Association of Monocyte Chemoattractant Protein-1 (MCP-1) 2518A/G Gene Polymorphism with Proliferative Diabetic Retinopathy in Patients with Type 2 Diabetes

Mona El-Tokhy[1], Eman Ramadan Abdel Gwad[1], Walid Abdel Ghaffar[2], Ola Serag[1]

[1]Department of Clinical & Chemical Pathology and [2]Department of Ophthalmology, Faculty of Medicine, Benha University, Egypt.
olaserag86@yahoo.com

Abstract: Background: MCP-1 is a member of the CC chemokine family and acts chemotactically on monocytes and induces monocyte and macrophage infiltration into tissues. Hyperglycemia induces MCP-1 production in vascular endothelial cells and retinal pigmented epithelial cells, and has been implicated as a causal factor in the facilitation of vascular complications in diabetes. In the present study, we evaluated the association of a single nucleotide polymorphism (SNP) in the MCP-1 gene with proliferative diabetic retinopathy (PDR) in egyptian population with type 2 diabetes. **Patients and Methods:** We conducted a case-control study, which included 50 subjects with type 2 diabetes. SNP genotyping of c.2518A/G in the MCP-1 gene was performed using polymerase chain reaction followed by digestion with PvuII restriction enzyme. **Results:** The prevalence of c.2518A/G polymorphism in diabetic patients was 52% (A/A), 34% (A/G) and 14% (G/G). In patients with diabetic retinopathy, the prevalence of PDR was significantly higher ($p<0.001$) in diabetic subjects with the c.2518A/A genotype (61.5%; 16/26) compared to those with either the A/G or G/G genotypes (16.7% (4/24). **Conclusion:** Our new genetic findings suggest that the c.2518A/A genotype in MCP-1 could be used as a susceptibility gene to predict the development of PDR in type 2 diabetic patients.

[Mona El-Tokhy, Eman Ramadan Abdel Gwad, Walid Abdel Ghaffar and Ola Serag. **Association of Monocyte Chemoattractant Protein-1 (MCP-1) 2518A/G Gene Polymorphism with Proliferative Diabetic Retinopathy in Patients with Type 2 Diabetes.** *Life Sci J* 2015;12(12):22-26]. (ISSN:1097-8135). http://www.lifesciencesite.com. 4. doi:10.7537/marslsj121215.04.

Key Words: MCP-1 polymorphism, type 2 DM, proliferative diabetic retinopathy

1. Introduction

Diabetic microvascular complications are associated with long-term hyperglycemia in patients with diabetes. Diabetic retinopathy (DR) is divided into two major categories, non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR)[1]. PDR is a serious diabetic microvascular complication as a consequence of active angiogenesis in the retina[2]. It is a leading cause of visual loss with a substantial impact on the quality of life of diabetic patients. It has been observed that neovascularization plays a pivotal role in the development of PDR. This process involves the migration and proliferation of endothelial cells as well as the remodeling of the extracellular matrix [3-5]. The pathogenic mechanism of PDR remains to be fully elucidated, but several cytokines and chemokines, including vascular endothelial growth factor, intercellular adhesion molecule-1 and transforming growth factor have been suggested in the etiology of PDR [6-9].

MCP-1 is a member of the CC chemokine family and acts chemotactically on monocytes and induces monocyte and macrophage infiltration into tissues [10]. Several studies show that intraocular concentrations of MCP-1 are increased in PDR and correlated with the clinical stage in DR. MCP-1 has been shown to play a possible role as a modulator of PDR due to its ability to regulate arterial smooth muscle cell proliferation and induce retinal neovascularization[11-12].

Several studies have identified that genetic variations in the MCP-1 gene influences MCP-1 production and function. A biallelic A/G polymorphism in the 5' flanking region at position -2518 of the MCP-1 gene has been identified, which influences MCP-1 expression in response to an inflammatory stimulus. This genetic variability correlates with individual differences in monocyte MCP-1 production and may be responsible for clinical differences in disease severity [13-14].

Because of the importance of MCP-1 in the pathogenesis of PDR and the genetic single nucleotide polymorphism (SNP) evidence for role in the regulation of MCP-1 production, we postulated that there may be an association between MCP-1 c.2518A/G with the pathogenesis of PDR.

2. Patients and Methods

This study included 50 patients with type 2 diabetes mellitus. They were 17 males and 33 females between the age of 45 and 71 years (mean age 57.2±8.33).The patients were assessed through dilated pupils and classified using the criteria based

on the International Clinical Retinopathy Severity Scale into: 1) 10 patients with no apparent diabetic retinopathy (NDR); 2) 20 patients with non-proliferative diabetic retinopathy (NPDR); and 3) 20 patients with PDR. Inclusion criteria were, age at diagnosis of diabetes ≥30 years and a known duration of diabetes of ≥5 years. Diabetes was diagnosed according to WHO criteria [15]. All patients underwent medical history and biochemical tests. The study was performed according to the principles approved by the local ethics committee.

Determination of MCP-1 c.2518A/G polymorphism

Genomic DNA was extracted from EDTA blood leucocytes obtained from each patient using Gene JET Whole Blood Genomic DNA Purification Mini Kit according to the manufacture instructions *(Thermo Scientific, EU).* MCP-1 c.2518A/G polymorphism was identified by the polymerase chain reaction- restriction fragment length polymorphism (PCR- REFLP). The reaction mixture contains the following: 25 µl of Dream Taq Green PCR master Mix (2X),2.5 µl of each primer, 5 µl of genomic DNA, nuclease free water to a final volume 50 µl. The following primers *(Biosearch technologies, USA)* were used for amplification: forward 5'-CCGAGATGTTCCCAGCACAG-3' and reverse 5'-CTGCTTTGCTTGTGCCTCTT-3'. The DNA was amplified by Initial denaturation at 95°C for 3 minutes then cycling of denaturation at 95°C for 30 second, annealing at 53°C for 30 second, and extension at 72°C for 1 minute. After 40 cycles, the reaction was extended for an additional 15 minutes at 72°C. PCR reactions were performed with PicoReal instrument. The amplified PCR product (930 bp) was subsequently digested at 37°C for 10 minutes with PvuII *(Thermo Scientific, EU).* Agarose gel electrophoresis (1.2%) of the digested products was performed to determine the presence of the various genotypes: 1) A/A genotype yields only a single 930 bp band; 2) G/G genotype results in two bands (222 bp and 708 bp); and 3) A/G genotype results in three bands (222 bp, 708 bp and 930 bp). **Fig. (1)**

Statistical analysis

All statistical tests were performed with SPSS version 16 soft ware (Spss Inc, Chicago, ILL Company). Categorical data were presented as numbers and percentages while quantitative data were expressed as mean and standard deviation (SD). Chi square test (X2) or Fisher's exact test (Monte Carlo method) were used, Odds Ratios (ORs) and the corresponding 95% confidence interval (CI) were calculated when applicable. Z test and ANOVA were used as tests of significance. Significant ANOVA was followed by post hoc multiple comparisons using Bonferroni test to detect significant pairs. Binary logistic regression was used to detect the significant predictors of proliferative diabetic retinopathy. All p values <0.05 were considered statistically significant.

3. Results

Study population included 20 patients with proliferative diabetic retinopathy, 20 patients with NPDR and 10 diabetic patients with non-apparent diabetic retinopathy (NDR). Clinical and laboratory data are shown in Table 1. The PDR group showed longer duration of diabetes, higher levels of glycosylated hemoglobin (HbA1c), cholesterol , TG and LDL-C than NDR group. The NPDR group also had higher levels of HbA1c and LDL-C than NDR group.

Genetic variation in the SNP analysis of c.2518A/G in the MCP-1 gene was analyzed with the following distribution: A/A (52%), A/G (34%) and G/G (14%). In terms of severity of diabetic retinopathy (Table 2), the prevalence of PDR was significantly higher (p=0.001) in subjects with the c.2518 A/A genotype (61.5%; 16/26 patients) compared to those carrying either the A/G or G/G genotype [16.7% (4/24)]. The subjects with the AA genotype were 8 folds risky to develop PDR than those with both AG and GG genotype.

The A allele in the 2518A/G polymorphism was a susceptibility allele for diabetic retinopathy and the prevalence of PDR was statistically significant (p <0.05) in subjects with A allele (85%) compared to those with G allele (15%). Subjects with A allele were 4.05 folds risky to develop PDR than those with G allele (p <0.05,OR 4.05) Table (3).

Multivariate logistic regression analyses showed that independent risk factors for PDR were allele A (p <0.05, OR 10.2), diabetes duration (p<0.05, OR 9.2), TG (p <0.05, OR 10.09) and genotype (A/A) (p ≤ 0.001, OR 21.5) (Table 4) .

The demographics of the study population according to the MCP-1 c.2518A/G genotype are summarized in Table (5). The AA genotype had higher levels of HbA1c than GG genotype.

Table (1): Clinical and laboratory characteristics of diabetic subjects with non-diabetic retinopathy (NDR), non-proliferative retinopathy (NPDR) and proliferative retinopathy (PDR).

Variable	NDR group (n=10)		NPDR group (n=20)		PDR group (n=20)		F	*P*
	Mean	± SD	Mean	± SD	Mean	± SD		
Age (y)	57.2	8.33	55.05	6.50	56.1	4.41	0.42	0.65
Duration of DM (y)	7.7	1.76	10.9	3.6	13.2*	4.46	7.37	**0.002**
FBG (mg/dl)	147.4	11.52	145.1	36.32	170.3	48.67	2.37	0.104
PPBG (mg/dl)	186.4	22.89	203.8	54.45	221.6	48.96	1.92	0.16
HbA1c (%)	6.89	0.18	7.80*	0.55	8.00*	0.74	12.1	**<0.001**
Cholesterol (mg/dl)	174.5	11.58	189.0	18.04	206.3*†	26.11	8.4	**0.001**
TG (mg/dl)	137.5	9.04	145.6	12.03	157.5*†	19.26	6.66	**0.003**
HDL-C (mg/dl)	49.1	4.88	44.4	12.81	39.2	11.43	2.79	0.07
LDL-C (mg/dl)	69.5	12.73	115.1*	26.48	122.1*	35.77	11.89	**<0.001**
Creatinine (mg/dl)	0.96	0.15	1.05	0.39	1.08	0.31	0.45	0.64

*Significant in comparison with NDR group. † Significant in comparison with NPDR group

Table (2): Prevalence of diabetic proliferative retinopathy according to the MCP-1c.2518A/G SNP

			MCP-1 c.2518A/G SNP.		Total	*P*	OR (95% CI)
			AA	AG+GG			
Groups	**PDR**	**No.**	16	4	20	0.001	8 (2.1-30.3)
		%	61.5%	16.7%	40.0%		
	(NDR +NPDR)	**No.**	10	20	30		
		%	38.5%	83.3%	60.0%		
Total		**No.**	26	24	50		
		%	100.0%	100.0%	100.0%		

Table (3): Prevalence of PDR according to allele frequency

			Alleles		Total	X^2	*P*	OR (95% CI)
			A	G				
Groups	**PDR**	**No.**	34	6	40	7.89	**0.005**	**4.05** (1.48-11.1)
		%	85.0%	15.0%	100.0%			
	(NDR+NPDR)	**No.**	35	25	60			
		%	58.3%	41.7%	100.0%			
Total		**No.**	69	31	100			
		%	69.0%	31.0%	100.0%			

Table (4): Binary logistic regression analysis for the predictors of PDR.

Variable	Odds Ratio (Exp B)	95% CI	*P*
Genotype (AA)	21.5	3.2-142.4	**0.001**
Allele A	10.2	2.9-56.9	**0.003**
Duration of DM ≥10y	9.2	1.2-68.5	**0.03**
HbA1c >7%	3.8	0.36-40.1	0.26
Cholesterol ≥200 mg/dl	1.28	0.15-11.6	0.82
TG ≥150 mg/dl	10.09	1.06-95.4	**0.044**
LDL-c ≥ 120 mg/dl	2.17	0.25-18.8	0.46

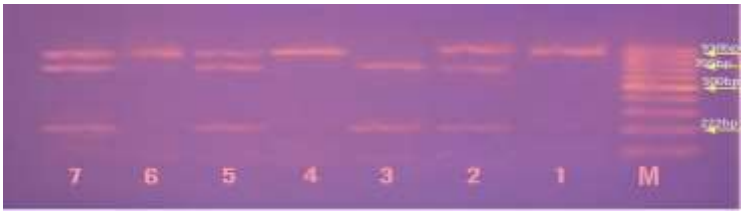

Fig. (1): Genotyping of the MCP-1 gene -2518 A/G polymorphism

•M: PCR marker (DNA ladder[100 bp]) .
•Lane 3 Homozygous (G/G) with two bands at 222 and 708 bp.
•Lanes 1,4,6 Homozygous (A/A) with one band at 930 bp.
• Lanes 2, 5, 7 Heterozygous (A/G) with three bands at 222, 708 and 930 bp.

Table (5): Clinical and laboratory characteristics of patients with type 2 diabetes according to the MCP-1 c.2518A/G SNP

Variable	AA (n=26)		AG (n=17)		GG (n=7)		F	*P*
	Mean	± SD	Mean	± SD	Mean	± SD		
Age(y)	57.1	6.18	55.9	5.62	51.2	5.46	2.71	0.077
Duration of DM(y)	11.5	4.81	11.2	3.38	9.8	3.62	0.42	0.66
FBG (mg/dl)	165.7	43.56	148.1	35.30	136.2	28.36	2.03	0.14
PPBG (mg/dl)	216.8	43.53	197.7	53.92	181.8	20.02	2.02	0.14
HbA1c (%)	7.9‡	0.83	7.5	0.49	7.2	0.34	3.5	**0.038**
Cholesterol (mg/dl)	199.8	26.20	183.1	20.49	191.8	12.40	2.7	0.077
TG (mg/dl)	151.8	17.62	144.6	16.24	147.4	12.89	0.98	0.38
HDL-C (mg/dl)	40.8	8.90	46.7	15.45	44.0	7.54	1.39	0.25
LDL-C (mg/dl)	116.8	39.95	108.3	29.07	87.2	14.55	2.09	0.13
Creatinine (mg/dl)	1.15	.66	.95	.13	1.07	.27	1.21	0.31

‡ significant in comparison with GG group.

4. Discussion

The pathogenesis of PDR remains poorly understood. Several studies have implicated a number of cytokines and chemokines in the activation of a complex network of pathways to promote cellular migration and proliferation. In conjunction with these factors, it appears that genetics play a critical role in the regulation of PDR, since only a subgroup of diabetic patients develop PDR in spite of similar glycemic control and duration of diabetes [7-10].

Among the genetic factors being considered as a susceptibility gene for PDR, MCP-1 is known to be associated with diabetic microvascular or macrovascular complications. MCP-1 is a chemokine that exerts several effects on monocytes and macrophages including induction of superoxide anion, cytokine production, and adhesion molecule expression. Moreover, intraocular MCP-1 levels have been shown to be significantly increased in diabetic retinopathy (DR) and associated with clinical stage of DR [16,17].

In the present study, the prevalence of PDR was significantly higher in diabetic patients with the c.2518A/A genotype compared to those with either the A/G or G/G genotype. Also, Jeon and his collegues (2013) reported that the 2518A/A genotype in MCP-1 could be used as a susceptibility gene to predispose Koreans exhibiting type 2 diabetes for the development of PDR [18].

However, the relationship between SNP polymorphism c.2518A/G in the MCP-1 gene with diabetic retinopathy remains controversial. Katakami and coworkers (2010) reported that the G allele in the c.2518A/G polymorphism is a susceptibility allele for diabetic retinopathy in a Japanese population of diabetic patients [13]. In another Japanese study, Yoshioka *et al.* (2009) showed that there was no association between c.2518A/G polymorphism of the MCP-1 gene with diabetic retinopathy in a group of Japanese patients with type 2 diabetes [19]. Dong *et al.* (2014) reported that MCP-1 c.2518 G/G was a susceptibility gene to predict DR, especially high-risk PDR in type 2 diabetes patients in the northern Han population of China [20]. A study carried out by Jiang and associates (2015), in chinese population, found that the MCP-1−2518 GG genotype was significantly associated with DR[21].

The results of the present work suggest that the c.2518A/A genotype of the MCP-1 gene could be a potential risk factor for PDR in egyptian type 2 diabetic patients. The difference in the nucleotide that was associated with PDR between the current study and the Katakami, *et al.* [13]; Dong, *et al.* [20] and Jiang, *et al.* [21] may be attributed to ethnic variation, and the number of patients enrolled in each study.

There are some limitations to our study that could impact our conclusions. First, this was a cross-sectional study, and we did not measure plasma MCP-1 in this study. For more accurate results, prospective studies will be needed to investigate the role of MCP-1 polymorphism in PDR in type 2 diabetic patients, and a larger prospective genome wide association study that includes family history will be required to fully clarify the pathogenesis of PDR in type 2 diabetic patients.

In conclusion, this study suggests that the c.2518A/G polymorphism in the MCP-1 gene can be used as a novel method to detect susceptibility to PDR development in egyptians with type 2 diabetes.

References

1. *Klein R, Klein BE, Moss SE, et al. (1984):* The Wisconsin Epidemiologic Study of Diabetic Retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol; 102:520-526.*

2. *Antonetti DA, Klein R and Gardner TW (2012):* Diabetic retinopathy. *N Engl J Med; 366:1227–1239.*
3. *Adamiec J, Oficjalska-Młyńczak J and Misiuk-Hojł M (2009):* Proliferative diabetic retinopathy-the influence of diabetes control on the activation of the intraocular molecule system. *Diabetes Res Clin Pract; 84:46–50.*
4. *Leal EC, Santiago AR and Ambrósio AF (2005):* Old and new drug targets in diabetic retinopathy: from biochemical changes to inflammation and neurodegeneration. Curr Drug Targets CNS Neurol Disord; 4:421-434.
4. *Fong DS, Aiello L, Gardner TW, et al. (2004):* American Diabetes Association Retinopathy in diabetes. *Diabetes Care; 27(1):84–87.*
5. *Simó R, Carrasco E, García-Ramírez M, et al. (2006):* Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. *Curr Diabetes Rev; 2:71-98.*
6. *Crawford TN, Alfaro DV 3rd, Kerrison JB, et al. (2009):* Diabetic retinopathy and angiogenesis. *Curr Diabetes Rev; 5:8-13.*
7. *Hernández C, Burgos R, Cantón A, et al. (2001):* Vitreous levels of vascular cell adhesion molecule and vascular endothelial growth factor in patients with proliferative diabetic retinopathy: a case-control study. *Diabetes Care; 24: 516-521.*
8. *Beránek M, Kanková K, Benes P, et al. (2002):* Polymorphism R25P in the gene encoding transforming growth factor-beta (TGF-beta1) is a newly identified risk factor for proliferative diabetic retinopathy. *Am J Med Genet; 109:278-283.*
9. *Charo IF and Taubman MB (2004):* Chemokines in the pathogenesis of vascular disease. *Circ Res; 95(9):858–866.*
10. *Harada C, Okumura A, Namekata K, et al. (2006):* Role of mono-cyte chemotactic protein-1 and nuclear factor kappa B in the pathogenesis of proliferative diabetic retinopathy. Diabetes Res ClinPract; 74:249–256.
11. *Kolattukudy PE and Niu J (2012):* Inflammation, endoplasmic reticulum stress, autophagy, and the monocyte chemoattractant protein-1/CCR2 pathway. *Circ Res; 110:174–189.*
12. *Katakami N, Matsuhisa M, Kaneto H, et al, (2010):* Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism as a potential risk factor for diabetic retinopathy in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract; 89:9 –12.*
13. *Malecki MT, Cyganek K, Mirkiewicz-Sieradzka B, et al. (2008):* Alanine variant of the Pro12Ala polymorphism of the PPAR gamma gene might be associated with decreased risk of diabetic retinopathy in type 2 diabetes. *Diabetes Res Clin Pract; 80:139-145.*
14. *Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003):* Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care; 26(1):5–20.*
15. *Mitamura Y, Takeuchi S, Matsuda A, et al. (2001):* Monocyte chemotactic protein-1 in the vitreous of patients with proliferative diabetic retinopathy. *Ophthalmologica; 215:415–418.*
16. *Rovin BH, Lu L and Saxena R (1999):* A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun; 259:344-348.*
17. *Jeon HJ, Choi HJ, Park BH, et al. (2013):* Association of monocyte chemoattractant protein-1 (MCP-1) 2518A/G polymorphism with proliferative diabetic retinopathy in Korean type 2 diabetes. *Yonsei Med J; 54(3):621-625.*
18. *Yoshioka K, Yoshida T, Kogure A, et al. (2009):* Monocyte chemoattractant protein-1 −2518 polymorphism is not associated with diabetic retinopathy in Japanese type 2 diabetes. *Diabetes Metab Syndr: Clin Res Rev; 3:166–168.*
19. *Dong L, lv XY, Wang BJ, et al. (2014):* Association of monocyte chemo attractant protein-1 (MCP-1) 2518A/G polymorphism with proliferative diabetic retinopathy in northern chinese type 2 diabetes. *Graefes Arch Clin Exp Ophthalmol; 252:1921–1926.*
20. *Jiang Z, Hennein L, Xu Y, et al. (2015):* Elevated serum monocyte chemoattractant protein-1 levels and its genetic polymorphism is associated with diabetic retinopathy in Chinese patients with Type 2 diabetes. *Diabet Med. doi:10.1111/dme.12804.*

11/26/2015