S, Hafezi-Moghadam A, Sakamoto T. (2010). Stromal cell-derived factor-1 is essential for photoreceptor cell protection in retinal detachment. *Am J Pathol*.177(5):2268-77

Patterson C, Parrella MA, Endege WO, Yoshizumi M, Lee Mu-En, Haber E. (*1996),* Dowregulation of Vascular Endothelial Growth Factor Receptors by Tumor Necrosis Factor-alpha in Cultured Human Vascular Endothelial Cells. *J Clin Invest. 98,2, 490-96*.

Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G (2009) EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 373:2027-3

Peczyńska J, Urban M, Urban B, Głowińska B, Florys B. (2004). Assessment of growth factor levels in adolescents with type 1 diabetes mellitus and the beginning of diabetic microangiopathy. *Pediatr Endocrinol Diabetes Metab,* 10(1):41-8.

Pérez-Muñuzuri A, Fernández-Lorenzo JR, Couce-Pico ML, Blanco-Teijeiro MJ, Fraga-Bermúdez JM. (2010). Serum levels of IGF1 are a useful predictor of retinopathy of prematurity. *Acta Paediatr,* 99(4):519-25

Polykandriotis E, Arkudas A, Beier JP, Dragu A, Rath S, Pryymachuk G, Schmidt VJ, Lametschwandtner A, Horch RE, Kneser U. (2011). The impact of VEGF and bFGF on vascular stereomorphology in the context of angiogenic neo-arborisation after vascular induction. *J Electron Microsc* (Tokyo).

Poulaki V, Joussen AM, Mitsiades N, Mitsiades CS, Iliaki EF, Adamis AP. (2004). Insulin-like growth factor-I plays a pathogenetic role in diabetic retinopathy. *Am J Pathol.* 165(2):457-69.

Praidou A, Androudi S, Brazitikos P, Karakiulakis G, Papakonstantinou E, Dimitrakos S. (2010). Angiogenic growth factors and their inhibitors in diabetic retinopathy. *Curr Diabetes Rev,* 6(5):304-12

Querques G, Bux AV, Martinelli D, Iaculli C, Noci ND. (2009). Intravitreal pegaptanib sodium (Macugen) for diabetic macular oedema. *Acta Ophthalmol.*;87:623–30

Raczyńska K, Zorena K, Myśliwska J, Myśliwiec M, Raczyńska–Woźniak D, Balcerska A. (2008). Analysis of the Pro-Angiogenic Factor Influencing the Development of

Retinopathy in Children with Diabetes Mellitus Type 1. *Polish Journal of Environmental Studies,* Vol.17, 1A: 132-136.

Rakic JM, Lambert V, Devy L, Luttun A, Carmeliet P, et al. (2003). Placenta growth factor, a member of the VEGF Family, contribute to the development of choroidal neovascularization. *Invest Ophthalmol Vis Sci.* 44(7):3186–3193.

Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S, (2004) CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol,* 25, 201-9

Rosenfeld PJ. (2011). Bevacizumab versus ranibizumab for AMD. N Engl J Med. 2011 May 19;364(20):1966-7. Epub

Rosenthal R, Malek G, Salomon N, Peill-Meininghaus M, Coeppicus L, Wohlleben H, Wimmers S, Bowes Rickman C, Strauss O. (2005). The fibroblast growth factor receptors, FGFR1 and FGFR2, mediate two independent signalling pathways in human retinal pigment epithelial cells. *Biochem. Biophys. Res. Commun*. 337, 241–247.

Rosenson RS, Fioretto P, Dodson PM. (2011). Does microvascular disease predict macrovascular events in type 2 diabetes? Atherosclerosis. [Epub ahead of print]

Roy S, Trudeau K, Roy S, Behl Y, Dhar S, Chronopoulos A. (2010). New insights into hyperglycemia-induced molecular changes in microvascular cells. *J Dent Res* 89:116-27

Saika S, Yamanaka O, Okada Y, Tanaka S, Miyamoto T, Sumioka T, Kitano A, Shirai K, Ikeda K. (2009). TGF beta in fibroproliferative diseases in the eye. Front Biosci (Schol Ed). 1:376-90.

Sall JW, Klisovic DD, O'Dorisio MS, Katz SE. (2004). Somatostatin inhibits IGF-1 mediated induction of VEGF in human retinal pigment epithelial cells. *Exp Eye Res.* 79(4):465-76.

Sanchez RN, Chan CK, Garg S, Kwong JM, Wong MJ, Sadun AA, Lam TT. (2003). Interleukin-6 in retinal ischemia reperfusion injury in rats. *Invest Ophtalmol Vis Sci.* 44:4006-4011.

Sansó Sureda A Rocamora Durán V Sapiña Camaró A Royo Escosa V Bosque López MJ. (2011). Ustekinumab in a patient with Crohn's disease and anti-TNF-α-induced psoriasis. *Gastroenterol Hepatol.* [Epub ahead of print]

Santilli F, Spagnoli A, Mohn A, Tumini S, Verrotti A, Cipollone F, Mezzetti A, Chiarelli F. (2001). Increased vascular endothelial growth factor serum concentrations may help to identify patients with onset of type 1 diabetes during childhood at risk for developing persistent microalbuminuria. *J Clin Endocrinol Metab.* 86(8):3871-6.

Sappington RM, Calkins DJ. (2006). Pressure-induced regulation of IL-6 in retinal glial cells: involvement of the ubiquitin/proteasome pathway and NFkappaB. *Invest Ophthalmol Vis Sci.* 47(9):3860-9.

Sari R, Balci MK, Apaydin C. (2010). The relationship between plasma leptin levels and chronic complication in patients with type 2 diabetes mellitus. *Metab Syndr Relat Disord*. 8(6):499-503.

Savino F. (2008). Adiponectin: an intriguing hormone for paediatricians. *Acta Pediatr,* 97:701-705.

Schalwijk CG, Chaturvedi N, Schram M. (2006). Adiponectin is inversely associated with renal function in type 1 diabetic patients. *J Endocrinol Metab* 91: 129-135

Sfikakis PP, Grigoropoulos V, Emfietzoglou I, Theodossiadis G, Tentolouris N, Delicha E, Katsiari C, Alexiadou K, Hatziagelaki E, Theodossiadis PG. (2010). Infliximab for

diabetic macular edema refractory to laser photocoagulation: a randomized, double-blind, placebo-controlled, crossover, 32-week study. *Diabetes Care.* 33(7):1523-8

Sgadari C, Angiolillo AL, Tosato G. *(*1996). Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* ,87:3877–82.

Shi C, Lu J, Wu W, Ma F, Georges J, Huang H, Balducci J, Chang Y, Huang Y. (2011). Endothelial Cell-Specific Molecule 2 (ECSM2) Localizes to Cell-Cell Junctions and Modulates bFGF-Directed Cell Migration via the ERK-FAK Pathway. *PLoS One*, 6(6):e21482

Siebert J, Reiwer-Gostomska M, Myśliwska J, Marek N, Raczyńska K, Glasner L.(2010). Glycemic control influences serum angiogenin concentrations in patients with type 2 diabetes.*Diabetes Care*. 33:1829-30

Siebert J, Reiwer-Gostomska M, Babinska Z, Myśliwska J, Myśliwski A, Skopińska – Różewska E, Sommer E, Skopiński P. (2007). Low serum angiogenin level concentrations in patients with type 2 diabetes. *Diabetes Care*, 30 :3086–3087

Simó R, Carrasco E, García-Ramírez M, Hernández C. (2006). Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. *Curr Diabetes Rev*; 2: 71-98.

Simo R, Lecube A, Segura RM, Garcia Arumi J, Hernandez C. (2002). Free insulin growth factor-I and vascular endothelial growth factor in the vitreous fluid of patients with proliferative diabetic retinopathy. *Am J Ophthalmol*, 134: 376–382.

Starita M. Patel, Katz B. and Adamis A.P. (2007). Vascular endothelial growth factor and the potential therapeutic use of pegaptanib (macugen) in diabetic retinopathy, *Dev. Ophthalmol.* 39 pp. 122–148

Stoker M, Gherardi E, Perryman M, Gray J. (1987). Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature,* 327:239–242.

Strieter RM, Burdick MD, Gomperts BN, Belperio JA, Keane MP (2005). CXC chemokines in angiogenesis. *Cytokine Growth Factor Rev.*16: 593-609.

Subramanian P, Crawford SE, Becerra SP. (2011). Assays for the antiangiogenic and neurotrophic serpin pigment epithelium-derived factor. *Methods Enzymol.* 499:183-204.

Sukhanov S, Higashi Y, Shai SY, Vaughn C, Mohler J, Li Y, Song YH, Titterington J, Delafontaine P. (2007). IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol.* 27(12):2684-90.

Sumioka T, Fujita N, Kitano A, Okada Y, Saika S. (2011). Impaired angiogenic response in the cornea of mice lacking tenascin C. *Invest Ophthalmol Vis Sci.* 52(5):2462-7.

Sun X, Huang L, Zhang M, Sun S, Wu Y. (2010). Insulin like growth factor-1 prevents 1-mentyl-4-phenylphyridinium-induced apoptosis in PC12 cells through activation of glycogen synthase kinase-3beta. *Toxicology*. 271(1-2):5-12

Suzuma I, Hata Y, Clermont A, Pokras F, Rook SL, Suzuma K. (2001) Cyclic stretch and hypertension induce retinal expression of vascular endothelial growth factor and vascular endothelial growth factor receptor-2: potential mechanisms for exacerbation of diabetic retinopathy by hypertension. *Diabetes,* 50: 444–454.

Suzuki Y, Nakazawa M, Suzuki K, Yamazaki H, Miyagawa Y. (2011). Expression profiles of cytokines and chemokines in vitreous fluid in diabetic retinopathy and central retinal vein occlusion. *Jpn J Ophthalmol*. 55(3):256-63.

Tan KC, Xu A, Chow WS, Lam MC, Ai VH, Tam SC, Lam KS. (2004). Hypoadiponectinemia is associated with impaired endothelium-depentend vasodilation. *Journal of Clin Endocrinol and Metabolism.* 100: 2473- 2476.

Tello-Montoliu A, Patel JV, Lip GY. (2006). Angiogenin: a review of the pathophysiology and potential clinical applications. *J Thromb Haemost*, 4(9):1864-74.

Thomas MC, Söderlund J, Lehto M, Mäkinen VP, Moran JL, Cooper ME, Forsblom C, Groop PH. (2011). ; on behalf of the FinnDiane Study Group. Soluble receptor for AGE (RAGE) is a novel independent predictor of all-cause and cardiovascular mortality in type 1 diabetes. *Diabetologia.* [Epub ahead of print]

Tombran-Tink J. (2010). PEDF in angiogenic eye diseases. *Curr Mol Med.* 10(3):267-78.

Trinchieri, G. (1998). *Int. Rev. Immunol*, 16(3-4), 365-396.

Unoki N, Murakami T, Nishijima K, Ogino K, van Rooijen N, Yoshimura N (2010). SDF-1/CXCR4 contributes to the activation of tip cells and microglia in retinal angiogenesis.. Invest Ophthalmol Vis Sci..,51: 3362-7

Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. (1995). Inhibition of angiogenesis in vivo by interleukin 12. *J Natl Cancer Inst.* 87:581–6.

Volpert O.V., Zaichuk T., Zhou W., Reiher F., Ferguson T.A., Stuart P.M., Amin M., Bouck N.P. (2002). Inducer-stimulated Fas targets activated endothelium for destrustion by anti-angiogenic thrombospndin-1 and pigment epithelium-derived factor. *Nat. Med.*, 8: 349-357

Wa C, Cerny RL, Clarke WA, Hage DS. (2007). Characterization of glycation adducts on human serum albumin by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Chim Acta* 385: 48-60

Wedrychowicz A, Dziatkowiak H, Nazim J, Sztefko K. (2005). Insulin-like growth factor-1 and its binding proteins, IGFBP-1 and IGFBP-3, in adolescents with type-1 diabetes mellitus and microalbuminuria. *Horm Res*. 63(5):245-5

Wilson, J.; Balkwill, F (2002). *Semin. Cancer Biol,* 12(2), 113-120.

Wirostko B, Wong TY, Simo´ R. (2008). Vascular endothelial growth factor and diabetic complications. *Prog Retin Eye Res*, 27: 608–621.

Wulster-Radcliffe MC, K.M. Ajuwon, J. Wang, J.A. Christian, M.E. Spurlock, (2004). Adiponectin differentially regulates cytokines in procine macrophages. *Biochem. Biophys. Res. Commun,* 316. 924– 929.

Xia M, Sui Z. (2009). Recent developments in CCR2 antagonists. *Expert Opin Ther Pat* 19: 295–303

Yamagishi S, Amano S., Inagaki Y., Okamoto T., Takeuchi M., Inoue H. (2003). Pigment epithelium-derived factor inhibits leptin-induced angiogenesis by suppressing vascular endothelial growth factor gene expression through anti-oxidative properties. Microvasc. Res, 65: 186-190.

Yamagishi SI, Maeda S, Matsui T, Ueda S, Fukami K, Okuda S. (2011). Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. *Biochim Biophys Acta.* Apr 1. [Epub ahead of print]

Yingchuan F, Chuntao L, Hui C, Jianbin H. (2010). Increased Expression of TGF-beta1 and Smad 4 on Oxygen-Induced Retinopathy in Neonatal Mice. *Adv Exp Med Biol.* 664:71-7.

Yoshida S, Yoshida A, Ishibashi T. (2004). Induction of IL-8, MCP-1, and bFGF by TNF-alpha in retinal glial cells: implications for retinal neovascularization during post-ischemic inflammation. *Graefes Arch Clin Exp Ophthalmol.* 242(5):409-13.

Yoshida S, Ishikawa K, Matsumoto T, Yoshida A, Ishibashi T, Kono T. (2010). Reduced concentrations of angiogenesis-related factors in vitreous after vitrectomy in patients with proliferative diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol*. 248(6):799-804

Yoshida S, Sotozono C, Ikeda T, Kinoshita S. (2001). Interleukin−6 [IL−6] production by cytokine−stimulated human Müller cells. *Curr Eye Res.*, 22, 341–347.

Yoshimura T, Leonard EJ. (1992). Human monocyte chemoattractant protein-1: structure and function. *Cytokines.* 4:131-52.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM , 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature,* 372: 425–432.

Zhang W, Rojas M, Lilly B, Tsai NT, Lemtalsi T, Liou GI, Caldwell RW, Caldwell RB. (2009). NAD(P)H oxidase-dependent regulation of CCL2 production during retinal inflammation. *Invest Ophthalmol Vis Sci*.50: 3033-40

Zheng L, Szab C and Kern TS. (2004) "Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-κB," *Diabetes*, 53: 2960–2967.

Zietz B, Buechler C, Kobuch K, Neumeier M, Schölmerich J, Schäffler A. (2008). Serum levels of adiponectin are associated with diabetic retinopathy and with adiponectin gene mutations in Caucasian patients with diabetes mellitus type 2. *Exp Clin Endocrinol Diabetes.* 116(9):532-6

Zhou Y, Wei Y, Wang L, Wang X, Du X, Sun Z, Dong N, Chen X. (2011). Decreased adiponectin and increased inflammation expression in epicardial adipose tissue in coronary artery disease. *Cardiovasc Diabetol*. 12;10(1):2.

Zong H, Ward M, Stitt AW.(2011). AGEs, RAGE, and Diabetic Retinopathy. *Curr Diab Rep.*;11(4):244-52.

Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Hak Ł, Lipowski P, Raczyńska K. (2007). Serum TNF-Alpha Level Predicts Nonproliferative Diabetic Retinopathy in Children. *Mediators Inflamatation.* 1, 92196.

Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Lipowski P, Raczyńska K (2007). Interleukin-12, vascular endothelial growth factor and tumor necrosis factor-alpha in the process of neoangiogenesis of diabetic retinopathy in children. *Klin Oczna,* 109(4-6):155-9.

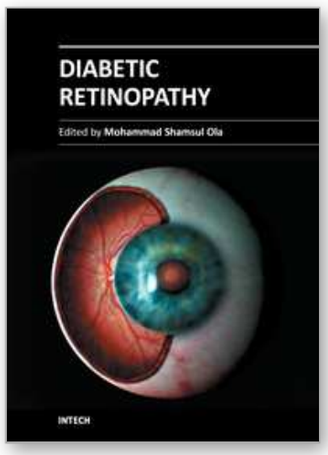
Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Raczyńska-Woźniak D, Raczyńska K. (2007). Inflammatory and angiogenic factors in children with diabetic retinopathy. *Family Med. Prim. Care Rev.* 4: 1007-1010.

Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Lipowski P, Raczyńska K. (2008). Interleukin 12 and Tumor Necrosis Factor-α equilibrium is prerequisite for clinical course free from late complications in type 1 diabetes mellitus children. *Scandinavian Journal Immunology*, 67, 2, 204-8.

Zorena K, Myśliwska J, Myśliwiec M, Balcerska A, Lipowski P, Raczyńska-Woźniak D, Raczyńska K. (2009). Modulatory factors responsible for neoangiogenesis in young patients with long-standing diabetes mellitus type 1. *Recent Patents Endocr. Metabol. Immun. Drug Disc.*vol. 3, nr 2, 144-149.

Zorena K, Myśliwiec M, Myśliwska J, Balcerska A, Wiśniewski P, Kula M, Raczyńska D. (2010). The status of the CCL2/MCP-1 and CXCL10/IP-10 chemokines in children and youths with type 1 diabetes mellitus. *Pediatric Endocrinology*, 4: 9-18.

Zorena K, Myśliwska J, Myśliwiec M, Rybarczyk-Kapturska K, Malinowska E, Wiśniewski P, Raczyńska K. (2010). Association between vascular endothelial growth factor and hypertension in children and adolescents type I diabetes mellitus. *J Hum Hypertens*, 24(11):755-62



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The aim of this book is to provide a comprehensive overview of current concepts in pathogenesis, diagnosis and treatments of diabetic retinopathy. It provides a collection of topics written by excellent authors, covering discussions on advances in understanding of pathophysiology, immunological factors and emerging concepts, relating to clinical aspects and treatment strategies. The contents of the book will not only provide a resource for our knowledge but also improve diagnosis and treatment options for those patients who suffer vision loss due to diabetic retinopathy.

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