Iraqi Journal of Biotechnology, 2014,Vol.13, No.2 , 48-57

Pigment Epithelium Derived Factor and Vascular Endothelial Growth Factor in Diabetic Retinopathy

Seenaa Badr Al-Awadi[1] Salwa Jaber Al-Awadi[2] Abdulhussein Alwan Algenabi[3]

[1]College of Medicine, University of Babylon, Hilla, Iraq
[2]Nahrain Forensic DNA training center, Nahrain University, Baghdad, Iraq
[3]College of Medicine, Kufa University, Najaf, Iraq

Abstract: Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus. Vascular endothelial growth factor (VEGF) is a major mediator of vascular permeability and angiogenesis and also an important mediator of retinal ischemia-associated intraocular neovascularization. Pigment epithelium-derived factor (PEDF) is a strong inhibitor of angiogenesis. The objective of this study was to demonstrate the correlation between VEGF and PEDF in DR. A total of 117 subjects (healthy, diabetic without retinopathy and diabetic retinopathy) were studied. Serum VEGF and PEDF were measured. Result revealed a significant positive correlation between PEDF and VEGF (OR=0.820, p<0.01) in all subjects so the concentrations of PEDF and VEGF predict adverse outcomes, and their measurement may facilitate risk estimation, and PEDF-based interventions might be considered.

Keywords: Diabetic retinopathy, Pigment epithelium-derived factor, vascular endothelial growth factor.

العامل المستمد من ظهارة الصباغ وعامل النمو البطاني الوعائي في اعتلال الشبكية السكري

سيناء بدر العوادي[1] سلوى جابر العوادي[2] عبدالحسين علوان الجنابي[3]

[1]كلية الطب، جامعة بابل، حلة، العراق
[2]مركز الدنا، جامعة النهرين، بغداد، العراق
[3]كلية الطب ،جامعة الكوفة، نجف، العراق

الخلاصة: اعتلال الشبكية السكري (DR) هي المضاعفات الأكثر شيوعا للأوعية الدموية الدقيقة في داء السكري. عامل النمو البطاني الوعائي (VEGF) هو الوسيط الرئيسي لنفاذية الأوعية الدموية وأيضا وسيط مهم في شبكية العين المرتبط نقص التروية العين اتساع الأوعية الدموية. عامل المستمدة من ظهارة الصباغ (PEDF) هو المانع القوي لنمو الأوعية الدموية. ان الهدف من هذه الدراسة لإثبات العلاقة بين VEGF وPEDF في DR. تمت دراسة 117 شخصا (اصحاء، المصابون بالسكري مع اعتلال الشبكية وبدون اعتلال الشبكية السكري) وقياس PEDF وVEGF في الدم. وجد ان هناك علاقة إيجابية ذات دلالة إحصائية بين PEDF و VEGF (OR=0.820, p<0.01) . تركيز PEDF وVEGF تتنبأ بالنتائج السلبية، وقياسها قد يسهل تقدير المخاطر، ويمكن الاستفادة من التدخلات الدوائية لل PEDF.

Introduction

Diabetic retinopathy (DR) is one of the most common complications of diabetes, affecting millions of working adults worldwide, in which the retina progressively damaged leading to blindness (1).

In the year 2000, there were around 171 million people with diabetes globally, and by 2030, it is estimated that this number would increase to 366 million (2). As the number of persons with diabetes increases, the development of microvascular complications like retinopathy also rises. DR is responsible for 4.8% of the 37 million cases of blindness throughout the world (3). The mechanisms underlying the development of DR are not fully understood; however, with early detection and treatment, visual loss may be limited. The magnitude of damage caused by these microvascular complications of diabetes stresses the need for sensitive markers of screening for retinopathy. DR is characterized by gradually progressive alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vasopermeability, and in response to retinal nonperfusion, pathologic intraocular proliferation of retinal vessels (4, 5).

The two broad categories of DR are:

a. Non Proliferative Diabetic Retinopathy (NPDR) (6, 7)

b. Proliferative Diabetic Retinopathy (PDR) (8).

There are many methods to diagnose DR, such as ophthalmoscopy, fluorescent angiography, and fundus photography but all of these ophthalmic diagnostic approaches must be conducted by efficient ophthalmologists and require invasive and expensive procedures. The identification of peripheral blood biochemical parameters including angiogenic profile for DR could be helpful for early detection and management of patients with DR before vision loss.

VEGF is a 45-KDa homodimeric glycoprotein (9), has initially drawn much attention as an important mediator of retinal ischemia-associated intraocular neovascularization (10). VEGF a major mediator of vascular permeability and angiogenesis (11), therefore may play a pivotal role in mediating the development and progression of DR. Diabetic retinopathy is characterized by vascular permeability, increased tissue ischemia, angiogenesis and increase oxidative stress. VEGF expression is induced by hypoxia and certain cytokines (12, 13, 14).

VEGF is expressed in multiple cells and tissues including skeletal and cardiac muscle (15), osteoblasts, macrophages, keratinocytes (16), brown adipose tissue, CD34 stem cells (17), endothelial cells, fibroblasts, and vascular smooth muscle cells (18). VEGF dimers bind to two related receptor tyrosine kinases, VEGF R1 (also called Flt1) and VEGF R2 (Flk-1/KDR), and induce their homodimerization and autophosphorylation (19, 21, 22). These receptors have seven extracellular immunoglobulin-like domains and an intracellular split tyrosine kinase domain. They are expressed on vascular endothelial cells and a range of non-endothelial cells. Although VEGF affinity is highest for binding to VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity (19, 20).

PEDF is a 50-kDa glycoprotein initially isolated from fetal human retinal pigment epithelial cells (23) and was later found to be expressed in various tissues and cells (24,25), including

endothelial cells, osteoblasts (26,27), plasma (28), and liver(29).PEDF is a member of the serpin super-family of serine protease inhibitors(23). However, unlike many serpins, PEDF does not inhibit serine proteases(30).It is a multifunctional secreted protein (23) that has anti-angiogenic, antivasopermeability (31), antiinflammatory (32), antifibrosis (33), antitumorigenic (34) and neurotrophic (35) functions.

PEDF inhibit the migration of endothelial cells in vitro in a dose-dependent manner and was more effective than angiostatin, thrombospondin-1, and endostatin (36). These results placed PEDF among the most potent natural inhibitors of angiogenesis.

PEDF expression is upregulated by angiostatin (37, 38). Hypoxia leads to the downregulation of PEDF (38). This effect is due to the fact that hypoxic conditions cause matrix metalloproteinases (MMPs) to proteolytically degrade PEDF (39). Secreted PEDF binds a receptor on the cell surface termed PEDF-R (40). PEDF enhances gamma-secretase activity, leading to the cleavage of the VEGF receptor 1 (VEGFR-1) transmembrane domain (41). This action interferes with VEGF signaling thereby inhibiting angiogenesis (42).

Aims of the Study

Determination the level of VEGF and PEDF in sera of patients with diabetic retinopathy.

Assessment the relation between VEGF and PEDF.

Subjects and Methods

The study was conducted in the city of Hilla, from December 2011 to February 2013; this case-control study enrolled 117 subjects which attended different medical centers including Al-Hilla teaching general hospital, and Marjan medical city. Informed consent was obtained from all participants; the practical side of the study was performed at general health laboratory in Hilla and lab of clinic in Al-Hilla teaching general hospital

64 Patients with DR were divided into 2 groups, group (1): 42 NPDR patients with age mean 53.8 ± 8 years and group (2): 22 PDR patients with age mean 51.8 ±10 years

those were recruited from the Ophthalmological Clinic, and had underwent complete ophthalmological examination, including best corrected visual acuity, and slit-lamp examination with high power condensing lens (78,90diopter) was done after pupillary dilation by tropicamide 1% ophthalmic drops. The examination was performed by senior ophthalmologist.

Control include 53 subjects : 29 diabetic non retinopathy(DNR) with age mean 49.3 ±13 years and 24 healthy volunteers (HC) with no history of diabetes, or any major clinical disorders with age mean 47.15±13 years.

Serum VEGF and PEDF were measured using ELISA Kits (BIOO Scientific - U.S.A and BioProducts MD-U.S.A.

Statistical Analysis

Statistical analysis were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA) and statistical significance was defined as $P< 0.05$

Results

There was a significant difference in serum VEGF level between studied groups ($P = 0.004$), and the highest level is in PDR group as figure (1) reveals.

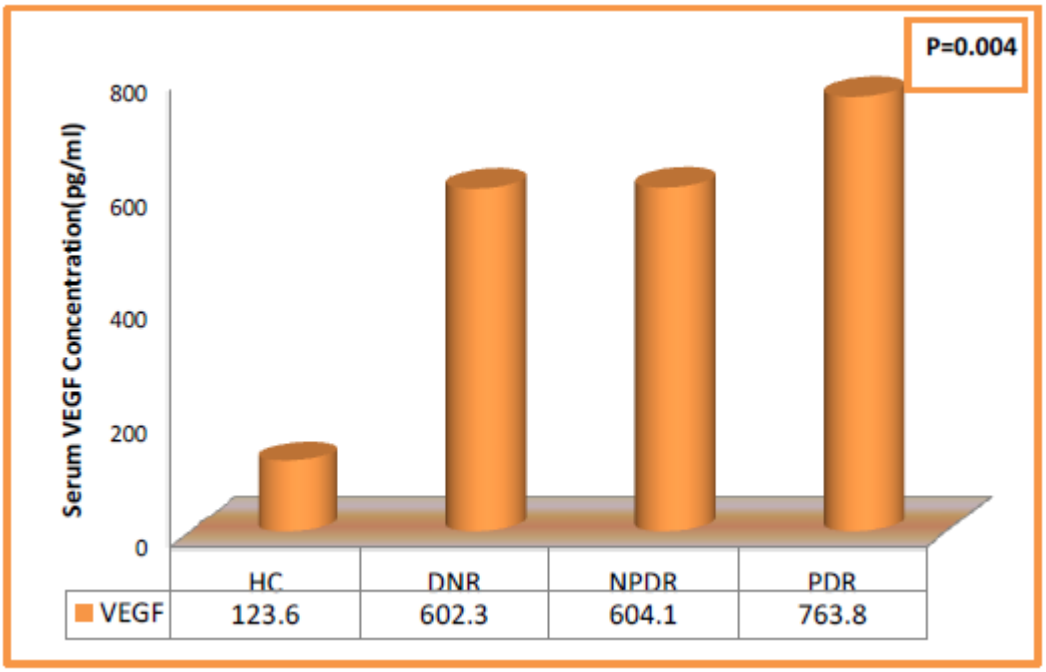

Figure (1): Mean of serum VEGF concentration in studied groups

Also this study revealed that serum level of PEDF was significantly different between groups (HC, DNR, NPDR, PDR) (p=0.001) as shown in figure (2) and significantly higher in PDR group compare to HC group (p=0.002), DNR group (p=0.002) and NPDR group (P=0.001).

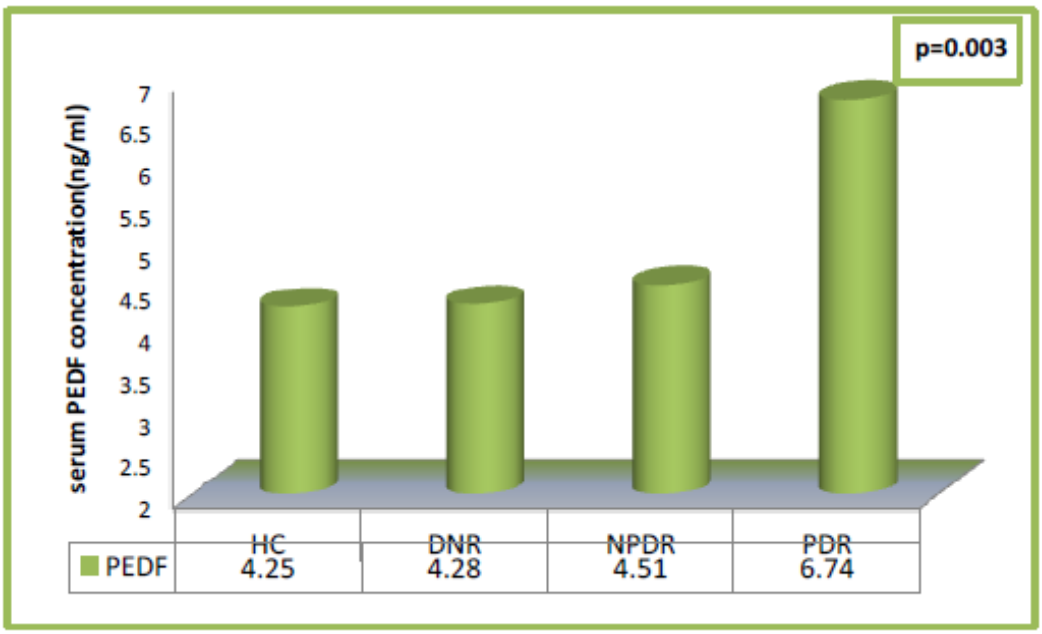

Figure (2): Concentration of serum PEDF (mean) in studied groups

In addition there was significant positive correlation between serum level of VEGF and PEDF ($P < 0.001$, $r = 0.820$) in all subjects as figure (3) shown.

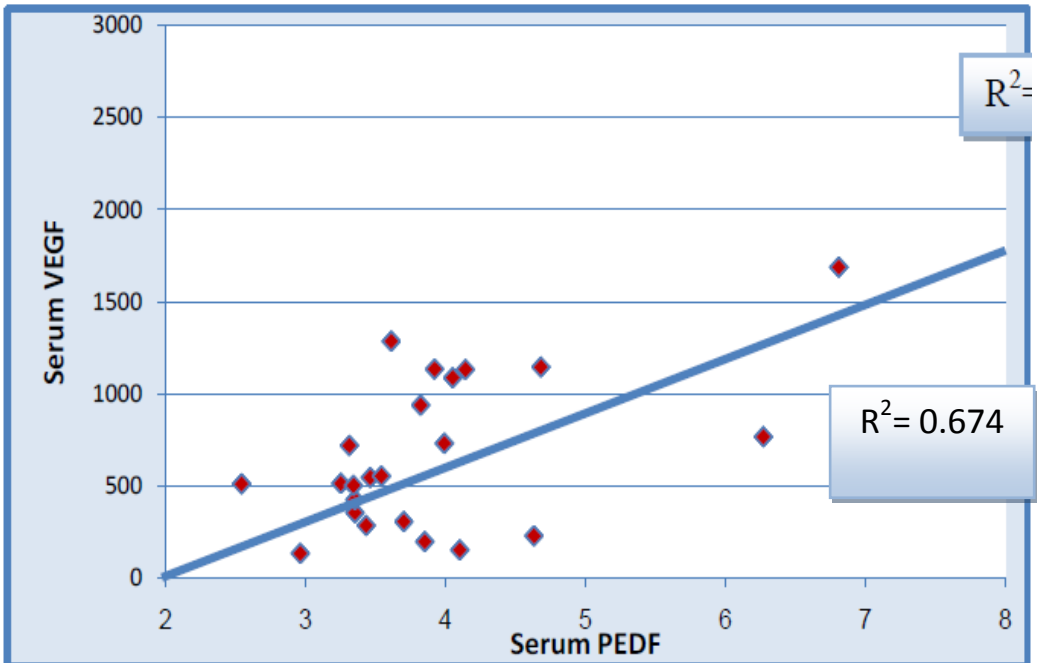

Figure (3): Correlation between serum level of VEGF and PEDF

Discussion

VEGF plays an important role in the pathogenesis of diabetic microvascular complications, as VEGF involved in the process of new blood vessel formation. Increased production of this cytokine (VEGF) can result from hyperglycemia and subsequent advanced glycation end products (43, 44).

Hyperglycemia causes an increased glucose flux through polyol pathway and generation of AGEs. Those processes result in enhanced production of diacylglycerol which is an activator of protein kinase C (PKC) (45, 46). In the earlier stage of the disease, this PKC has been implicated in induction of various cytokines and angiogenic factors including VEGF (47,45,46,47,48,49).

Also, hyperglycemia causes alterations of retinal blood flow (50), loss of pericytes and formation of microaneurysms which are followed by the closure of retinal capillaries that leads to local nonperfusion along with an increase of vascular permeability and hypoxia. The hypoxia, in turn, causes the release of several inflammatory mediators and angiogenic factors. VEGF is one of factors that its expression is induced by hypoxia (51-53). Both hyperglycemia and hypoxia are predominant factors in DM which explain the high levels of VEGF in DNR and DR groups compared to healthy control. Those results show a similarity to Ying Yang et al. result, Faten et al., Sydorova M. et al. and Hasanain M .et al. results (54-57).

PEDF is synthesized in a wide range of human tissues including the lung, brain, kidney, and especially the liver (29), which may contribute to the high levels of PEDF in the blood. PEDF is most likely associated with the metabolism in patients with diabetes mellitus and may be associated with vascular damage. Vascular endothelial growth factor (VEGF) is a strong angiogenic factor, and many studies have demonstrated that VEGF induces the progression of diabetic retinopathy.

Advanced glycation end products (AGEs) in diabetic patients are also involved in the leukostasis and microthrombosis that result in PDR; it has been suggested that PEDF counteracts the effects of VEGF (58), and it also been suggested that PEDF significantly inhibits AGE activity(59) thus, increased levels of PEDF in the blood of patients with the PDR may be a response to counteract the activity of VEGF and AGEs. Previous studies demonstrated that the level of PEDF was lower in eyes with diabetic retinopathy, especially in eyes with PDR (60-63). These findings indicated that the decrease of PEDF in the eyes might be involved in the progression of diabetic retinopathy and the degree of retinal neovascularization because, PEDF is a potent anti-angiogenic and antiinflammatory cytokine (64,65), PEDF may be consumed in the eye with diabetic retinopathy to counteract the angiogenic and inflammatory responses of the endothelial cell. Our study is consistence with study done by Nahoko Ogata et al.(66) which found The plasma level of PEDF in the PDR group was significantly higher than that of controls.

In addition presence of positive correlation between VEGF and PEDF documents our suggestion that one of the causes for elevation of PEDF is to counteract the effects of VEGF (58).

The VEGF and PEDF level in the blood is elevated in diabetic patients, especially in those with PDR compared to healthy control so their concentrations predict adverse outcomes, and their measurement may facilitate risk estimation, and PEDF-based interventions might be considered.

References

1. Mohamed Q, Gillies MC, Wong TY. "Management of diabetic retinopathy: systematic review". JAMA 2007; 298:902–916.
2. Wild S, Roglic G, Green A, Sicree R, King H." Global prevalence of diabetes: estimates for the year 2000 and projections for 2030". Diabetes Care 2004, 27:1047-1053.
3. WHO."Magnitude and causes of Visual impairment". 2010.
4. Nguyen TT., Wang JJ., Wong TY.: Retinal vascular changes in pre-diabetes and pre-hypertension: new findings and their research and clinical implications. Diabetes Care, 2007; 30:2708-2715.
5. Nguyen TT., Wong TY.: Retinal vascular manifestations of metabolic disorders. Trends Endocrinol Metab. 2006; 17:262-268.
6. Early Treatment Diabetic Retinopathy Study Research Group. "Fundus photographic risk factors for progression of diabetic retinopathy". ETDRS Report No. 12. Ophthalmology 1991;98:823-33.
7. Aris N. Kollias, Michael W.: Diabetic Retinopathy Early Diagnosis and Effective Treatment. Int. 2010; 107(5):75-84.
8. Early Treatment Diabetic Retinopathy Study Research Group. "Fluorescein angiographic risk factors for progression of diabetic retinopathy". ETDRS Report No. 13. Ophthalmology 1991;98:834-40.
9. Ferrara N., Davis-Smyth T.: The biology of vascular endothelial growth factor. Endocr. Rev., 1997; 18, 4-25.
10. Duh E., Aiello L.P.: Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. Diabetes, 1999; 48, 1899-1906.
11. Takahashi H., Shibuya M.: The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clin. Sci. 2005; 109, 227-241.
12. Angelo, L.S. and R. Kurzrock Clin. Cancer Research. 2007; 13:2825.
13. Byrne, A.M. et al. J. Cell. Molecular. Medicine. 2005; 9:777.
14. Robinson, C.J. and S.E. Stringer .J. Cell. Sci. 2001; 114:853.
15. Sugishita Y. et al.: Biochem. Biophys. Res. Commun., 2000; 268:657.
16. Diaz B.V. et al. :Regulation of Vascular Endothelial Growth Factor Expression in Human Keratinocytes by Retinoids. J. Biol. Chem., 2000; 275: 642.
17. Bautz F. et al.: Exp. Hematol., 2000; 28: 700.
18. Nauck M. et al.: Induction of vascular endothelial growth factor by platelet-activating factor and platelet-derived growth factor is downregulated by corticosteroids Am. J. Respir. Cell. Mol. Biol., 1997; 16: 398.
19. Byrne A.M. et al.: Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J. Cell. Mol. Med., 2005; 9: 777.
20. Robinson C.J., S.E. Stringer: The splice variants of vascular endothelial growth factor (VEGF) and their receptorsJ. Cell. Sci., 2001; 114: 853.
21. Angelo L.S., R. Kurzrock: Vascular Endothelial Growth Factor and Its Relationship to Inflammatory Mediators Clin. Cancer Res., 2007; 13: 2825.
22. Kowalewski M.P. et al.: Molecular cloning of VEGFR2 (KDR) in canine corpus luteum. Accession ABB., 2005; 82619.
23. Filleur S, Nelius T, de Riese W, Kennedy RC. "Characterization of PEDF: a multi-functional serpin family protein". J. Cell. Biochem. 2009,106 (5): 769–75.
24. Karakousis PC, John SK, Behling KC, Surace EM, Smith JE, HendricksonA, Tang WX, Bennett J, Milam AH " Localization of pigment epithelium derived factor (PEDF) in developing and adult human ocular tissues". Mol Vis 2001, 7:154-163.
25. Ogata N, Wada M, Otsuji T, Jo N, Tombran-Tink J, Matsumura M" Expression of pigment epithelium-derived factor in normal adult rat eye and experimental choroidal neovascularization". Invest Ophthalmol Vis Sci 2002, 43(4):1168-1175.
26. Tombran-Tink J, Barnstable CJ" Therapeutic prospects for PEDF:more than a promising angiogenesis inhibitor". Trends Mol Med 2003, 9(6):244-250.
27. Tombran-Tink J" The neuroprotective and angiogenesis inhibitory serpin, PEDF: new insights into phylogeny, function, and signaling". Front Biosci 2005, 10:2131-2149.

28. Petersen SV, Valnickova Z, Enghild JJ." Pigment-epithelium-derived factor (PEDF) occurs at a physiologically relevant concentration in human blood: purification and characterization". Biochem J 2003; 374:199–206.

29. Sawant S, Aparicio S, Tink AR, Lara N, Barnstable CJ, Tombran-Tink J. "Regulation of factors controlling angiogenesis in liver development: a role for PEDF in the formation and maintenance of normal vasculature". Biochem Biophys Res Commun 2004; 325:408–13.

30. Becerra SP, Sagasti A, Spinella P, Notario V" Pigment epitheliumderived factor behaves like a noninhibitory serpin. Neurotrophic activity does not require the serpin reactive loop". J Biol Chem 1995; 270(43):25992-25999.

31. H. Liu, J. G. Ren, W. L. Cooper, C. E. Hawkins, M. R. Cowan, and P. Y. Tong, "Identification of the antivasopermeability effect of pigment epithelium-derived factor and its active site," Proceedings of the National Academy of Sciences of the United States of America, 2004; 101, 17, 6605–6610.

32. S. X. Zhang, J. J.Wang, G. Gao, C. Shao, R.Mott, and J. X.Ma,"Pigment epithelium-derived factor (PEDF) is an endogenous antiinflammatory factor," FASEB Journal, 2006; 20, 2, 323–325.

33. M.Matsuoka, N. Ogata, T. Otsuji, T. Nishimura, K. Takahashi, and M. Matsumura, "Expression of pigment epithelium derived factor and vascular endothelial growth factor in choroidal neovascular neovascular membranes and polypoidal choroidal vasculopathy," British Journal of Ophthalmology, 2004;88. 6, 809–815.

34. H. Yang and H. E. Grossniklaus, "Constitutive overexpression of pigment epithelium-derived factor inhibition of ocular melanoma growth and metastasis," Investigative Ophthalmology & Visual Science, 2010; 51, 1. 28–34.

35. T. Yabe, D. Wilson, and J. P. Schwartz, "NFκB Activation Is Required for the Neuroprotective Effects of Pigment Epithelium-derived Factor (PEDF) on Cerebellar Granule Neurons," Journal of Biological Chemistry, 2001; 276, 46, 43313–43319.

36. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, BouckNP " Pigment epithelium-derived factor: a potent inhibitor of angiogenesis".Science. 1999 285:245–248.

37. Yang H, Xu Z, Iuvone PM, Grossniklaus HE. "Angiostatin decreases cell migration and vascular endothelium growth factor (VEGF) to pigment epithelium derived factor (PEDF) RNA ratio in vitro and in a murine ocular melanoma model". Mol Vis 2006;12: 511–7.

38. Gao G, Li Y, Gee S, Dudley A, Fant J, Crosson C, Ma JX. "Down-regulation of vascular endothelial growth factor and up-regulation of pigment epithelium-derived factor: a possible mechanism for the anti-angiogenic activity of plasminogen kringle 5". J Biol Chem 2002;277 (11): 9492–7.

39. Notari L, Miller A, Martínez A, Amaral J, Ju M, Robinson G, Smith LE, Becerra SP. "Pigment epithelium-derived factor is a substrate for matrix metalloproteinase type 2 and type 9: implications for downregulation in hypoxia". Invest. Ophthalmol. Vis. Sci. 2005;. 46 (8): 2736–47.

40. Notari L, Baladron V, Aroca-Aguilar JD, Balko N, Heredia R, Meyer C, Notario PM, Saravanamuthu S, Nueda ML, Sanchez-Sanchez F, Escribano J, Laborda J, Becerra SP. "Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor". J. Biol. Chem. 2006; 281 (49): 38022–37.

41. Cai J, Jiang WG, Grant MB, Boulton M ."Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1". J. Biol. Chem. 2006; 281 (6): 3604–13.

42. Bernard A, Gao-Li J, Franco CA, Bouceba T, Huet A, Li Z ."Laminin receptor involvement in the anti-angiogenic activity of pigment

epithelium-derived factor". J. Biol. Chem. 2009; 284 (16): 10480–90.

43. Yamagishi S., Amano S., Inagaki Y., Okamoto T., Koga K., Sasaki N., Yamamoto H., Takeuchi M., Makita Z.: Advanced glycation end products-induced apoptosis and over expression of vascular endothelial growth factor in bovine retinal pericytes. Biochem. Biophys. Res. Commun., 2002 25; 290: 973-8.

44. Treins C., Giorgetti-Peraldi S., Murdaca J., Van Obberghen E.: Regulation of vascular endothelial growth factor expression by advanced glycation end products. J. Biol. Chem., 2001; 276: 43836.

45. Brownlee M.: Biochemistry and molecular cell biology of diabetic complications. Nature, 2001; 414:813-820.

46. Geraldes P., Hiraoka-Yamamoto J., Matsumoto M., et al.: Activation of PKC delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. Nat. Med., 2009;15: 1298-1306.

47. Das Evcimen N., King GL.: The role of protein kinase C activation and the vascular complications of diabetes. Pharmacol Res., 2007; 55: 498-510.

48. Aiello LP., Davis MD., Girach A., Kles KA., Milton RC., Sheetz MJ., Vignati L., Zhi XE., PKCDRS2Group: Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology, 2006; 113: 2221-2230.

49. Avignon A., Sultan A.: PKC-B inhibition: a new therapeutic approach for diabetic complications. Diabetes Metab., 2006; 32:205-213.

50. Aiello LM., Aiello LP., Cavallerano JD.: Ocular complications of diabetes mellitus. Diabetes Mellitus. 14th ed. Philadelphia: Lippincott Williams & Wilkins, 2005; 901-924.

51. Millauer B., Shawver LK., Plate KH., Risau W., Ullrich A: Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1mutant. Nature,1994; 10:576 -579.

52. Cooper ME., Vranes D., Youssef S., Stacker SA., Cox AJ., Rizkalla B., Casley DJ., Bach LA., Kelly DJ., Gilbert RE.: Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. Diabetes, 1999; 48: 2229-2239.

53. Sone H., Kawakami Y., Okuda Y., Kondo S., Hanatani M., Suzuki H., Yamashita K.: Vascular endothelial growth factor is induced by long-term high glucose concentration and up-regulated by acute glucose deprivation in cultured bovine retinal pigmented epithelial cells. Biochem. Biophys.Res. Commun., 1996; 5:193-198.

54. Ying Yang, Bradley T Andresen, Ke Yang, Ying Zhang, Xiaojin Li, Xianli Li, HuWang: Association of vascular endothelial growth factor 634C/Gpolymorphism and diabetic retinopathy in type 2 diabetic. Han Chinese Experimental Biology and Medicine, 2010; 235:1204-1211.

55. Faten A. Zakareia, Abdulmageed A. Alderees, Khalid Abdualla Al Regaiy, Fawziah A. Alrouq: Correlation of electroretinography b-wave absolute latency, plasma levels of human basic fibroblast growth factor, vascular endothelial growth factor, soluble fatty acid synthase, and adrenomedullin in diabetic retinopathy. Journal of Diabetes and Its Complications, 2010; 24: 179-185.

56. Sydorova M., Lee MS.: Vascular endothelial growth factor levels in vitreous and serum of patients with either proliferative diabetic retinopathy or proliferative vitreoretinopathy. Ophthalmic Res., 2005; 37:188-190.

57. Hasanain M. Ahmed, Mahdi Alsihlawi, Fadhil Abdulameer: The relevance of serum level of VEGF in type 2 diabetic retinopathy. Kufa Med.Journal, 2012;15:106-113.

58. Liu H, Ren JG, Cooper WL, Hawkins CE, Cowan MR, Tong PY." Identification of the antivasopermeability effect of pigment epithelium-derived factor and its active site". Proc Natl Acad Sci USA 2004; 101:6605–6610

59. Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S ." Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive

oxygen species generation". Diabetologia 2003;46:284–287.

60. Spranger J, Osterhoff M, Reimann M, Mohlig M, Ristow M, Francis MK, Cristofaro V, Hammes HP, Smith G, Boulton M, Pfeiffer AF ." Loss of antiangiogenic pigment epithelium-derived factor in patients with angiogenic eye diseases". Diabetes 2001 50:2641–2645

61. Ogata N, Tombran-Tink J, Nishikawa M, Nishimura T, Mitsuma Y, Sakamoto T, MatsumuraM." Pigment epithelium-derived factor in the vitreous is low in diabetic retinopathy and high in rhegmatogenous retinal detachment".Am J Ophthalmol , 2001. 132:378–382

62. Ogata N, Nishikawa M, Nishimura T, Mitsuma Y, Matsumura M ."Unbalanced vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor in diabetic retinopathy". Am J Ophthalmol 2001.134:348–353.

63. Boehm BO, Lang G, Volpert O, Jehle PM, Kurkhaus A, Rosinger S, Lang GK, BouckN." Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retinopathy". Diabetologia 2003. 46:394–400.

64. Zhang SX, Wang JJ, Gao G, Shao C, Mott R, Ma JX." Pigment epithelium-derived factor (PEDF) is an endogenous antiinflammatory factor". FASEB J 2006; 20:323-5.

65. Tombran-Tink J, Barnstable CJ. PEDF:" a multifaceted neurotrophic factor". Nat Rev Neurosci 2003; 4:628-36.

66. Ogata N, Matsuoka M, Matsuyama K, Shima C, Tajika A,Nishiyama T, Wada M, Jo N, Higuchi A, Minamino K,Matsunaga H, Takeda T, Matsumura M. Plasmaconcentration of pigment epithelium-derived factor in patients with diabetic retinopathy. J Clin Endocrinol Metab 2007; 92:1176-9.