Vascular Endothelial Growth Factor (VEGF) Gene Insertion/Deletion Polymorphism and Diabetic Retinopathy in Patients with Type 2 Diabetes

Hanan Fouad[1]; Mona A. Abdel Hamid[2]; Amira A. Abdel Azeem[*3]; Hany M. Labib[4] and Nervana A. Khalaf[5]

Medical Biochemistry Department [1,] Faculty of Medicine, Cairo University, Biochemistry [2], Ophthalmic Genetics [3], Ophthalmology [4]and Clinical Pathology[5] Departments, Research Institute of Ophthalmology, Cairo, Egypt
*azeem.amira@yahoo.com

Abstract: Background: Vascular endothelial growth factor (VEGF) appears to play a central role in mediating microvascular pathology in diabetic retinopathy (DR). Aim of the study: To assess the possible association of the insertion/deletion (I/D) polymorphism of VEGF gene with diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. Subjects and Methods: This cross-sectional case-control study enrolled 87 unrelated subjects with type 2 diabetes mellitus, 43 diabetic patients without signs of retinopathy but did have type 2diabetes for more than 10 years and 44 patients with diabetic retinopathy. The control group involved 44 normal subjects without diabetes. Total genomic DNA was isolated from peripheral blood leukocytes. PCR analysis was conducted to detect the insertion/deletion gene polymorphism of the 18 bp fragment at position 2549 of the promoter region of VEGF. The frequency of D and I VEGF alleles and genotype distribution were compared in diabetics with retinopathy, diabetics without retinopathy and the control subjects. Results: There was no significant difference in genotype distribution (D/D, I/D and I/I), (p=0.43) and in (D and I) allele frequency (p=0.093) of diabetic patients with retinopathy, diabetics without retinopathy and control subjects. The distribution of the VEGF, D/D genotype was higher in patients with diabetic retinopathy compared with diabetic group without retinopathy and healthy controls (40.9% *vs*. 27.9% and 22.7% respectively), however the difference was still not statistically significant with Chi-Square= 3.637 and p value = 0.162. Despite the insignificant results, this study adjusted OR of 2.25 (95% CI, 0.672- 7.538) for D/D genotype versus I/I genotype between diabetic patients with retinopathy and controls with p value = 0.185 and the OR of 1.6 (95% CI, 0.873- 2.891) for the D allele versus I allele between diabetic patients with retinopathy and controls with p= 0.129, while the OR of the D allele versus I allele in diabetic patients without retinopathy and controls was only 1.2 and the p value was 0.539. In multivariate analysis only increased triglyceride level was the independent risk factor for diabetic retinopathy among Egyptian patients with type 2 diabetes. Conclusion: Our study suggested that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with retinopathy in Egyptian type 2 diabetic patients, however a moderate risk (i.e., OR, < 2 for D/D genotype and < 1.5 for D allele) could not be excluded. Only increased triglyceride level was the independent risk factor in the development of diabetic retinopathy detected in this study.
[Hanan Fouad; Mona A. Abdel Hamid; Amira A. Abdel Azeem; Hany M. Labib and Nervana A. Khalaf. Vascular Endothelial Growth Factor (VEGF) Gene Insertion/Deletion Polymorphism and Diabetic Retinopathy in Patients with Type 2 Diabetes. Journal of American Science 2011;7(3):199-205]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Vascular endothelial growth factor (VEGF), insertion/deletion polymorphism, diabetic retinopathy, type 2 diabetes, Egyptian patients.

1. Introduction:

Many diabetic patients, especially those with poor glycemic control, develop diabetic retinopathy, which remains the major cause of blindness among diabetic adults [1]. Diabetic retinopathy is characterized by increased vascular permeability, tissue ischemia with angiogenesis [2]. Growth factors may play an important role in modifying and accelerating the tissue damage caused by hyperglycemia [3].

Vascular endothelial growth factor (VEGF) is a potent multifunctional cytokine which plays a key role in the pathogenesis of diabetic microvascular complications [4]. VEGF is produced from many cell types within the eye and it is a highly conserved homodimeric glycoprotein which promotes angiogenesis and is a potent mediator of microvascular permeability [5].

Several studies have shown that VEGF expression is increased in patients with diabetic retinopathy [6, 7, 8] and others documented that VEGF levels are markedly elevated in vitreous of the eyes of individuals with proliferative diabetic retinopathy (PDR) [9, 10]. In addition, VEGF induction of vascular permeability may contribute to the development of non-proliferative diabetic retinopathy (non-PDR) [11].

The genetic variations in the VEGF gene can influence levels of VEGF protein expression [2]. The human VEGF gene is located on chromosome 6 (6p21.3) and is highly polymorphic. Of particular interest is an insertion/deletion (I/D) polymorphism of the 18 bp fragment at–2549 position of the promoter region that has been implicated in a number of diseases, especially those with angiogenic basis [12, 13].

The aim of this study was to investigate the impact of genetic I/D polymorphism of VEGF on diabetic retinopathy in an Egyptian population with type 2 diabetes.

2. Subjects and Methods:

Subjects:

This cross-sectional case-control study enrolled 87 unrelated Egyptian diabetic patients (43 men, 44 women) with age ranged from 60 to 75 years. They were classified as having type 2 diabetes according to the current American Diabetes Association criteria for the diagnosis and classification of diabetes [14]. Patients were recruited from the Ophthalmology Clinic of the Research Institute of Ophthalmology. Control subjects were 44 healthy volunteers with no history of diabetes, or any major clinical disorders and had normal fasting blood sugar and HbA_{1C}

Methods:

Family history of DR for all patients and controls and the duration of diabetes for patients were registered. All patients underwent a complete ophthalmological examination, including best corrected visual acuity, slit-lamp examination, intraocular pressure measurement using Goldmann applanation tonometry, indirect ophthalmoscopy and biomicroscopy. Fundusg fluorescein angiography was done in needed cases using Topcon fundus camera TRC. 50 EX on image-net. Five ml of 10% sodium fluorescein was injected in the antecubital vein and photography was carried out. Retinopathy was diagnosed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria: the presence of microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading and new vessels [15]. Patients were classified into 2 groups: group (1): 43 diabetic patients without retinopathy, group (2): 44 diabetic patients with diabetic retinopathy (DR) and 44 healthy control group. Informed consent was obtained from participants after a clear explanation of potential risk of the study.

HbA_{1C} was measured with a cation exchange chromatography method to assess glycaemic control. The procedure is a microchromatographic methodology for the quantitation of glycosylated haemoglobin (non-diabetic reference 5.5 % - 7.7%) (GLYCO Hb Quick column procedure) [16]. Serum total cholesterol and triglycerides were measured using enzymatic methods. [17, 18].

Determination of the VEGF genotypes

Genomic DNA was extracted from peripheral blood leucocytes using QIA amp DNA mini kit (QIAGEN, Inc., Germany). The I/D polymorphism was analysed using the following primers: forward 5'-GCTGAGAGTGGGGCTGACTAGGTA-3' and reverse 5'-GTTTCTGACCTGGCTATTTCCAGG-3'. Genomic DNA (300 ng) was amplified in a final volume of 30 µl using the following conditions: denaturation at 95°C for 6 min followed by 35 cycles at 94°C for 1 min., 57°C for 1.5 min. and 72°C for 2 min. A final extension was at 72°C for 10 min. The amplification products were separated by electrophoresis on 2.5% agarose gel stained with ethidium bromide. For the VEGF I/D polymorphism two bands were observed, 211 bp for D allele and 229 bp for I allele.

Statistical analysis:

The data were analyzed using the statistical package SPSS (version 15). They were expressed as mean ± standard deviation for quantitative variables and as number and percentage for qualitative values. Statistical differences between categorical data like gender, genotype distribution and family history of diabetic retinopathy were tested using Chi Square test. For qualitative variables, independent sample t test and ANOVA (analysis of variance) with post Hoc Bonferroni test were used for normally distributed variables as age, FBS, HbA_{1c}, total cholesterol and triglycerides**.** Associations of genotypes and alleles were assessed as OR and 95% confidence intervals (CI). Differences by univariate methods ($\chi 2$ test, unpaired Student t test) were analyzed together in a logistic regression analysis to test for significant risk factors for diabetic retinopathy. Values less than or equal to 0.05 were considered statistically significant.

3. Results:

Table (1) presents the clinical and biochemical variables of the studied diabetic subjects and controls. There was no statistically significant difference in age and gender between the control group, group (1) and group (2). Also, there was no significant difference between group (1) and group (2) in the duration of the disease. However there was a statistically significant increase of family history of retinopathy in group (2) compared to group (1) and

a statistically significant increase of fasting blood sugar, HbA_{1c}, total cholesterol and triglycerides was noted in patients compared to controls.

Table (2) showed that there was no statistically significant difference in genotype distribution and allele frequencies of the I/D polymorphism between the three groups in general (p-values were 0.43 and 0.093 respectively), in spite of increased the frequency of D allele in patients with diabetic retinopathy compared to both diabetic patients and controls (61.4% vs. 54.65% and 50% respectively). Further analysis showed that the distribution of the VEGF, D/D genotype was higher in patients with diabetic retinopathy compared with diabetic group without retinopathy and healthy controls, (40.9% *vs*. 27.9% and 22.7% respectively) however the difference was still not statistically significant with Chi-Square= 3.637 and p value = 0.162 (table 3). The OR of D/D genotype versus I/I genotypes between diabetic patients with retinopathy and controls was 2.25 (95% CI 0.672-7.538) with p value =0.185 (table 4) and the OR for the D allele versus I allele between diabetic patients with retinopathy and controls was 1.6 (95% CI 0.873-2.891) with p= 0.129 (table 5), while (table 6) showed that the OR for the D allele versus I allele in diabetic patients without retinopathy and controls was only 1.2 and the p value was 0.539 (95% CI 0.64-2.29). Logistic regression analysis which included significant variables (family history of DR, fasting blood sugar, HbA1c% total cholesterol and triglycerides) plus age, gender (F vs. M) and duration of diabetes showed that only triglycerides was an independent risk factor for diabetic retinopathy in patients with type 2 diabetes with p value =0 .047 and 95% CI, 1.0- 1.033.

Table (1): Clinical and biochemical characteristics of studied subjects

	Controls (n=44)	Group 1 (n=43)	Group 2 (n=44)	p value
Gender Male **n (%)** Female **n (%)**	 20(45.5) 24(54.5)	 22(51) 21(49)	 21(48) 23(52)	0.866
Age (years)	69.05 (±5.23)	70.63 (±3.02)	70.50 (±4.11)	0.155
Duration of disease (years)	-	11.93 (±2.58)	11.82 (±2.16)	0.826
Family history of diabetic retinopathy, n (%)	-	18(41.9%)	28(63.6%)	0.042*
FBS (mg/dl)	102.23 (±9.52)	239.35 (±45.98)	242.68 (±46.35)	<0.001*
HbA_{1c} (%)	6.17 (±1.0)	9.63 (±1.87)	10.23 (±1.37)	<0.001*
Total cholesterol (mg/dl)	176.66 (±19.31)	192.60 (±34.92)	199.16 (±47.80)	0.012*
Triglycerides(mg/dl)	169.05 (±24.68)	172.60 (±33.05)	188.57 (±33.63)	0.008*

Table (2): Genotype and allele distribution of VEGF gene I/D polymorphism in type 2 diabetic patients and controls

	Controls (n=44)	Group 1 (n=43)	Group 2 (n=44)	p value
Gene polymorphism, n (%) **II** **ID** **DD**	 10(22.7%) 24(54.6%) 10(22.7%)	 8(18.6%) 23(53.5%) 12(27.9%)	 8(18.2%) 18(40.9%) 18(40.9%)	0.43
Allelic frequency, n (%) **I** **D**	 44 **(**50%) 44 (50%**)**	 39**(**45.34%) 47(54.65%**)**	 34 (38.6%) 54(61.4%)	0.093

Table (3): The distribution of the VEGF, DD versus ID and II genotypes among patients and controls

Genotype	DM& Retinopathy	DM	Control	Total	X^2	p value
DD Count (%)	18 (40.9)	12 (27.9)	10(22.7)	40(30.5)	3.637	0.162
ID&II Count (%)	26 (59.1)	31 (72.1)	34(77.3)	91(69.5)		
Total Count (%)	44 (100)	43 (100)	44(100)	131(100)		

DM= diabetes mellitus

Table (4): DD versus II genotypes in diabetic patients with retinopathy and controls

Genotype	DM& Retinopathy	Control	Total	p-value	OR	95% Confidence Interval
DD Count (%)	18 (69.2)	10 (50)	28(60.9)	0.185	2.25	0.672- 7.538
II Count (%)	8 (30.8)	10 (50)	18(39.1)			
Total Count (%)	26 (100)	20 (100)	46 (100)			

Table (5): Allele distribution of VEGF gene I/D polymorphism in patients with diabetic retinopathy and controls

Alleles	DM& Retinopathy	controls	Total	P value	OR	95% Confidence Interval
D No (%)	54 (61.4)	44 (50)	98 (55.7)	0.129	1.6	0.873- 2.891
I No (%)	34 (38.6)	44 (50)	78 (44.3)			
Total	88 (100)	88 (100)	176 (100)			

Table (6): Allele distribution of VEGF gene I/D polymorphism in patients with diabetes without retinopathy and controls

Alleles	DM	controls	Total	P value	OR	95% Confidence Interval
D No (%)	47(54.7)	44 (50)	91(52.3)	0.539	1.2	CI= 0.64- 2.29
I No (%)	39 (45.3)	44 (50)	83(47.7)			
Total	86 (100)	88 (100)	174 (100)			

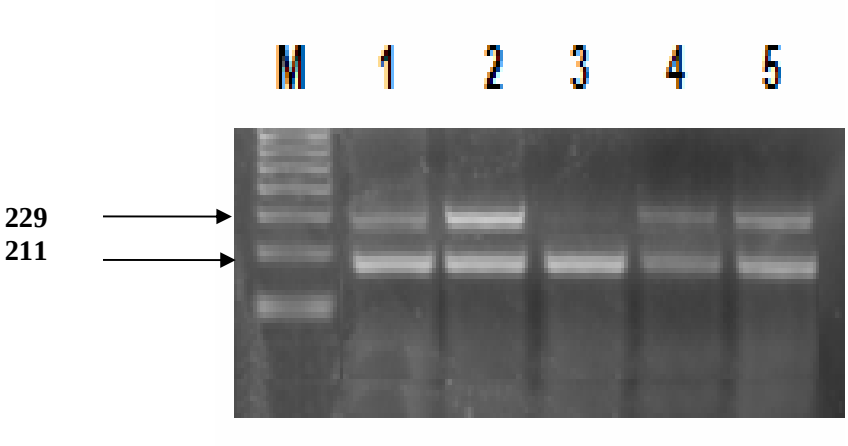

Figure (1): PCR amplification products of (I/D) VEGF gene polymorphism for patients with DR
Lane M: PCR marker 100 bp
Lanes 1, 2, 4 and 5 showed both 211 bp band of D allele and 229 bp band of I allele and lane3 showed only band of D allele.

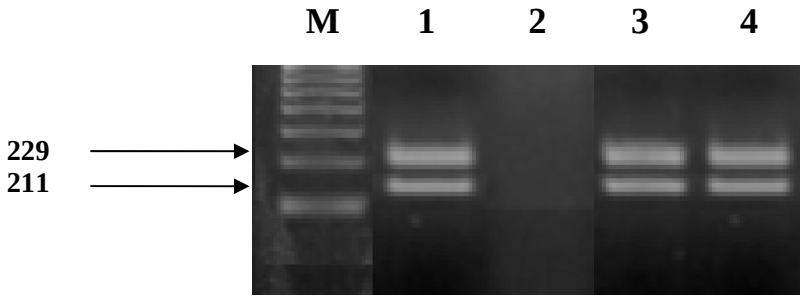

Figure (2): PCR amplification products of I/D - VEGF gene polymorphism of diabetic patients without retinopathy
Lane M: PCR marker 100 bp
Lanes: 1, 3 and 4 showed both 211bp band of D allele and 229bp band of I allele.

4. Discussion:

Diabetic retinopathy is characterized by increased vascular permeability, tissue ischemia and neovascularisation [19]. During hypoxia, hypoxia-inducible factors bind to the hypoxia-response elements and induce the expression of VEGF, which leads to the stimulation of angiogenesis and increases the permeability of the microvasculature [20]. Many cell types within the eye produce VEGF which is markedly elevated in the vitreous and aqueous fluids of patients with diabetic retinopathy [21].

Different polymorphisms in VEGF gene like I/D polymorphism in the promoter region, +405 G/C and -634 C/G polymorphisms in the 5'-untranslated region, have been studied in different ethnic groups [2, 11 and 13]. In this study we focused on the insertion/deletion (I/D) polymorphism of the 18 bp fragment at –2549 position of the promoter region. This region has been shown to be highly polymorphic and in addition, most of the hypoxia-responsive elements are present in this region. To our knowledge, the present study is the first attempt to examine the possible association between this polymorphism and diabetic retinopathy among Egyptian patients.

In spite of increased the frequency of the D/D genotype of the VEGF gene I/D polymorphism in patients with diabetic retinopathy than diabetic patients without retinopathy and controls (40.9% vs. 27.9% and 22.7%), it did not show significant difference in genotype distribution (D/D, I/D and I/I) ($p = 0.43$). However we found more than a two fold increased risk of DR associated with D/D genotype and more than one and half fold increased risk with D allele in patients with DR than controls. On the other hand, there was an inconsiderable increased risk

associated with D allele in diabetic patients than controls (OR =1.2).These results suggest that the D allele has a detectable role in the development of diabetic retinopathy, however it may probably need interaction with other genetic or environmental factors or with increasing sample size in future studies we may predict a more powerful significant effect of the D allele on the development of diabetic retinopathy.

The association of the D allele at –2549 in the promoter region of the VEGF gene with the susceptibility to diabetic retinopathy can be explained in part by the enhanced level of transcription compared with the I allele. This would likely result in elevated levels of VEGF in these patients compared with the subjects carrying the I allele. Buraczynska et al. (2007), suggested that the I/D polymorphism in the promoter region of the VEGF gene is associated with retinopathy but not nephropathy in type 2 diabetes patients [13]; however Yang and his colleagues (2003), found that the D/D genotype was significantly increased in patients with diabetic nephropathy (a diabetic microvascular complication) compared to those with no complications (40.2% vs. 22.7%, respectively) [22].

Our study showed a statistically significant increase of family history of diabetic retinopathy in patients with DR than diabetic patients without retinopathy. A previous study of two hundred and twelve Egyptian families having one patient or more with diabetes mellitus showed that family history is an important risk factor for the development of DR among Egyptian patients [23]. Glycosylated hemoglobin (HbA_{1c}) was also significantly higher in patients than controls ($P<0.001$). HbA_{1c} value >8.0% was significantly related with sight-threatening diabetic retinopathy in a screening programme in India.The Receiver Operating Characteristic (ROC) analysis showed that the cut-off value of 8.0 had 75.6% sensitivity and 58.2% specificity [24]. Nordwall et al. (2009) documented that good glycemic control remains crucial in prevention of late diabetic complications [25].

This study demonstrated also that serum triglycerides and total cholesterol were significantly elevated in patients with DR. This agreed with The Early Treatment Diabetic Retinopathy Study (ETDRS) that suggested that lipid lowering may decrease the risk of hard exudates formation and associated vision loss in patients with diabetic retinopathy. Preservation of vision may be an additional motivating factor for lowering serum lipid levels in persons with diabetic retinopathy and elevated serum lipid levels [26]. El Haddad and Saad (1998), also documented that lowering of blood lipids may be effective in lowering the incidence of retinopathy in controlled Omani diabetic patients [27]. On the other hand, Miljanovic et al. (2004) had failed to confirm this relationship [28]. By applying the multivariate logistic regression analysis we found that only increased level of triglycerides was the independent risk factor for diabetic retinopathy.

In conclusion, our study suggested that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with retinopathy in Egyptian type 2 diabetic patients, however moderate risk (i.e., OR, >2 for D/D genotype and > 1.5 for D allele) could not be excluded. Only increased triglyceride level was the independent risk factor in development of diabetic retinopathy detected in this study by logistic regression analysis. We recommend further investigations with increased sample size to predict a more significant effect of the D allele of VEGF gene on the development of diabetic retinopathy.

Corresponding author
Amira A. Abdel Azeem
Ophthalmic Genetics Departments, Research Institute of Ophthalmology, Cairo, Egypt
azeem.amira@yahoo.com

5. References:

1. Aiello LP and Wong J-S (2000): Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int 58: Suppl 77, S113–S119.
2. Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, Inoue I, Katayama S (2002): A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes, 51:1635–1639.
3. Gröne HJ (1995): Angiogenesis and vascular endothelial growth factor (VEGF): is it relevant in renal patients? Nephrol Dial Transplant 10:761–763.
4. Ferrara N and Davis-Smyth T (1997): The biology of vascular endothelial growth factor. Endocr Rev 18:4–25.
5. Zhou L, Sun H, Xu J, Kang J (2010): Level of vascular endothelial growth factor and interleukin-6 in aqueous humor in diabetic retinopathy patients. Yan Ke Xue Bao. 25(1):26-30.
6. Cavusoglu AC, Bilgili S, Alaluf A, Doğan A, Yilmaz F, Aslanca D, Karaca B, Yüksel B, Topaloglu E (2007): Vascular endothelial growth factor level in the serum of diabetic patients with

retinopathy. Ann Ophthalmol (Skokie) ; 39(3):205-208.
7. Yan H, Cui J, Yu JG, Han JD, Chen S, Zhang JK, Xu YH (2009): The expression of vascular endothelial growth factor of vitreous in patients with proliferative diabetic retinopathy. Zhonghua Yan Ke Za Zhi; 45 (3): 206-209.
8. Chiarelli F, Spagnoli A, Basciani F, et al (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: 650–656.
9. Kant S, Seth G, Anthony K. (2009): Vascular endothelial growth factor-A (VEGF-A) in vitreous fluid of patients with proliferative diabetic retinopathy. Ann Ophthalmol (Skokie); 41(3-4):170-173.
10. Ferrara N, Gerber HP (2001): The role of vascular endothelial growth factor in angiogenesis. Acta Haematol; 106 :148 –156.
11. Watson CJ, Webb NJA, Bottomley MJ, Brenchley PE (2000): Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 12:1232–1235.
12. Vincenti V, Cassano C, Rocchi M, Persico G. (1996): Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. *Circulation* 93:1493–1495.
13. Buraczynska M, Ksiazek P, Baranowicz-Gaszczyk I and Jozwiak I (2007): Association of the VEGF gene polymorphism with diabetic retinopathy in type 2 diabetes patients. Nephrol Dial Transplant 22: 827–832.
14. Report of the expert committee on the Diagnosis and Classification of diabetes mellitus (2003): Diabetes Care; 26: 3160–3167.
15. Early Treatment Diabetic Retinopathy Study Research Group (1991): Grading diabetic retinopathy from stereoscopic color fundus photographs - an extension of the modified Airlie House classification. ETDRS report number 10. Ophthalmology, 98: 786–806.
16. Maquart FX, Gillery P, Bernar JF, Mante TP, and Borel JP (1980): A method specifically measuring hemoglobin A_1c with disposable commercial ion exchange column. Clin. Chem. Acta, 108: 329-332.
17. Richmonds W (1973): Determination of serum cholesterol. Clin Chem, 19:1350-1356.
18. Fossati P and Prenciphe L (1982): Determination of serum triglycerides. Clin. Chem. 28: 207.
19. Witmer AN, Vrensen GF, Van Noorden CJF, Schlingemann RO. (2003): Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 22:1–29.
20. Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, Van Dorpe J, Hellings P, Gorselink M, Heymans S, Theilmeier G (2001): Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet; 28:131-138.
21. Watanabe D, Suzuma K, Suzuma I, Ohashi H, Ojima T,Kurimoto M, Murakami T, Kimura T, Takagi H (2005): Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Am J Ophthalmol 139: 476-481.
22. Yang B, Cross DF, Ollerenshaw M, Millward BA, Demaine AG. (2003): Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. *J Diabetes Complications*, 17:1–6.
23. Abdel Azeem AA, Abu EL Ela MH, Elbastawisy HI, Osman ZM (2010): Genetic study of diabetes mellitus and diabetic retinopathy among Egyptians. Journal of the Egyptian Ophthalmological Society, (103):1 [under press].
24. Raman R, Verma A, Pal SS, Gupta A, Vaitheeswaran K, Sharma T (2011): Influence of glycosylated hemoglobin on sight-threatening diabetic retinopathy: A population-based study. Diabetes Res Clin Pract. [under press]
25. Nordwall M, Arnqvist HJ, Bojestig M, Ludvigsson J (2009): Good glycemic control remains crucial in prevention of late diabetic complications-the Linköping Diabetes Complications Study. Pediatr Diabetes, 10 (3): 168-176.
26. Rodriguez-Fontal M, Kerrison JB, Alfaro DV, Jablon EP (2009): Metabolic control and diabetic retinopathy. Curr Diabetes Rev; 5(1): 3-7.
27. El Haddad OA, Saad MK (1998): Prevalence and risk factors for diabetic retinopathy among Omani diabetics. Br J Ophthalmol; 82(8):901-906.
28. Miljanovic B, Glynn RJ and Nathan DM et al (2004): A prospective study of serum lipids and risk of diabetic macular oedema in type 1 diabetes. Diabetes 53, 2883-2892.

2/15/2011