# EVALUATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR LEVEL IN TEAR AND SERUM AMONG DIABETIC PATIENTS

*by*

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**DISCLAIMER**

I hereby certify that the work in this my own except for the quotations and summaries which have been duly acknowledged.

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**ABSTRAK**

**Pengenalan:**

*Vascular endothelial growth factor* (VEGF) dipercayai memainkan peranan dalam patogenesis dan tahap keterukan diabetik retinopati (DR) di kalangan pesakit diabetik. Oleh itu, dengan mengkaji paras VEGF dalam serum darah dan air mata pesakit diabetik akan memberikan kita gambaran yang lebih mendalam tentang peranan VEGF dalam patogenesis DR.

**Objektif:**

Objektif kajian ini adalah untuk menilai paras kandungan VEGF dalam serum darah dan air mata di kalangan pesakit diabetes mellitus (DM) jenis ke 2 dengan pelbagai tahap DR. Kajian ini juga akan melihat kolerasi paras VEGF di antara serum darah dan air mata di kalangan pesakit ini.

**Kaedah:**

Kajian keratan rentas telah dijalankan di salah satu hospital tertiari di Malaysia iaitu di Hospital Universiti Sains Malaysia (USM) di antara Ogos 2016 dan Mei 2018. Kajian ini telah melibatkan pesakit DM jenis ke 2 dengan pelbagai tahap DR [tiada DR, retinopati tidak proliferatif (NPDR), retinopati proliferative (PDR)]. Sampel air mata diambil menggunakan kertas Schirmers manakala sampel serum darah diambil dari salur darah vena. Paras VEGF di dalam serum darah dan air mata diukur menggunakan teknik assay enzim imunosorben.

**Keputusan:**

Sejumlah 88 pesakit DM jenis ke 2 (tiada DR: 30 pesakit, NPDR: 28 pesakit, PDR: 30 pesakit) terlibat dalam kajian. Kajian ini telah menunjukan purata VEGF air mata lebih tinggi yang signifikan dalam kumpulan NPDR dan PDR (114.9 SD 8.6 pg / mL dan 149.5 SD 10.4 pg / mL mengikut aturan) berbanding pesakit yang tiada DR (41.2 SD 11.3 pg / mL, $p < 0.001$). Walaubagaimanapun, tiada perbezaan signifikan purata VEGF serum darah di kalangan tiga kumpulan ini. Kajian ini juga mendapati terdapat korelasi yang sederhana paras VEGF di antara serum darah dan air mata ($p = 0.015$, $r = 0.263$).

**Kesimpulan:**

Di kalangan pesakit DM jenis ke 2, paras VEGF air mata di kalangan pesakit DR adalah lebih tinggi berbanding dengan pesakit yang tidak mempunyai DR. Paras VEGF air mata juga didapati ada kaitan dengan tahap keterukan DR. Selain itu kajian ini juga menunjukan terdapat korelasi yang sederhana paras VEGF antara serum dan air mata. Paras VEGF di dalam air mata mungkin memainkan peranan dalam meramalkan tahap keterukan DR dan membuka jalan bagi penilaian DR secara bukan invasif. Kajian kohort yang lebih besar diperlukan untuk penilaian selanjutnya.

**ABSTRACT**

**Introduction**

Vascular endothelial growth factor (VEGF) has been postulated to play a role in the pathogenesis and progression of diabetic retinopathy (DR). Thus, detection of VEGF level in ocular tissue may perhaps provide insight into a role of VEGF in pathogenesis of DR.

**Objective:**

The objective of this study was to evaluate the VEGF in serum and tears between different type of DR severity among type 2 diabetes mellitus (DM) patients and to determine the correlation between serum and tears VEGF levels.

**Methods:**

A comparative cross-sectional study was conducted at a tertiary hospital in Malaysia, Hospital Universiti Sains Malaysia (USM) between August 2016 and May 2018 involving type 2 DM patients with different status of DR [no DR, non-proliferative DR (NPDR), proliferative DR (PDR)]. Tear samples were collected using no.41 Whatman filter paper (Schirmer's strips) and 5 mls of blood sample were drawn by venous puncture. VEGF levels in tears and serum were measured by enzyme-linked immunosorbent assay.

**Results:**

A total of 88 type 2 DM patients (no DR: 30 patients, NPDR: 28 patients, PDR: 30 patients) were included into the study. Mean tear VEGF were significantly higher in NPDR and PDR groups (114.9 SD 8.6 pg/mL and 149.5 SD 10.4 pg/mL respectively) as compared to no DR (41.2 SD 11.3 pg/mL, $p < 0.001$). There was no significant difference of mean serum VEGF between the three groups. There was a fair correlation between serum and tears VEGF ($p = 0.015$, $r = 0.263$).

**Conclusion:**

Among type 2 DM patients, VEGF levels in tears were higher amongst patients with DR compared to those without DR. Tear VEGF levels were also significantly associated to the severity of DR. There was a fair correlation between serum and tears VEGF. Detection of VEGF in tears is a good non-invasive predictor test for severity of DR. A large cohort study is needed for further evaluation.

# CHAPTER 1

# INTRODUCTION

## 1.1 Diabetes Mellitus

Diabetes mellitus (DM) was first described by the ancient Egyptian around 1500 B.C. and only later in 1776 Matthew Dobson successfully demonstrated the evidence of the high glucose concentration in the urine in diabetic patients (Polonsky, 2012). Despite many advances in the medical field, DM remains as an important global public health concern (Wild *et al.*, 2004). It is more alarming as diabetes is projected to affect approximately 7.7% of the world's population (439 million adults) by 2030 (Shaw *et al.*, 2010).

The International Diabetes Federation (IDF) estimates that the prevalence of DM in South East Asia will increase by two folds by the year 2025 (IDF and WHO, 2000). According to The World Health Organization (WHO), it is estimated by the year of 2030, Malaysia would have a total of 2.48 million people with diabetes (WHO, 2005). In 1986, the first National Health and Morbidity Survey I (NHMS I) reported a DM prevalence of 6.3%. This had risen to 8.3% in the NHMS II's 1996 report. Recently, the NHMS 2011 reported diabetes prevalence figures of 15.2% and 20.8% for adults above the age of 18 and 30 years old respectively in Malaysia (NHMS IV, 2011). NHMS I and II involved subjects aged above 30 years while the NHMS III was conducted among subjects above 18 years of age (Feisul and Soraya, 2013). Among adults above the age of 18 years old, the prevalence was highest among Indians (24.9%) followed by Malays (16.9%) and Chinese (13.8%) (NHMS IV, 2011).

The prevalence of type 2 DM is increasing in the young with 2% and 4.9% of those between ages 18-19 years and 20-24 years respectively (NHMS IV, 2011). In terms of diabetes control, only 23.8% of patients in primary care and 12.7% in tertiary institutions were able to achieve their specified glycaemic targets (Feisul and Soraya, 2013; Mafauzy *et al.*, 2016). Up to 21.4% of type 2 DM patients in primary care were on insulin compared to 65.4% in tertiary institutions

(Feisul and Soraya, 2013; Mafauzy *et al*., 2016). In years to come, it is anticipated that a proportionate increment in diabetic retinopathy (DR) is unavoidable.

### 1.2 Effect of Diabetes Mellitus

DM is a complex and multisystem disease with various end organ complications. This damage is referred to as diabetic related complications. Generally, the detrimental effects of hyperglycemia are divided into macrovascular and microvascular complications (Patel *et al.*, 2012). Indeed, there is increasing evidence that micro- and macro-vascular disease share similar risk factors (Liao *et al.*, 2004; Nguyen *et al.*, 2007) and possibly even a common pathophysiological basis (Krentz *et al.*, 2007; Stokes *et al.*, 2005). Hyperglycemia can promote vascular complications by multiple postulated mechanisms. Elevated glucose can foster the formation of advanced glycation end products (AGEs) protein cross-linking and reactive oxygen species formation which damages vessel endothelial cell wall (Piga *et al*. 2007). This causes the thickening of capillary basement membranes and arteriolar hyalinosis. Hyperglycemia can also activate multiple pro-inflammatory target genes in endothelial cells and stimulate oxidative stress (Otsuka *et al.* 2005). This has been strongly implicated in the endothelial cell proliferation and formation of atherosclerosis.

#### 1.2.1 Systemic Effects

The fundamental pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body (Michael, 2008). Atheroma formation is thought to result from chronic inflammation of the endothelial lining of vessel wall (Hansson, 2005). In response to endothelial injury and inflammation, oxidized lipids from low density lipoprotein particles accumulate in the endothelial wall of

arteries (Michael, 2008). The net result of this process is the formation of a lipid-rich atherosclerotic plaque which impairs blood flow and cause vascular infarction (Boyle, 2007).

Type 2 DM increases the risk of cardiovascular disease which can present as angina, myocardial infractions, congestive cardiac failure and sudden death (Martín-Timón, *et al.* 2014). This accounts for up to two-third of deaths in type 2 DM (Carnethon, *et al*. 2010). In addition, type 2 DM, independent of chronic heart disease (CHD) may lead to diabetic cardiomyopathy. Hyperglycemia seems to be central to the pathogenesis of diabetic cardiomyopathy. Elevared blood glucose trigger a series of maladaptive stimuli that result in myocardial fibrosis and collagen deposition (Aneja, *et al.* 2008). It has been shown that hyperglycemia and its related consequences is an important risk for CHD and related mortality (DCCT, 1993; Stamler *et al.*, 1993; Adlerberth *et al.*, 1998).

In addition, patients with diabetes are also at high risk of developing microvascular complications. Pathological changes in the diabetic microvasculature will alter organ perfusion (Orasanu and Plutzky, 2009). This includes organs heavily dependent on their microvasculature supply, namely the retina, kidneys, and peripheral nervous system. Diabetic nephropathy is a major cause of chronic kidney disease (CKD) contributing to 57% of patients requiring dialysis in 2007 in Malaysia (Lim *et al.*, 2007). Diabetic nephropathy is also a major risk factor for cardiovascular morbidity and mortality (Alzaid 1996). This cardiovascular risk increases in a continuous fashion along with progression of nephropathy from normal to overt proteinuria levels (Mattock, *et al.* 1992). In a large study, the reduction of albuminuria with therapeutic interventions resulted in protection against cardiovascular disease as well as the development of progressive renal impairment (UKPDS, 1998).

Other microvascular complications like diabetic peripheral neuropathy may be asymptomatic in a large proportion of cases (Boulton *et al.*, 2005). Neuropathy causes loss of protective sensation and loss of coordination of muscle groups in the foot and leg (Rice, *et al.* 2014). This increases mechanical stress during ambulation, unnoticed injuries, structural foot deformity, such as hammertoes, bunions, metatarsal deformities, or Charcot foot leading to the formation of foot ulcerations (Rice, et al. 2014). In the 2006 NHMS III, prevalence of lower limb amputation among patients with diabetes was 4.3% (NHMS III, 2006). Foot ulcerations and amputations are major causes of morbidity and mortality in patients with diabetes.

### 1.2.2 Ocular Effects

DM can lead to several ocular complications such as DR, glaucoma, cataract, and ocular surface diseases. DR is the commonest major complication of DM and is currently the leading cause of preventable blindness in the developed countries (Klonoff and Schwartz, 2000; IDF and WHO, 2000). In Malaysia, the prevalence of DR from the 2007 Diabetic Eye Registry was 36.8% (Goh, 2007), a similar prevalence in the Singapore Malay Eye Study 2006 (35%) was documented (Wong *et al.*, 2008). Other unpublished data obtained from local primary care screening centers showed a combined prevalence ranging between 12.3% and 16.9% (Maziah *et al.*, 2009; Kamilah *et al.*, 2009). In patients with type 1 and type 2 diabetics with disease duration of over twenty years, the prevalences of DR are 95% and 60%, respectively (Garg and Davis, 2009). However, type 2 DM is responsible for a higher percentage of patients with visual loss (Kumari, *et al.* 2008).

It is estimated that an approximately 1.5% of adults with diabetes have proliferative diabetic retinopathy (PDR) (Zhang *et al.*, 2010). The DR study on the other hand showed that about half of all eyes with untreated PDR developed permanent vision loss (ie, visual acuity of

<20/800 for at least 4 months) due to serious complications such as tractional retinal detachment, vitreous hemorrhage, and neovascular glaucoma (DRS, 1979). PDR is characterized by retinal neovascularization, serum leakage, and fibrovascular proliferation. This results in vitreous hemorrhage and traction retinal detachment (Mazhar *et al.*, 2011; Fong *et al.*, 2004).

Other complications include dry eye syndrome (DES) and diabetic keratopathy. DES is common in the diabetic population; 54% prevalence of asymptomatic and symptomatic DES (Manaviat *et al.*, 2008). Chronic hyperglycemia, diabetic periphery neuropathy, decreased insulin levels, microvasculopathy, and systemic hyperosmotic disturbances are risk factors for diabetes-associated DES (Zhang *et al*., 2016). The severity of the dry eye disease also has been found to be correlated with the severity of DR (Nepp *et al.*, 2000). DM can trigger acceleration of ocular surface abnormalities which have been termed diabetic keratopathy (Schultz *et al.*, 1981). Several abnormalities have been shown in diabetic keratopathy. This includes thickened basement membrane, hemidesmosome alteration, increased epithelial fragility and decreased epithelial healing rates (Sanchez-Thorin, 1998). These abnormalities can lead to recurrent epithelial erosion (Schultz et al., 1981).

Diabetic cataract is considered a complication of DM, diabetic patients are 2-5 times more at risk for cataract formation and are more likely to get it at an earlier age (Klein *et al*. 1995). Cataract formation in diabetics seems to be related to hyperglycemia and hastened senile lens opacity (Klein, *et al.* 1995). Several pathogenic mechanisms that may precipitate formation of diabetic cataracts are increased osmotic stress caused by activation of the polyol pathway, non-enzymatic glycation of lens proteins, and increased oxidative stress (Srivastava, *et al.* 2005). A combination of cortical, nuclear, and posterior subcapsular cataract was the most common

form of the mixed cataract (20%), followed by the combined posterior subcapsular cataract and cortical (16%) (Raman, *et al.* 2010).

### 1.3 Angiogenesis in Diabetes Mellitus

Systemic vascular endothelial growth factor (VEGF) and the interplay between membrane-bound VEGF receptors (R) and the soluble form of VEGF-R1 are key to angiogenesis, vasculogenesis, neurogenesis and hemodynamics (Wirostko *et al.*, 2008). These cellular processes are regulated by complicated negative and positive feedback loops, many of which are disrupted and altered in diabetes (Wirostko *et al.*, 2008). Mahdy et. al. (2010) revealed that there was a significant increase in the serum VEGF in the diabetic patients; the difference was between micro- and macrovascular diabetic complications compared with uncomplicated diabetic group. In addition, various cytokines, such as interleukin-6, insulin growth factors, advanced glycation end products including the VEGF, have been identified to play a role in the ocular angiogenesis and pathogenesis of DR (Singh *et al.*, 2008; Mitamura *et al.*, 2005; Shams *et al.*, 2006). In the pathogenesis of PDR, the pro-angiogenic cytokine VEGF is considered the primary factor involved in neovascularization (Abu El-Asrar *et al.*, 2013). Figure 2 summarizes the role of VEGF in the development of PDR.

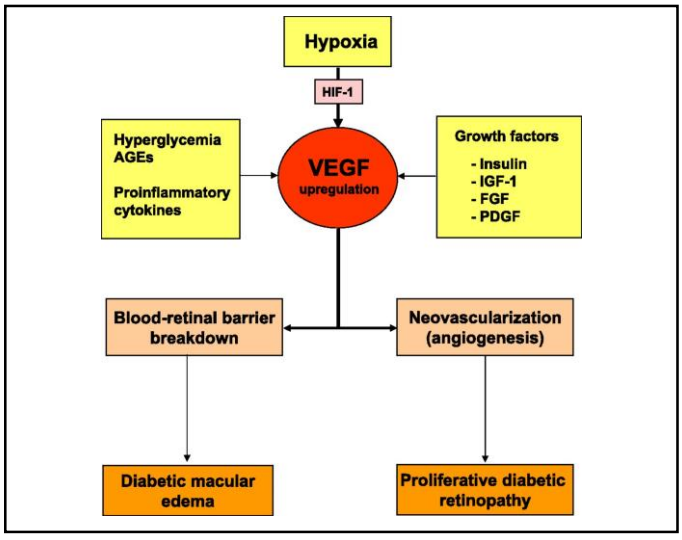


Figure 2. VEGF role in the development of PDR (Simó R *et al.*, 2014)

### 1.3.1 Vascular Endothelial Growth Factor (VEGF)

In 1983, Dvorak and colleagues discovered a key mediator involved in angiogenesis. It had a potent capacity to induce vascular leakage and was named vascular permeability factor (VPF) (Senger *et al.*, 1983). Six years later, Ferrara and colleagues identified a similar molecule which promotes the proliferation of endothelial cells and called it VEGF (Ferrara *et al.*, 1989). VEGF are a subfamily of growth factors that function as signaling proteins for both vasculogenesis and angiogenesis. Primarily, VEGF is secreted from retinal pigmented epithelial cells, pericytes, astrocytes, müller cells, glial cells, and endothelial cells (D'Amore 2007). VEGF has several members including VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PGF) (Gupta *et al.*, 2013). Figure 3 depicts a VEGF molecule.

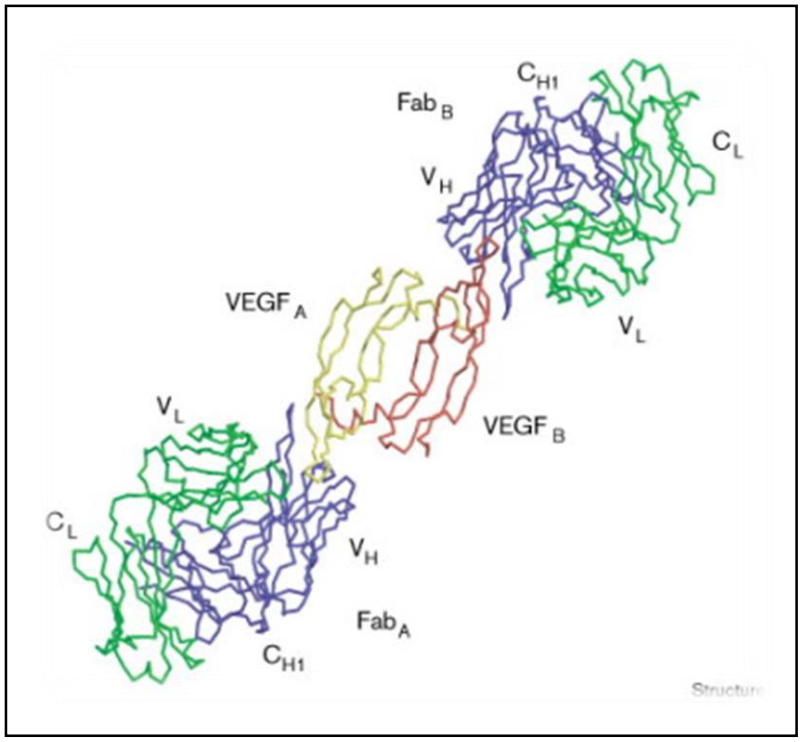


Figure 3. VEGF isoforms molecular structure (Muller *et al*., 1998)

VEGF-A is a 36-46 kDa glycosylated protein derived by alternate splicing of messenger ribonucleic acid (mRNA) from a single, 8-exon VEGF gene (Tischer *et al*., 1991). The molecule is expressed as five mRNA splice variants – isoforms 121, 145, 165, 189, and 206, depending upon the number of amino acids present (Penn *et al*, 2008). VEGF 165 the principle isoform is critical for development and pathological angiogenesis as well as vasculogenesis (Shibuya 2013). VEGF acts by binding to VEGF R1, VEGF R2, and Neuropilin-1 receptor as it regulates vasculogenesis and leakage (Figure 4).

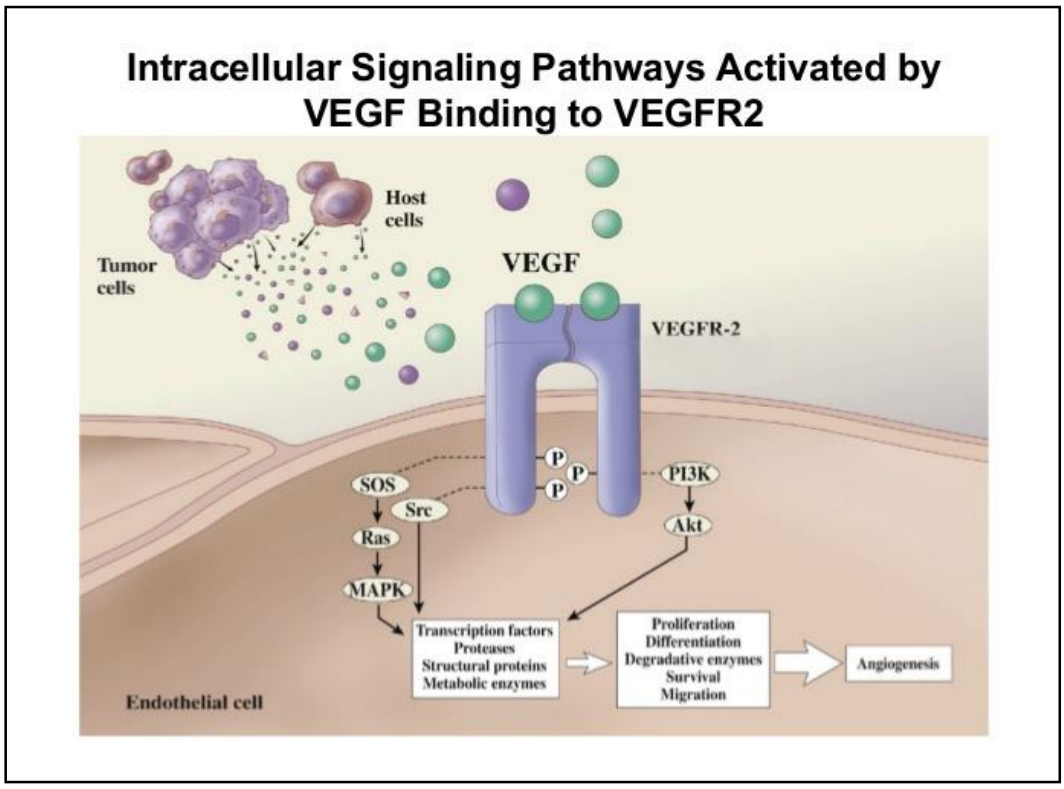


Figure 4. Signaling pathways activated by VEGF (Yulong *et al*., 1999)

Physiologically, VEGF is required for regulating the proliferation, migration, and survival of embryonic endothelial cells (Wang et al., 2008). However, in a disease state, it is involved in tumor development and vascular leakage (Yulong et al., 1999). Two independent studies

demonstrated that hypoxia could upregulate VEGF expression (Miller et al., 1994; Shweiki et al., 1992). It was found that higher level of VEGF in vitreous of patients with active diabetic proliferative retinopathies and venous occlusive disorders (Aiello et al., 1994). In addition, hypertension (Boursiquot *et al*., 2017), dyslipidemia (Zechariah *et al*., 2013) and smoking status (Ugur *et al*., 2018) are known factors affecting VEGF level.

### 1.3.2 VEGF in Diabetic Retinopathy

VEGF is an important factor in the development of both PDR and diabetic macular oedema by increasing the phosphorylation of proteins involved with tight-junctions such as zonula occludens and altering retinal capillary permeability (Simó *et al.*, 2008; Antonetti *et al.*, 1999). A study done by Alieo et al, suggested that VEGF in response to an ischemic state is as a primary initiator of PDR and as a potential mediator of non-proliferative diabetic retinopathy (NPDR) (Aiello *et al.*, 2000). Various studies on ocular VEGF levels have found a significant elevation in intraocular VEGF from the vitreous and aqueous samples. The level of VEGF also correlates with disease presence and its severity (Adamis *et al.*, 2006; Aiello *et al.*, 1994; Burgos *et al.*, 1997).

## 1.4 Ocular Tear Film

The exposed part of the ocular globe is covered by a thin, fluid film - the preocular tear film. Tear film is comprised of three dynamic layers; mucin, aqueous and lipid. Contacting the corneal epithelium is a hydrophilic mucus layer approximately 0.02 to 0.05 μm. As the underlying cornea itself is extremely hydrophobic, this layer is believed to provide a wetting substrate for the aqueous layer above it, which is about 6 to 9 μm thick (Holly and Lemp, 1977). The most superficial is the lipid layer, which reduces tear evaporation (Creech *et al.*, 1998). It also serves to protect against irritants, allergens, environmental extremes of dryness

and temperature, potential pathogens and pollutants. Tear components have antimicrobial function include lysozyme, lactoferrin, lipocalin, secretory immunoglobulin A (IgA) and complement (Lemp et al., 1970). Fleming and Allison (1922) also showed that lysozyme present in human tears can kill Gram-positive bacteria. This enzyme, which is secreted by the main and accessory lacrimal glands, accounts for up to 20–30% of total protein in both basal and reflex tears (Aho *et al.*, 1996). Lactoferrin has a high capacity to bind divalent cations including iron thus depriving bacteria of this essential nutrient for growth and production of some toxins, although iron-independent antimicrobial actions (Farnaud and Evans, 2003). Secretory IgA is the major antibody present in the tear film. IgA is produced by plasma cells residing in the lacrimal gland and accessory lacrimal glands (Knop and Knop, 2005). Thus, like lactoferrin, ocular surface IgA promotes the clearance of pathogens (Mantis *et al.*, 2011). Besides that, numerous functionally active complement factors are secreted by the lacrimal gland and distributed via the tears over the ocular surface (Klenker *et al*., 2007). Activation of the complement pathway generates fragments involved in acute inflammatory responses, fragments that act as opsonins, which facilitate formation of membrane attack complexes that can lyse pathogens (Willcox *et al.*, 1997).

### 1.4.1 Ocular Tear Film VEGF

Till date there is no studies investigating VEGF levels in tear amongst patients with type 2 DM. Other studies have shown that tear VEGF level can increase in cases of retinal vein occlusion (RVO) patients, chronic extended contact user and corneal neovascularization disease (Kasza *et al.*, 2015; Magone *et al.*, 2004; van Setten, 1997). However, other inflammatory protein biomarkers have been studied. Tear proteins such as lipocalin 1, lactotransferrin, lacritin, lysozyme C, lipophilin A and immunoglobulin lambda chain were identified as possible

biomarker with significantly higher levels patients with DR (Csősz *et al.*, 2012; Torok *et al.*, 2013).

### 1.4.2 Tear Sampling Techniques

There are primarily 2 techniques use to collect tear sample. A cross-sectional study done on 383 adult patients seeking for primary health care to evaluate whether tears could be used as tool for health screening. In this study, the pain score was elicited and compared between tear collection using Schirmer's strips, previous experience of antecubital venous puncture and finger prick test. They found that the pain score for Schirmer's tear collection was significantly lower than pain score for antecubital venous puncture. In addition, 70% agreed for their tears being collected to screen for eye problems (Quah *et al., 2014)*. Therefore, to collect the human tear by using Schirmer's strip is less invasive and less painful as compared to plasma collection.

Glass capillary tube is another method for tears collection. The capillary tube is rested in the lateral tears meniscus and minimizes contact with bulbar conjunctiva (Lam *et al.*, 2015). Tears are immediately collected by capillary tube. The average protein concentration obtained by the microcapillary tube showed the values was lower than the tears collection by schirmer strip (Farias *et al.*, 2013). However, Choy *et al* suggested that capillary tube for human tears collection was less invasive as compared with schirmer strip (Choy *et al.*, 2011). Nevertheless, human tears are relatively easier to be collected simultaneously from eyes by using Schirmer's strip and it's convenient as well.

**Rationale of the study**

DR remains a major cause of worldwide preventable blindness. The microvasculature of the retina responds to hyperglycemia through several biochemical changes. VEGF has been postulated to also play a role in the pathogenesis and progression of DR (Aiello *et al*., 2000; Adamis *et al*., 2006). Previous studies evaluating the VEGF levels in the serum of DR patients have reported conflicting results (Guo *et al.,* 2014; Deguchi *et al.,* 2009; Ozturk *et al.,* 2009; Fadhil, 2012). Serum levels of VEGF may be affected by the presence of other systemic illness which is common in diabetics (Yulong *et al.,* 1999; Papaioannou *et al.,* 2009). Identification of the serum VEGF might not reflect the changes in the retina due to the relatively small size of affected tissue such as retina and amount of the biomarker released in the large circulating blood volume (Nath *et al.,* 2017). Hence, identification and quantification of VEGF in ocular disease may needs highly sensitive assay systems.

Quantification of VEGF level in the ocular tissue, i.e. the aqueous or vitreous, may be more representative of DR. This is because VEGF levels in vitreous or aqueous are in close proximity to the retinal itself and better reflecting the pathophysiological process. However, measurement of aqueous or vitreous VEGF levels is relatively invasive and is associated with all the antecedent risks of surgical complication. Thus, aqueous and vitreous collection is only ethically possible if performed during surgical intervention such as cataract and vitreoretinal surgery. Studies has showed that VEGF were found in epithelial structures of the eye, such as cornea epithelium, ciliary epithelium, lens epithelium and retinal pigment epithelium (Setten *et al.,* 1997; Ford *et al*., 2012). VEGF in tears may reflect the VEGF level in cornea epithelium because cornea epithelium is directly in contact with tears and is continuously debrided into tears due to turn over process. Tear fluid might contain VEGF from epithelial cells covering the eye surface. Furthermore, VEGF normally residing in the blood can get into the tear fluid

through increased permeability of the conjunctival vessels (Zhou et al., 2009). Similarly, VEGF level in aqueous and vitreous may be closely similar to VEGF level in retina. Increased VEGF levels has been reported in the aqueous and vitreous fluid samples in DR patients (Adamis *et al.,* 2006; Aiello *et al.*, 1994; Burgos *et al.*, 1997). The levels of VEGF also correlate with disease presence and its severity. Thus, VEGF levels in tears may reflect the severity of the DR progression in the retina.

Although tear analysis is of increasing interest in ophthalmology, yet no studies have investigated tear VEGF levels in relation to the ischemic process and hence the severity of DR. Detection of VEGF levels in tears may thus be a less invasive, safe and acceptable method of screening DR. This might also act as a predictor for the severity of DR and to identifying patients who are at increased risk of progression from NPDR to PDR.

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