

The Relationship between Serum Homocysteine Levels and Nonproliferative Diabetic Retinopathy in Type 2 Diabetes Mellitus

Tip 2 Diabetes Mellitus'da Serum Homosistein Düzeyleri ile Nonproliferatif Diyabetik Retinopati İlişkisi

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ABSTRACT

Objective: To evaluate the relationship between serum homocysteine levels and the presence of nonproliferative diabetic retinopathy (NPDR) in type 2 Diabetes Mellitus (T2DM) patients

Methods: One-hundred patients with a diagnosis of T2DM and 30 healthy control subjects whose age and sex were similar were included in this study. In diabetic patients retinopathy was assessed by ophthalmological examination. Homocysteine, fasting glucose, HbA1C, triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein levels were analyzed in the blood samples in both groups. Also microalbumin levels were analyzed in 24-hour urine samples. T2DM patients were further divided into two groups according to the presence of retinopathy as patients with NPDR (Group 1, n=32) and without retinopathy (Group 2, n=68).

Results: There was no statistically significant difference in the homocysteine levels between the T2DM group and the control group (13.13±4.35µmol/l and 12.29±4.81µmol/l, respectively, p>0.05). Although homocysteine levels were higher in the patients with diabetic retinopathy (Group 1) than the diabetic patients without any diabetic complication (Group 2), the difference was not statistically significant (13.21±4.23mmol/l and 12.96±4.60mmol/l, respectively, p>0.05).

Conclusion: There was no increase in serum homocysteine levels in T2DM when there was no additional diabetic or cardiovascular complication other than NPDR. Our study, by demonstrating that serum homocysteine level was irrelevant to the presence of NPDR, suggests that homocysteine does not play a role at the early stages of retinopathy.

Key Words: Type 2 diabetes mellitus, nonproliferative diabetic retinopathy, serum homocysteine level

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ÖZET

Amaç: Tip 2 Diabetes Mellitus (T2DM) hastalarında nonproliferatif diyabetik retinopati (NPDR) gelişimi ile serum homosistein düzeyleri arasındaki ilişkinin incelenmesi.

Gereç ve Yöntem: T2DM tanısı bulunan yüz hasta ile yaş ve cinsiyet benzer 30 sağlıklı bireyden oluşan kontrol grubu çalışmaya dahil edildi. Diyabetik hastalara yapılan oftalmolojik muayene ile retinopati değerlendirildi. Her iki grubun kan örneklerinden homosistein, açlık kan şekeri, HbA1c, trigliserid, total kolesterol, yüksek dansiteli lipoprotein ve düşük dansiteli lipoprotein ölçümleri yapıldı. Ayrıca 24 saatlik idrar örneklerinde mikroalbumin ölçüldü. T2DM hastaları retinopati olan (Grup 1, n=32) ve retinopati olmayanlar (Grup 2, n=68) olarak iki grupta değerlendirildi.

Bulgular: T2DM hasta grubu ile kontrol grubunda serum homosistein düzeyleri açısından anlamlı bir fark saptanmadı (sırasıyla 13.13 ± 4.35 µmol/l ve 12.29 ± 4.81 µmol/l, p>0.05). Homosistein düzeyleri diyabetik retinopati bulunan hastalarda (Grup 1), diyabetik herhangi bir komplikasyonu olmayanlara (Grup 2) göre yüksek bulunsada, aradaki fark istatistiksel olarak anlamlı değildi (sırasıyla 13.21 ± 4.23 mmol/l ve 12.96 ± 4.60 mmol/l, p>0.05).

Sonuç: NPDR dışında eşlik eden bir diyabetik ve kardiyovasküler komplikasyon olmadığı durumlarda T2DM'de serum homosistein düzeylerinde artış olmamaktadır. Çalışmamızda serum homosistein düzeyleri ile NPDR gelişimi arasında da ilişki saptanmaması retinopatinin başlangıç aşamasında homosisteinin rolünün olmadığını düşündürmektedir.

Anahtar Sözcükler: Tip 2 diabetes mellitus, nonproliferatif diyabetik retinopati, serum homosistein düzeyi

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a multisystemic disease characterized with hyperglycemia which leads to micro and macro vascular complications. Prevention of chronic vascular complications of T2DM is a huge health problem with a global priority (1).

Diabetic retinopathy (DR), a chronic microvascular complication of DM, is seen in 20% of T2DM patients at the time of the diagnosis and in 60% of T2DM patients after 20 years. It is one of the leading causes of blindness in developed countries (2). Hyperinsulinemia, hyperglycemia, nonenzymatic glycolization, oxidative stress and alterations in aldose reductase pathway are believed to cause DR. However, currently no data can properly explain why the retinal microcirculation is severely and early affected despite a good metabolic control (3).

Homocysteine (Hcy) is an amino acid which is not involved in protein structure. It is produced during the methionine metabolism. It has recently been accepted as a component of oxidative system because of the discovery of free radical like effects. Hyperhomocysteinemia causes many harmful effects on the body. Hcy plays an atherogenic role by directly accumulating in endothelium, and also it reduces nitric oxide (NO) level, produces free radicals, increases oxidative stress, increases the level of oxidized low density lipoprotein (Ox-LDL), increases vascular smooth muscle cells, and leads to vascular endothelial dysfunction (4). So far, increased Hcy levels have been reported to be an independent risk factor for cardiovascular diseases (5-8). However, data about its relationship with chronic microvascular complications of DM is limited. For this reason we aimed to measure total Hcy (THcy) levels in T2DM patients and to evaluate its relationship with nonproliferative diabetic retinopathy (NPDR).

MATERIAL AND METHODS

One-hundred patients with T2DM (without retinopathy or with NPDR) and 30 healthy individuals were included in this study as T2DM group and the control group, respectively. T2DM group was further divided into two groups according to the presence of retinopathy as patients with NPDR (Group 1, n=32) and those without retinopathy (Group 2, n=68).

Patients with type 1 diabetes mellitus (T1DM), hypertension, coronary artery disease (CAD), congestive heart failure, chronic renal failure, liver function abnormalities, active malignancy or malignancy history, active infection, vitamin B12 or folic acid deficiency, malnutrition or patients smoking cigarettes, or on medications such as metformin, antibiotic, anti-inflammatory drugs, anticonvulsants such as phenytoin or carbamazepine, vitamin B12, B6, E or folic acid were excluded from the study. Also, T2DM patients with diabetic neuropathy, nephropathy, or proliferative retinopathy (PDR) or macroangiopathy (CAD, stroke, peripheral vascular problems) were not included. Informed consents were obtained from all patients.

Body mass index (BMI) in patient and control groups were calculated by dividing the body weight to the square of height in meters (kg/m^2). Blood pressures were measured with a standard mercury sphygmomanometer with a cuff around the right arm after 10 minutes of resting period in a comfortably seated position. Patients with an arterial blood pressure of 130/80 mmHg or more were not included in the study.

Patients' renal functions were evaluated by serum creatinine, microalbuminuria and creatinine clearance measurements. After ruling out urinary infection and hematuria, albumin clearance rate was calculated in 24-hour urine samples. The results above 30 mg/day obtained at least two times were accepted as a part of the diabetic nephropathy process, and those patients were excluded from the study.

Ophthalmological examination including indirect ophthalmoscopy of fundus through the dilated pupil was performed by the ophthalmologists in the study. Presence of microaneurysm, retinal hemorrhage, hard exudate, or macular edema was regarded as NPDR, whereas neovascularization on retina or optic disc, vitreous hemorrhage, fibrous proliferation, retinal traction and previous photocoagulation marks were regarded as PDR and those cases were excluded from the study.

Cases having peripheral neuropathy were detected and excluded through symptoms and signs such as numbness, dysesthesia or paresthesia in hands or feet, hypersensitivity to tactile stimulus, burning pain, neuropathic foot ulcer, loss of deep tendon reflexes and also by the monofilament test and electromyography in some cases when required.

Cases with a history of stroke documented by computerized tomography or magnetic resonance imaging, coronary artery disease (positive exercise test, angina history, abnormal coronary angiography or myocardial infarction history) and peripheral vascular disease namely pulseless extremities, abnormal Doppler ultrasound measurements, claudication history, vascular surgery or amputation were excluded.

Blood samples were taken after 10 hours of fasting from the antecubital vein of T2DM patients and the control group without a tourniquet. Fasting blood glucose (FBG) was measured by the hexokinase method, tryglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL) were measured by the enzymatic method in an Abbott-Aeroset otoanalyzer (Toshiba, Tochigi-Ken, Japan) using the device's original kits. Low density lipoprotein (LDL) was calculated by the Friedwald formula [total cholesterol - (HDL cholesterol + tryglyceride/5)]. HbA1C was measured by the HPLC (Agilent HPLC 1100 system) method. Microalbuminuria was measured in 24 hour urine samples by the immuno-turbidimetric method (DAKO, Denmark). Hcy levels were measured in an HPLC machine by fluorescence detector and chromsystems kits (Munich, Germany) (normal range: 5-13 $\mu\text{mol}/\text{l}$).

Statistical analysis was performed by SPSS 11.5 software. Results are shown as mean value \pm standard deviation (MV \pm SD). Differences in the groups were evaluated by a student's t test and differences in proportions of groups were evaluated by a chi-square test. Additionally, Pearson's correlation was used for correlation analysis. $p < 0.05$ was accepted as statistically significant.

RESULTS

In our study, the T2DM and healthy control groups had similar age and sex distribution ($p > 0.05$). In T2DM group, the mean duration of diabetes was 9.78 ± 7.19 years. Demographic and clinical properties of both groups are given in Table 1. Diabetic patients were under either insulin therapy or oral anti-diabetic agents other than metformin.

BMI, FBG, HbA1C and TG levels of patients in T2DM group were higher than the control group in a statistically significant way ($p < 0.001$). THcy levels were also higher in the T2DM group than the control group, but in a statistically non-significant way (respectively $13.13 \pm 4.35 \mu\text{mol}/\text{l}$ and $12.29 \pm 4.81 \mu\text{mol}/\text{l}$, $p > 0.05$).

The 32% of T2DM patients had NPDR (Group 1). The 68% of T2DM patients (Group 2) did not have any micro or macro diabetic complications. Group 1 and Group 2 were similar in terms of age (55.78 ± 7.39 years and 53.32 ± 9.17 years, respectively) and sex distribution (20 women / 12 men and 35 woman / 33 men, respectively) ($p > 0.05$). The duration of diabetes in Group 1 including the patients with NPDR was significantly higher than the T2DM patients in Group 2 who did not have any complications; 12.56 ± 7.40 years and 5.89 ± 5.17 years, respectively ($p < 0.001$) similar to the HbA1C levels which were $8.82 \% \pm 1.47$ and $7.93 \% \pm 1.88$, respectively in groups ($p = 0.02$). There was no statistically significant difference between the two groups in terms of other parameters ($p > 0.05$).

Although THcy levels were higher in NPDR patients (Group 1) than the T2DM patients without any complication (Group 2), that difference was not statistically significant ($13.21 \pm 4.23 \mu\text{mol}/\text{l}$ and $12.96 \pm 4.60 \mu\text{mol}/\text{l}$, respectively, $p > 0.05$). Clinical properties and statistical analysis of the two groups are given in Table 2.

In our study, there was a statistically significant and positive correlation between THcy, on one hand, and age and serum creatinine levels, on the other ($r = 0.177$, $p = 0.044$; $r = 0.257$, $p = 0.003$, respectively), whereas a significantly negative correlation was found between THcy and HDL cholesterol levels ($r = -0.203$, $p = 0.020$).

Table 1: Demographic and clinical properties of type 2 diabetes mellitus patients and the control group.

	T2DM (n=100)	Control group (n=30)	p value
Age (years)	54.11 ± 8.68	54.20 ± 2.57	NS
Sex (F/M)	55/45	17/13	NS
BMI (kg/m ²)	28.71 ± 4.16	25.57 ± 4.11	<0.001
FBG (mg/dl)	189.50 ± 66.86	88.77 ± 8.63	<0.001
HbA1C (%)	8.21 ± 1.80	5.22 ± 0.47	<0.001
Creatinine (mg/dl)	0.91 ± 0.19	0.85 ± 0.18	NS
TC (mg/dl)	201.20 ± 44.24	193.07 ± 21.43	NS
TG (mg/dl)	163.51 ± 82.61	106.93 ± 43.73	<0.001
HDL (mg/dl)	48.34 ± 10.42	50.27 ± 12.29	NS
LDL (mg/dl)	121.47 ± 17.03	119.53 ± 34.45	NS
THcy (µmol/l)	13.13 ± 4.35	12.29 ± 4.81	NS

T2DM: Type 2 Diabetes Mellitus, BMI: Body Mass Index, FBG: Fasting Blood Glucose, TC: Total Cholesterol, TG: Trygliceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, THcy: Total Homocysteine, NS: Not Significant

Table 2: Clinical properties and laboratory findings of diabetes patients with and without nonproliferative diabetic retinopathy (respectively Group 1 and Group 2).

	Group 1 (n=32)	Group 2 (n=68)	p value
Age (years)	55.78 ± 7.39	53.32 ± 9.17	NS
DM duration (years)	12.56 ± 7.40	5.89 ± 5.17	p <0.001
BMI (kg/m ²)	28.42 ± 3.57	28.84 ± 4.43	NS
FBG (mg/dl)	201.44 ± 63.26	183.88 ± 68.22	NS
HbA1C (%)	8.82 ± 1.47	7.93 ± 1.88	p = 0.02
Creatinine (mg/dl)	0.89 ± 0.19	0.92 ± 0.20	NS
TC (mg/dl)	203.50 ± 54.87	200.12 ± 38.67	NS
TG (mg/dl)	172.00 ± 80.93	159.51 ± 83.68	NS
HDL (mg/dl)	46.84 ± 9.87	49.04 ± 10.67	NS
LDL (mg/dl)	120.66 ± 36.53	119.00 ± 33.69	NS
THcy (µmol/l)	13.21 ± 4.23	12.96 ± 4.60	NS

DM: Diabetes Mellitus, BMI: Body Mass Index, FBG: Fasting Blood Glucose, TC: Total Cholesterol, TG: Trygliceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, THcy: Total Homocysteine, NS: Not Significant

DISCUSSION

In our study, evaluating the relationship between NPDR and serum THcy levels in T2DM patients, serum THcy levels in patients with NPDR were found to be higher than those without any diabetic complications. However this difference was not statistically significant (p>0.05).

Although T2DM is directly related to premature atherosclerosis and microvascular complications, the relationship between hyperhomocysteinemia and macroangiopathic and microangiopathic complications is currently under investigation. Elevated serum levels of homocysteine, which is a sulfur aminoacid produced in methionine metabolism, is proposed to contribute to diabetic retinopathy development by causing vascular endothelial dysfunction through oxidative stress in vascular endothelium and failure in vasodilatation. Vascular endothelial growth factor (VEGF) increases vascular permeability and causes edema; exudation and neovascularization lays the ground for diabetic retinopathy development. In some in-vitro studies, Hcy has been shown to increase the synthesis of VEGF

F, a proangiogenic factor, therefore, was reported to have a pivotal role in DR development and progression (9, 10).

In T2DM the mechanism and timing of changes in Hcy levels should be evaluated carefully. Diabetes does not have a direct effect on serum Hcy levels and Hcy is generally low or normal in diabetes patients. It was clearly shown that altered renal functions in T2DM elevate serum Hcy levels (11). In T1DM patients, unless there was an accompanying nephropathy, no relationship was found between Hcy and DR of different stages (12-14). Kidney plays a major role in the maintenance of Hcy levels. In the presence of diabetic nephropathy, Hcy levels may increase. Hcy may cause an impairment of renal endothelial and mesangial cells by increasing oxidative stress and eventually lead to microalbuminuria (15).

In our study, there was no statistically significant difference in THcy levels between T2DM patients and the control group. This may be due to the exclusion of T2DM patients with microalbuminuria and renal function disorder.

Among studies evaluating the relationship between Hcy and diabetic retinopathy, those supporting the presence of this relationship are relatively fewer. Vaccaro et al. detected a relationship between PDR and Hcy in T1DM patients with a more than 10-year- DM history (16). Chiarelli et al. also found similar results (17). Yücel et al., in their study of 40 T2DM patients from Turkey, reported that there was no significant difference in plasma Hcy levels between the preproliferative DR group and the control group, but they reported an increase in Hcy levels in the neovascular glaucoma group (18). The studies proposing high Hcy levels as a risk factor for DR attributed that risk to mutations in genes coding methylenetetrahydrofolate reductase enzyme (MTHFR), which plays a role in converting homocysteine to methionine. In a study of 112 Japanese patients with T2DM, Neugebauer et al. emphasized that the Hcy metabolism dysfunction due to MTHFR gene mutation was related to diabetic retinopathy (19). Looker et al. found a relationship between elevated Hcy concentrations, on one hand, and nephropathy and DR incidence, on the other, in 396 T2DM patients (20). In a study of T1DM patients, Saeed et al. found that THcy levels were significantly higher in the retinopathy group than the group without retinopathy. But in this study retinopathy was not classified (21). In our study, we think that we were able to evaluate the relationship between Hcy and diabetic retinopathy more specifically by excluding other factors that could contribute to the diabetic retinopathy development such as hypertension, CAD, congestive heart failure, microalbuminuria, chronic renal failure, liver function disorder, and cigarette smoking. In particular, we hope that the classification of retinopathy and the evaluation of the relationship between NPDR and Hcy makes our study different from other related studies.

Among the authors searching relationships between retinopathy and Hcy levels in diabetes, Agardh and Matteucci did not detect a relationship between DR and Hcy in T1DM patients. However, they found that serum folate, creatinine, urea, urinary albumin excretion, systolic blood pressure, DM duration, sex, age, smoking, lipoprotein(a) and nephropathy are all related to Hcy levels (13,14,22). Stabler, Abdella, Yang and Chico et al. did not find any relationship between DR and Hcy in patients with T2DM (23-26). Agullo-Ortuno et al. reported associations between high Hcy levels and macroangiopathy, retinopathy and nephropathy prevalence in patients with T1DM (n=57), whereas there was no association in patients with T2DM (n=32) (27). Smulders et al. did not detect a significant relationship between DR and Hcy levels after fasting or methionine load in 85 patients with T2DM (28).

In our study, Hcy levels of diabetic patients were within normal range, and were also similar between the two groups. Moreover, our study suggests that there is no association between Hcy and retinopathy because there was no significant difference in THcy levels between patients with NPDR (Group 1) and T2DM patients without any diabetic complication (Group 2).

The statistically significant positive correlation shown in our study between age and Hcy levels is also supported by the literature (29). Norlund et al. reported a significant association between age and Hcy levels. They also pointed out that the increase in Hcy levels with aging was partially related to worsening of renal functions which is associated with plasma cystatin C levels rather than the creatinine concentration (30). In our study, in concurrence with the results of Guttormsen et al., there was a positive correlation between creatinine and Hcy (31). The correlation between Hcy and creatinine in our study - although it only includes patients with normal renal functions - may be explained by the transfer of methyl groups liberated during the conversion of methionine to homocysteine, and into the synthesis of creatinine from its precursors.

Hcy levels of more than 16 µmol/l in T2DM patients were reported to increase DR risk (32) whereas in another study which assessed Hcy ≥15 µmol/l as hyperhomocysteinemia there was no association between DR and Hcy (33). The fact that the THcy levels in our patients with T2DM and control group were below 15 µmol/l may be the reason for detecting no association between Hcy and DR.

Hyperglycemia is a major determining factor for diabetic microvascular complications (23). As shown in many studies performed on patients with diabetes, good glycemic control can markedly delay the NPDR development and procrastinate the progression to PDR (18, 19). We showed that glycemic control was significantly worse and DM history was significantly longer in the NPDR developing group (Group 1) than in the T2DM group without retinopathy or other complications (Group 2).

There was no significant association between Hcy, on one hand, and TC, TG and LDL levels, on the other, in our study in accordance with Abdella et al. However, the association between fasting plasma glucose and HbA1C reported in their study was not found in ours (24).

In experimental studies, it was shown that Hcy stimulates the cholesterol and apolipoprotein B-100 synthesis and secretion in hepatic cell cultures. Hcy-related increase in cholesterol synthesis may play role in atherosclerosis pathogenesis. The significant negative correlation detected in our study between Hcy and HDL is different from what is reported in the literature.

Not having detected a significant relationship between THcy levels and NPDR presence in our study, we can, nevertheless, explain our different results as possibly being due to preanalytical alterations interfering with THcy measurements. Those alterations were minimized by an appropriate protocol of sampling in our study; however, the possibility of ex vivo generation of homocysteine could not be totally eliminated.

Nonetheless, the cut-off value of hyperhomocysteinemia varies widely among studies between 11.7-16 $\mu\text{mol/l}$. Therefore, variability of selected cut-off values might have contributed to incongruous results with other studies. In our study, mean THcy levels were $13.13 \pm 4.35 \mu\text{mol/l}$ in T2DM patients with and without retinopathy. This level was higher than the cut-off value set in previous studies. Also different methods of serum sampling, storage and measurements might have caused discrepancies among studies.

Many factors such as age, sex, cigarette smoking, folic acid, B12, B6, renal status, which may affect DR development and Hcy levels, were controlled in our study. However, not considering the genetic factors, depression and dementia which are currently associated with diabetes and aging may be a limitation of our study.

We believe that this study will contribute to the debate on whether or not hyperhomocysteinemia causes diabetic microvascular complications, especially NPDR. It will also inform the discussions about the necessity of screening for hyperhomocysteinemia and the screening criteria.

CONCLUSION

It may be concluded that in T2DM patients with no other accompanying complications of diabetes, an increase in Hcy levels should not be expected. Our study also suggests that serum Hcy levels in NPDR patients are not related to retinopathy. Other studies reporting an association between Hcy levels and retinopathy should be further evaluated to establish whether the elevated Hcy level is a cause or a result of other factors leading to retinopathy.

Conflict of Interest

No conflict of interest was declared by the authors.

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