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Association between plasma homocysteine and diabetic retinopathy in type II diabetes mellitus

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Original Article

Abstract

BACKGROUND: Diabetic retinopathy (DR) is one of the complications occurs in patients with diabetes mellitus (DM) and is the leading cause of new onset blindness. This study aimed to determine the possible association between plasma homocysteine (Hcy) levels and the development and progression of DR.

METHODS: This case-control study enrolled diabetic patients who referred for ocular consultation from the Diabetes Clinic of Tohid Hospital in Sanandaj, Iran, in 2013. Patients with type 2 DM (n = 156) were randomly assigned to evaluate the association between Hcy and DR. Participants were randomly divided into two groups; with or without DR. Patients in both groups were matched for confounding factors. Detection and grading of retinopathy was performed by indirect ophthalmoscopy and fluorescein angiography. Glycosylated hemoglobin (HbA1c) was measured by Enzyme-linked Immunosorbent Assay and fasting plasma Hcy levels measured by chromatography. Plasma Hcy more than 15 µmol/l was defined as hyperhomocysteinemia.

RESULTS: The results showed that there were no significant differences in Hcy levels in diabetic patients with or without retinopathy. Also, we found that there was no association between HbA1c level and plasma Hcy. In addition, data analysis indicates that no association was observed between disease duration and Hcy levels.

CONCLUSION: In conclusion, we found that there was not a significant association between plasma Hcy level and DR in patients with type II DM.

KEYWORDS: Diabetes Mellitus, Diabetic Retinopathy, Hyperhomocysteinemia

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Introduction

Diabetic retinopathy (DR) is one of the complications of diabetes mellitus (DM), and the main cause of new onset blindness among 20-74 years old patients in Western countries.[1,2] The risk of blindness in diabetic patients is 25 times greater than non-diabetic population.[1] According to the literature the prevalence of DR varies from 6.7% to 35%.[3,4] It has been reported that over 60% of patients with type II DM develop retinopathy within one or two decades after diagnosis.[5] DR results from microvascular decompensation beginning with basement membrane thickening, leading to vascular occlusion and eventual neovascularization. The duration of diabetes, poor glycemic control, and hypertension (HTN) are documented risk factors in the development and progression of DR.[6] DR is potentially sight threatening, thus the need to locate more effective diagnostic and therapeutic techniques to reduce

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this problem is obvious. Finding a measurable risk factor may have two advantages; the identification of patients who are at greater risk and decreasing the risk of this sight threatening condition by lowering this risk factor, if possible. Recently homocysteine (Hcy) has received much attention. Hcy is an emerging risk factor for cardiovascular and non-diabetic ocular vaso-occlusive diseases.[3] Evidence has now accumulated showing that an elevated plasma Hcy concentration is a risk factor for vascular disease in both normal patients and those with renal disease. Elevated concentrations may induce endothelial dysfunction or abnormalities of coagulation factors and platelets.[7,8]

Hcy is a sulfur amino acid with a free thiol group, not present in dietary protein. This amino acid is a secondary by-product of methionine from cysteine metabolism. The mechanisms of Hcy pathogenesis in vascular damage are unclear. High Hcy levels can cause endothelial damage, with increased thrombosis and atherosclerosis. Hyperhomocysteinemia has been reported in both type 1 and type 2 DM patients. In patients with type 2 DM, the relation between hyperhomocysteinemia, macrovascular complications and renal disease is not completely understood; however, a higher prevalence of macrovascular complications in diabetic patients with hyperhomocysteinemia is associated with a higher prevalence of renal disease. The relationship between retinopathy and Hcy has not been clarified. Although numerous studies have shown an association between Hcy and DR, the results, thus far, have been equivocal.[9] Some studies support the concept that the hyperhomocysteinemia may contribute to the pathogenesis of retinal microangiopathy and DR.[10-15] According to the above studies and in order to find a reliable factor to predict the risk of developing retinopathy, in this study we were interested to evaluate the possible association between plasma Hcy level and DR in patients with type 2 DM.

Materials and Methods

This case-control study enrolled diabetic patients who referred for ocular consultation from the Diabetes Clinic of Tohid Hospital, Sanandaj, Iran, in 2011-2012. All examinations were carried out at the Department of Ophthalmology at the Kurdistan University of Medical Sciences. According to the previous studies 78 subjects were enrolled in each group.[3] All patients underwent complete ocular examinations that included a determination of visual acuity using the Snellen chart, slit lamp examination, intraocular pressure measurement, and precise fundus examination with fully dilated pupils, using an indirect ophthalmoscope and slit lamp biomicroscopy with a 90 D lens. In cases where exact grading of the extent of retinopathy was possible by indirect ophthalmoscope or slit lamp funduscopy, we performed a fundus fluorescein angiography, and fundus photographs were taken for precise detection of any micro-aneurysms or leaking vessels. All examinations were done by a physician who was blind to the study. The case group (n = 78) composed of those patients with DR, irrespective of grade. For each case individual, we selected a matching control individual from previously examined diabetic patients. The case and control groups were matched according to: age, diabetes duration, HTN, ischemic heart disease (IHD), hyperlipidemia, and smoking status (according to: medical records, laboratory analyses, and cardiologic consultation). We obtained complete medical histories from all participants by conducting interviews and reviewing medical records in the diabetes clinic.

Fasting blood samples were obtained from all case and control subjects in the same manner. Hcy and glycosylated hemoglobin (HbA1c) serum levels were measured by Enzyme-linked Immunosorbent Assay and chromatography using Statfax and Mycocard devices, respectively.

Patients who had undergone laser therapy and any intraocular injections for DR, those

with any retinal diseases that interfered with the grading of DR, any patients with renal dysfunction whose creatinine level was ≥ 1.5 mg/dl, and those who were taking any medications increased plasma Hcy levels.

SPSS for Windows (version 18.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data are expressed as the mean ± standard error of the mean of 78 persons per group. Student's t-test, chi-square or Kruskal-Wallis was used to analyze statistical significance. P values < 0.05 were considered to be significant in all analyses.

The Ethics Committee at the Kurdistan University of Medical Sciences reviewed and approved the study protocol. Signed informed consent was obtained from each individual.

Results

According to table 1, patients were correctly matched based on age, diabetes duration, hyperlipidemia, IHD, HTN, and smoking status. Data analysis indicates that there were not any significant differences between case and control groups in mentioned criteria.

Table 1. Comparison of variables in case and control groups

Matched variables	Case (%) (n = 78)	Control (%) (n = 78)	P
Hyperlipidemia	51.6	63.2	0.172
IHD	27.6	23.1	0.516
HTN	60.3	52.6	0.333
Smoking	4.3	4.0	0.824

HTN: Hypertension, IHD: Ischemic heart disease

Although the HbA1c level was significantly higher in the case group than the control (P = 0.002) but the results showed that there was not statistical differences in Hcy level between the case and control groups (Table 2). Furthermore, our findings in tables 3 and 4 show that there is not a significant difference between the grade of retinopathy and Hcy or HbA1c levels.

In order to find a possible association between diabetes disease duration and Hcy level the case subjects were divided into five groups including (i) less than 5 years, (ii) 6-10 years, (iii) 11-15 years, (iv) 16-20 years, and (v) more than 20 years. Data analysis indicated that there was no significant difference between disease duration and Hcy level (P = 0.300) (Table 5).

Table 2. Comparison of plasma homocysteine and glycosylated hemoglobin in case and control groups

Variables	Mean ± SD	P
Homocysteine (µmol/l)		
Case	10.65 ± 4.60	0.190
Control	9.78 ± 3.50	
HbA1c		
Case	9.44 ± 2.05	0.002
Control	8.41 ± 1.78	

HbA1c: Glycosylated hemoglobin; SD: Standard deviation

Table 3. Comparison of plasma homocysteine levels and retinopathy grade

Retinopathy grade	Subject	Mean ± SD (Hcy)	P
Mild NPDR	15	9.73 ± 3.62	0.481
Moderate NPDR	7	12.77 ± 2.97	
Severe NPDR	31	10.33 ± 4.07	
PDR	25	11.22 ± 5.93	

Hcy: Homocysteine; SD: Standard deviation
NPDR: Non-proliferative diabetic retinopathy
PDR: Proliferative diabetic retinopathy

Table 4. Comparison of plasma glycosylated hemoglobin levels and retinopathy grade

Retinopathy grade	Number of subject	Mean ± SD (HbA1c)	P
Mild NPDR	15	9.73 ± 1.49	0.516
Moderate NPDR	7	12.77 ± 1.35	
Severe NPDR	31	10.33 ± 2.12	
PDR	25	11.22 ± 2.33	

HbA1c: Glycosylated hemoglobin; SD: Standard deviation
NPDR: Non-proliferative diabetic retinopathy
PDR: Proliferative diabetic retinopathy

Table 5. Comparison between plasma homocysteine level and diabetes duration

Diabetes duration (year)	Number of subjects	Mean ± SD (Hcy)	P
0-5	8	11.17 ± 6.70	0.300
6-10	26	10.07 ± 3.01	
11-15	25	10.08 ± 3.74	
16-20	11	13.38 ± 7.07	
> 20	8	9.90 ± 4.78	

Hcy: Homocysteine; SD: Standard deviation

Discussion

The present study was aimed to evaluate the possible relationship between plasma total Hcy (tHcy) concentration and DR.

Our finding indicated that there was not any significant association between the DR degree and plasma Hcy level while there was a positive association between the presence of retinopathy and the amount of HbA1c (P = 0.002). Furthermore, the results showed that, there was no association between plasma Hcy levels and variables such as HTN, HbA1c, IHD, age, and diabetes duration (except for patients older than 70 years).

According to the literature we found that the association between Hcy plasma level and DR data is controversy.[10-15] Satyanarayana et al. conducted a cross-sectional case-control study to investigate the status of B-vitamins and Hcy in DR. They reported that mean plasma Hcy levels were found to be higher in the diabetic patients compared to normal subjects.[16]

Lim et al. showed that Hcy concentration of blood plasma, vitreous and aqueous in the patients with DR was approximately 30% higher than observed in the control subjects.[17] In addition Goldstein et al. reported that there was a significant elevation in Hcy levels in the non-proliferative diabetic retinopathy and proliferative diabetic retinopathy groups compared to the control group. Their findings have suggested that hyperhomocysteinemia might be associated with DR and partially explained the increased risk of microvascular angiopathy occurs in these patients.[18]

Ganapathy and colleagues used mutant mice with endogenously elevated Hcy levels due to a heterozygous deletion of the cystathionine-β-synthase gene and examined changes in retinal pathology following induction of diabetes. Their finding showed that elevated Hcy levels hastened cell loss in the retinal ganglion cell layer.[19] This was approved by Lee et al. that suggested that hyperhomocysteinemia caused by renal failure is often associated with diabetic nephropathy and retinopathy.[20]

On the other hand, some studies indicated no significant differences were noted in plasma Hcy levels between diabetic and control groups.[21,22] Another research has shown that Hcy levels in diabetic patients with pre-proliferative DR were not higher than the healthy normal group but were higher in the neovascular glaucoma group.[12]

In our study, patients with serum creatinine concentration greater than 1.5 mg/dl and those who were diagnosed with renal failure were excluded. It was a difference of our study with others that supported the idea of a positive relationship between DR and hyperhomocysteinemia. Therefore, we have concluded that in those studies renal failure acted as a confounding factor. In addition, Ozmen et al. showed that elevation of plasma tHcy concentration in type 2 DM is related to deterioration of renal function and diabetic nephropathy.[23] Furthermore, the association between Hcy and severity of kidney disease has been reported.[24] Therefore, we believe that retinopathy may develop simultaneously with nephropathy in the process of developing diabetic microvascular complications.

The low plasma Hcy level in all patients in comparison with similar studies[3,4] that may be related to intake regimen was one of the limitations of the present study. In addition, the main limitation of this study was the use of a cross-sectional design, which prevents determination of temporal direction and, therefore, of causal inference. Although retinopathy risk factors and the most important confounders of Hcy in this population specifically, age, sex, smoking status, and renal status were controlled.

In summary, we found that there was not a significant correlation between plasma Hcy level and DR. Nevertheless, it is necessary to perform more studies to clarify the possible association between Hcy and DR.

Conflict of Interests

Authors have no conflict of interests.

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References

1. Clark CM, Lee DA. Prevention and treatment of the complications of diabetes mellitus. N Engl J Med 1995; 332(18): 1210-7.
2. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol 1984; 102(4): 527-32.
3. Brazionis L, Rowley K, Itsiopoulos C, Harper CA, O'Dea K. Homocysteine and diabetic retinopathy. Diabetes Care 2008; 31(1): 50-6.
4. Van Hecke MV, Dekker JM, Nijpels G, Teerlink T, Jakobs C, Stolk RP, et al. Homocysteine, S-adenosylmethionine and S-adenosylhomocysteine are associated with retinal microvascular abnormalities: the Hoorn Study. Clin Sci (Lond) 2008; 114(7): 479-87.
5. Wright AD, Martin N, Dodson PM. Homocysteine, folates, and the eye. Eye (Lond) 2008; 22(8): 989-93.
6. Nguyen TT, Wong TY. Retinal vascular changes and diabetic retinopathy. Curr Diab Rep 2009; 9(4): 277-83.
7. Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, et al. Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. Circulation 1998; 97(2): 138-41.
8. Perna AF, Ingrosso D, Lombardi C, Acanfora F, Satta E, Cesare CM, et al. Possible mechanisms of homocysteine toxicity. Kidney Int Suppl 2003; (84): S137-S140.
9. de Luis D, Fernandez N, Aller R. Homocysteine in patients with diabetes mellitus. Med Clin (Barc) 2004; 122(1): 27-32.
10. Stabler SP, Estacio R, Jeffers BW, Cohen JA, Allen RH, Schrier RW. Total homocysteine is associated with nephropathy in non-insulin-dependent diabetes mellitus. Metabolism 1999; 48(9): 1096-101.
11. Agardh E, Hultberg B, Agardh CD. Severe retinopathy in type 1 diabetic patients is not related to the level of plasma homocysteine. Scand J Clin Lab Invest 2000; 60(3): 169-74.
12. Yucel I, Yucel G, Muftuoglu F. Plasma homocysteine levels in noninsulin-dependent diabetes mellitus with retinopathy and neovascular glaucoma. Int Ophthalmol 2004; 25(4): 201-5.
13. Soedamah-Muthu SS, Chaturvedi N, Teerlink T, Idzior-Walus B, Fuller JH, Stehouwer CD. Plasma homocysteine and microvascular and macrovascular complications in type 1 diabetes: a cross-sectional nested case-control study. J Intern Med 2005; 258(5): 450-9.
14. de Luis DA, Fernandez N, Arranz ML, Aller R, Izaola O, Romero E. Total homocysteine levels relation with chronic complications of diabetes, body composition, and other cardiovascular risk factors in a population of patients with diabetes mellitus type 2. J Diabetes Complications 2005; 19(1): 42-6.
15. Huang EJ, Kuo WW, Chen YJ, Chen TH, Chang MH, Lu MC, et al. Homocysteine and other biochemical parameters in Type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy. Clin Chim Acta 2006; 366(1-2): 293-8.
16. Satyanarayana A, Balakrishna N, Pitla S, Reddy PY, Mudili S, Lopamudra P, et al. Status of B-vitamins and homocysteine in diabetic retinopathy: association with vitamin-B12 deficiency and hyperhomocysteinemia. PLoS One 2011; 6(11): e26747.
17. Lim CP, Loo AV, Khaw KW, Sthaneshwar P, Khang TF, Hassan M, et al. Plasma, aqueous and vitreous homocysteine levels in proliferative diabetic retinopathy. Br J Ophthalmol 2012; 96(5): 704-7.
18. Goldstein M, Leibovitch I, Yeffimov I, Gavendo S, Sela BA, Loewenstein A. Hyperhomocysteinemia in patients with diabetes mellitus with and without diabetic retinopathy. Eye (Lond) 2004; 18(5): 460-5.
19. Ganapathy PS, Roon P, Moister TK, Mysona B, Smith SB. Diabetes accelerates retinal neuronal cell death in a mouse model of endogenous hyperhomocysteinemia. Ophthalmol Eye Dis 2009; 1: 3-11.
20. Lee CH, Chang HW, Wang IK, Lin CL, Chen TC, Wang PH, et al. Diabetes mellitus, hyperhomocystinemia and atherosclerotic vascular disease in Taiwanese chronic hemodialysis patients: a retrospective study. Ren Fail 2004; 26(3): 317-23.
21. Pavia C, Ferrer I, Valls C, Artuch R, Colome C, Vilaseca MA. Total homocysteine in patients with type 1 diabetes. Diabetes Care 2000; 23(1): 84-7.
22. Cotellessa M, Minniti G, Cerone R, Prigione F, Calevo MG, Lorini R. Low total plasma homocysteine concentrations in patients with type 1 diabetes. Diabetes Care 2001; 24(5): 969-71.
23. Ozmen B, Ozmen D, Turgan N, Habif S, Mutaf I, Bayindir O. Association between homocysteinemia and renal function in patients with type 2 diabetes mellitus. Ann Clin Lab Sci 2002; 32(3): 279-86.
24. Klein BE, Knudtson MD, Tsai MY, Klein R. The relation of markers of inflammation and endothelial dysfunction to the prevalence and progression of diabetic retinopathy: Wisconsin epidemiologic study of diabetic retinopathy. Arch Ophthalmol 2009; 127(9): 1175-82.