Serum Homocysteine Values are Associated with Diabetic Foot Ulcer

Serum Homosistein Değerleri Diyabetik Ayak ile İlişkilidir

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Abstract

Background: Diabetic foot ulcers and lower extremity amputations are leading causes of mortality and morbidity in diabetic patients. Diabetic neuropathy and peripheral vascular diseases are major reasons for the development of diabetic foot ulcer. Most of the studies showed that hyperhomocysteinemia is related with microvascular and macrovascular complications in diabetic patients. In addition, hyperhomocysteinemia is related to poor ulcer healing. In this study, we investigated the relationship of hyperhomocysteinemia with diabetic foot ulcers.

Materials and Methods: Fifty-one patients with diabetic foot ulcers, 35 diabetes patients without foot ulcers and 60 healthy individuals were enrolled in the study. Wagner classification of diabetic foot ulcers was used. Plasma homocysteine levels were measured by Florescence Polarization Immunoassay.

Results: The median age was 60.0±10.5 years (Range: 38-85 years) in diabetic foot ulcer group, 57.8±9.6 years (range: 41-74 years) in diabetic group and 55.6±6.8 years (range: 40-70 years) in control group (p=0.062). The groups were similar in terms of mean age and sex distribution. The mean homocysteine levels were 10.7 µmol/l (range: 7-22 µmol/l) in control group, 16.27 µmol/l (range: 8.1-31.8 µmol/l) in diabetic foot ulcer group, 13.38 µmol/l (range: 7/23.6 µmol/l) in diabetic group and were showing statistically significant difference between the groups (p<0.001). However, no difference was seen between diabetic foot ulcer group and diabetic group (p>0.05). Multiple logistic regression analysis showed that, peripheral neuropathy and hyperhomocysteinemia (OR 10.558, p=0.008 and OR 1.186, p=0.013, respectively) were independent risk factors for the development of diabetic foot ulcer.

Conclusion: Serum homocysteine values are associated with diabetic foot ulcer. *Türk Jem 2011; 15: 106-10*

Key words: Type 2 Diabetes Mellitus, diabetic foot ulcer, hyperhomocysteinemia

Özet

Amaç: Diyabetik ayak ülserleri ve alt ekstremite amputasyonları diyabet hastalarında ana morbidite ve mortalite nedenlerindendir. Diyabetik nöropati ve periferik vasküler hastalık diyabetik ayak ülser oluşumuna zemin hazırlarlar. Birçok çalışmada hiperhomosisteineminin diyabet hastalarında mikrovaskuler ve makrovaskuler komplikasyonlarla olan ilişkisi gösterilmiştir. Ayrıca hiperhomosisteinemi ülser iyileşmesini de geciktirmektedir. Bu çalışmada hiperhomosisteinemi ile diyabetik ayak ülserleri arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntemler: Diyabetik ayak ülseri olan 51 hasta, ülseri olmayan 35 diyabet hastası ve 60 saglikli kontrol çalışmaya dahil edildi. Diyabetik ayaklarda Wagner sınıflandırılması kullanıldı. Plazma homosistein ölçümleri Floresan Polarizasyon immun ölçümleri ile yapıldı.

Bulgular: Diyabetik ayak ülserli grupta median (min/max) yaş 61 (38/85) yıl, diyabetik grupta 57 (41/74) yıl ve kontrol grubunda ise 56 (40/70) yıl idi (p=0,062). Gruplar, ortalama yas ve cinsiyet açısından benzerdi. Homositein seviyeleri; kontrol grubunda 10,7 (7/22) µmol/l, diyabetik ayak ülseri olan grupta 16,27 (8,1/31,8) µmol/l, diyabetik hasta grubunda ise 13,38(7/23,6) µmol/l idi ve istatistiksel olarak anlamlıydı (p<0,001). Fakat ayni anlamlılık diyabetik ayak ülseri olan grup ile diyabetik hasta grubu arasında yoktu. Multiple logistic regresyon analizleri periferik nöropati ve hiperhomosisteineminin (sırasıyla OR 10,558/ p=0,008 ve OR 1,186 / p=0,013) diyabetik ayak ülser oluşumu için bağımsız risk faktörleri olduğunu gösterdi.

Sonuç: Serum homosistein seviyeleri diyabetik ayak ülserleri ile ilişkilidir. *Turk Jem 2011; 15: 106-10*

Anahtar Kelimeler: Tip 2 Diabetes Mellitus, diyabetik ayak ülseri, hiperhomosisteinemi

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Introduction

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health expenditure in many countries (1,2). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of kidneys, nerves, blood vessels resulting in foot ulcers (1).
Diabetic foot ulcers and lower-limb amputation account for morbidity, mortality and healthcare expenditures among diabetic patients and will occur in 5-10% of diabetic population and, up to 3% will have a lower extremity amputation(3). Ulceration is the most common precursor to amputation and is identified in more than two-thirds of lower limb amputations (4). Diabetic foot ulcer development involves several mechanisms, such as neuropathy, increased biomechanical stress, external trauma and peripheral artery disease (5).
Homocysteine is a non-protein amino acid containing sulphur and is an intermediate in the metabolism of methionine (6). In 1969, Mc Cully first described the association of homocysteine with vascular disease (7). In the follow-up period several studies showed that homocysteine levels are associated with wound healing, endothelial dysfunction, insulin resistance, prothrombotic states, macroangiopathy, nephropathy and neuropathy (6,8-10).
In this study, we evaluated the relationship of homocysteine with the development of diabetic foot ulcer.

Materials and Methods

Between October 2007 and May 2008, 51 patients with diabetic foot ulcers, 35 diabetic patients without foot ulcers and 60 healthy controls were included in this study. Patients were recruited from visitors to an outpatient clinic. Group sample sizes of 35 and 51 achieve 98% post-hoc power for homocysteine.
Exclusion criteria were: presence of Type 1 diabetes mellitus, serious hepatic disease, cardiac or renal failure, diseases with acute inflammation, malignancy, thyroid disorders and psychiatric disorders. In addition, medications (metformin, fenofibrate) and/or vitamin supplements (vitamin B6, 12 and folic acid) were not allowed, so that homocysteine values could not be influenced. Local Ethics Committee approved the study protocol. Informed consent was obtained from each participant.
Blood samples were taken in the morning after an overnight fast. Serum total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, creatinine, vitamin B12, folic acid, thyroid stimulating hormone, glycolized hemoglobin, high sensitivity C-reactive protein, erythrocyte sedimentation rate, complete blood count, microalbuminuria, and homocysteine levels were measured. Biochemical parameters were studied using a Roche/Hitachi Modular autoanalyzer, complete blood count was studied using a Roche Symex autoanalyzer.
Blood samples were drawn into tubes containing tripotassium EDTA. Plasma was separated and frozen at -80 0C until assay. Plasma homocysteine levels were measured by florescence polarization method in Abbott AxSYM system using AxSYM Homocysteine reagent (Abbott, Wiesbaden, Germany).
Foot ulcer is defined as full-thickness skin defect that do not heal within 14 days (11). Wagner classification was used for diabetic foot ulcers. Infection was defined as at least two of the following: purulent discharge, local heat increment, local erythema, lymphangitis, edema, fever and bad odour (4,5).
Retinopathy was diagnosed by ophthalmologic evaluation (12).
Diabetic nephropathy (microalbuminuria) was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (RIA), was 30-300 mg/24-hours in at least two out of three 24-hours urine collections over a three-month period. Creatinine clearance was calculated by Cockroft-Gault formula (GFR) [GFR=(140-age) x weight (kg)/ Plasma creatinine x 72 (in women x 0,85)](13).
Macrovascular disease was defined as presence of ischemic heart disease, stroke, transient ischemic attack or peripheral artery disease (4,12). Peripheral artery disease was diagnosed by absence of both feet pulses and/or ankle-brachial pressure index (ABPI) lower than 0,90 (4,5,11,14). ABPI measurement was made using a hand-held Doppler ultrasound (Hadeco ES-101EX, 8 Mhz).
Peripheral sensory neuropathy was defined as insensitivity to the 5.07 (10-g) Semmes-Weinstein monofilament at any one of ten sites on either foot (dorsal midfoot, plantar aspect of foot including pulp of the first, third, and fifth digits, the first, third and fifth metatarsal heads, the medial and lateral midfoot and the calcaneus). No other methods were employed in order to diagnose patients with peripheral diabetic neuropathy.

Statistical Analysis

Statistical analysis was made by using SPSS 11.0 for Windows. Pearson's Chi-Square test was used for categorical data and the Mann-Whitney U test was used for assessing numeric data. Logistic multiple regression analysis was used for evaluating peripheral neuropathy and serum homocysteine values as independent risk factors for diabetic foot ulcer.

Results

A total of 86 patients and 60 controls were enrolled in the study. There were 25 females (71.4%), 10 males (28.6%) in the diabetic group without foot ulcer; 26 males (51 %), 25 females (49%) in the diabetic group with foot ulcers; 29 females (48.3%), 31 males (51.7%) in the control group.
The median age was 60.0±10.5 years (Range: 38-85 years) in the diabetic group with foot ulcer, 57.8±9.6 years (range: 41-74 years) in the diabetic group without foot ulcer and 55.6±6.8 years (range: 40-70 years) in the control group (p=0.062). No significant difference was observed between the groups in terms of age.
In the diabetic group with foot ulcers, 14 patients (27.5%) had grade 1 ulcer, 14 (27.5%) - grade 2, 9 (17.6%) - grade 3, 11 (21.6%) - grade 4 and 3 patients (5.9%) had grade 5 ulcer.
Anthropometric and clinical characteristics of the diabetic patients are shown in Table 1, laboratory parameters are shown in Table 2. Significant differences were seen in diabetes duration, retinopathy, neuropathy, macrovascular disease and infection between the patient groups. (Table 1). HbA1c, HDL-cholesterol, Folic Acid, Vitamin B12, CRP and ESR were significantly different between patient groups (Table 2).

Table 1. Anthropometric and clinical features of diabetic patients

	Diabetic Patients without foot ulcers Median (Minimum/Maximum)	Diabetic Patients with foot ulcers Median (Minimum/Maximum)	P
Numbers(F/M)	35 (25/10)	51(25/26)	0.062
Age(year)	57(41/74)	61(38/85)	0.384
BMI (kg/m^2)	29.9(21.5/44.6)	27.2(15.2/42.8)	0.143
Waist circumference (cm)	101(73/132)	98(67/127)	0.613
Hip circumference (cm)	98(85/134)	98(78/135)	0.809
Diabetes duration (year)	9 (2/25)	13(1/30)	0.048*
Smoking (%)	14.3	21.6	0,394
Systolic Blood Pressure (mmHg)	120(100/160)	120(110/180)	0.452
Diastolic Blood Pressure (mmHg)	80(60/90)	80(60/100)	0.825
Hypertension (%)	57.1	58.8	0.877
Retinopathy (%)	60	82.4	0.021*
Nephropathy (%)	77.1	86.3	0.273
Macrovascular disease (%)	40	62.7	0.038*
Neuropathy (%)	62.9	96.1	<0.001***
Infection (%)	0	49	<0.001***

* Statistically significant difference was determined between two groups (p<0.05).
*** Statistically significant difference was determined between two groups (p<0.001).
Diabetes duration, retinopathy, neuropathy, macrovascular disease and infection were related to diabetic foot ulcers.

Table 2. Laboratory findings of diabetic patients

	Diabetic Patients without foot ulcers Median (Minimum/Maximum)	Diabetic Patients with foot ulcers Median (Minimum/Maximum)	P
HbA_{1c} (%)	12 (6/18)	13.38(7.0/23.6)	<0.001***
Total Cholesterol (mg/dl)	178(108/260)	160(96/299)	0.127
LDL- Cholesterol (mg/dl)	115(51/181)	95(44/202)	0.222
HDL- Cholesterol (mg/dl)	39(24/104)	34(7/85)	0.034*
Triglycerides (mg/dl)	126(50/413)	147(46/566)	0.895
Creatinine (mg/dl)	0.8(0.5/1.5)	0.8(0.4/2.2)	0.574
Uric acid (mg/dl)	4.6(3/9)	5(2/10)	0.117
Creatinine Clearance(ml/min)	84(43/182)	85(29/170)	0.482
Folic acid (ng/ml)	13.6(2.2/20)	7.4(3.2/19.5)	<0.001***
VitaminB12 (pg/ml)	446(147/1343)	502(142/2000)	0.033*
TSH (mIU/ml)	0.87(0.3/6.1)	1.14(0.1/4.2)	0.485
Leucocytes (x1000/ mm3)	8200(5000/15000)	8800(3800/25000)	0.227
CRP (mg/dl)	0.63(0.1/7.8)	2.6(0.1/26.2)	<0.001***
ESR (mm/hour)	26(5/101)	64(18/120)	<0.001***
Homocysteine (µmol/l)	9.5 (6/17)	16.27(8.1/31.8)	<0.001***

* Statistically significant difference was determined between two groups (p<0.05).
*** Statistically significant difference was determined between two groups (p<0.001).
HbA1c, HDL-Cholesterol, folic asid, VitaminB12, CRP and ESR were different between groups.

The median plasma homocysteine levels were 10.7 (7/22) µmol/l in the control group, 13.38(7/23.6) µmol/l in the diabetic group without foot ulcers and 16.27 (8.1/31.8) µmol/l in the diabetic group with foot ulcers and were significantly different (p<0.001, Figure 1). There was no difference in homocysteine levels according to diabetic foot stages in the diabetic foot group (Table 3).

Regression analyses showed that homocysteine levels and neuropathy were (b=0.171 and b=2.357, respectively) related and were independent risk factors (p=0.013 and p=0.008 respectively) for the development of diabetic foot ulcer. Patients who had hyperhomocysteinemia (OR:1.186; 95% CI:1.0-1.3) and neuropathy (OR:10.558; 95% CI:1.8-60.7) were likely to develop diabetic foot ulcers (Table 4).

Table 3. Plasma homocysteine levels according to diabetic foot grades

	N	Plasma Homocysteine Levels (µmol/l) Median (Minimum/Maximum)
Grade 1	14	16.58 (9.5/ 31.8)
Grade 2	14	14.04 (11.3/ 29.2)
Grade 3	9	16.10 (11.7/ 24.4)
Grade 4+5	14	18.19 (8.1/ 28.9)

No significant difference in plasma homocysteine levels were seen between diabetic foot ulcer stages (p=0.953).

Table 4. Risk factors for diabetic foot ulcer development

	B	P	OR	95% CI
Age (year)	-0.019	0.512	0.981	0.925 - 1.039
Diabetes duration (year)	0.057	0.273	1.059	0.956 - 1.173
Sex (F/M)	0.919	0.133	2.506	0.755 - 8.313
Retinopathy	0.169	0.815	1.184	0.287 - 4.887
Macrovascular disease	0.398	0.493	1.490	0.477 - 4.652
Homocysteine (µmol/l)	0.171	0.013*	1.186	1.036 – 1.357
Neuropathy	2.357	0.008**	10.558	1.836 - 60.714
Constant	-4.364	0.020		

* Statistically significant difference was determined between two groups (p<0.05).
** Statistically significant difference was determined between two groups (p<0.01).
Logistic Regression analyses showed that homocysteine levels and neuropathy were related and were independent risk factors for diabetic foot ulcer development.

Serum homocysteine differences between the groups according to gender and folic acid values were adjusted and covariance analysis was used. The effect of homocysteine levels on diabetic foot development was found to be rather meaningful (p= 0.004).

Discussion

Diabetic foot ulceration is one of the serious complications of diabetes causing major extremity amputations with considerable economic and public health implications (15,16).

In the development of diabetic foot ulceration, diabetic neuropathy and peripheral artery disease are the major risk factors where as microvascular disease, biomechanical abnormalities, limited joint movements, and infection are the minor ones. Diabetic foot ulceration is seen frequently in the sixth decade of life. Clinically, 45-60% of ulcerations are pure neuropathic, 10% are pure ischemic and 25-45% are neuroischemic (15).

Diabetic peripheral neuropathy is observed in 55-70% of diabetic patients and 90% of patients with diabetic foot ulcers. Similarly, peripheral neuropathy incidence was 62.9% in diabetic patients and 96.1% in diabetic foot ulcer group in our study (p<0.001). In a diabetic patient with neuropathy, foot ulceration development is more likely (OR: 10.5 ; CI:95%, 1.8-60.7) to develop. This suggests a strong relationship of neuropathy with foot ulceration.

The other major risk factor is peripheral artery disease and accompanies 60% of nonhealing diabetic foot ulcers (17). We found that macrovascular disease is seen in 62.7% of diabetic patients with foot ulcers and in 40% of patients with diabetes. This difference between the groups was significant (p=0.038). There was no significant difference between macrovascular disease and diabetic foot ulcer development with regression analysis which can be due to insufficient number of patients (p=0.493).

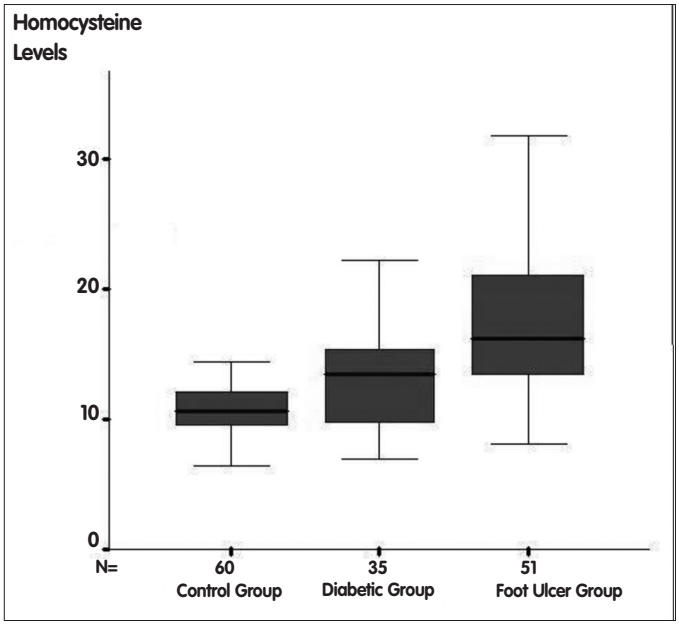

Figure 1. Median plasma homocysteine levels were 10.7 (7/22) µmol/l in the control group, 13.38(7/23.6) µmol/l in the diabetic group without foot ulcers, 16.27 (8.1/31.8) µmol/l in the diabetic group with foot ulcers and were significantly different (p<0.001)

In the literature, infection is observed in 35-50 % of diabetic foot ulcers which was 49% in our study (5).

The median age was 60.0±10.5 years (Range: 38-85 years) in the diabetic patients with foot ulcers which is consistent with other studies. This result showed that, although no significant difference was seen between the groups, diabetic foot ulcers were seen in the elderly patients. Male gender (51%) is related with diabetic foot ulcers, but no significant relationship was observed in our study.

In the diabetic foot ulcer group, retinopathy, neuropathy and macrovascular disease were seen more frequently than in the diabetic group (p<0.05). This may be due to the long duration of diabetes years (mean:13 years, range:1-30 years versus mean:9 years, range: 2-25, [p=0.048]) in the diabetic foot ulcer group.

It is well known that, increased plasma homocysteine level is a risk factor for cardiovascular disease (18,19,20,21-23). We designed this study to investigate the relationship of homocysteine levels with chronic complications of diabetes.

Diverse results are seen between homocysteine levels and diabetes (20). Elevated homocysteine levels are seen in patients with nephropathy, neuropathy, macrovascular diseases, and retinopathy in some studies (8,18,20,22,23). We found elevated homocysteine levels in the diabetic patients without foot ulcers and diabetic foot ulcer group compared to to controls [13.38 (7/23.6) µmol/l, 16.27 (8.1/31.8) µmol/l, 10.7 (7/22) µmol/l, respectively (p<0.05)]. Similarly with the literature, this significance was also observed in the diabetic foot ulcer patients which chronic complications are seen more frequently (p<0.05).

Some studies showed that elevated homocysteine levels are related to poor ulcer healing (9,10). Majors et al. (10) reported that homocysteine may alter normal thrombosis and delay or interfere with wound healing by impairing the interaction of fibronectin with fibrin.

Boykin et al. (9) reported a diabetic patient with intractable bilateral venous ulcerations, who had an elevated homocysteine level, which was healed after multivitamin therapy.

Similarly, we found that homocysteine levels were elevated in diabetic foot ulcer group. Also hyperhomocysteinemia had a linear relation with ulcer formation in the diabetic foot ulcer group. In a diabetic patient with hyperhomocysteinemia, foot ulceration development is more likely (OR:10.5; CI:95%, 1.8-60.7) to develop. Although not as strong as neuropathy, hyperhomocysteinemia could be related to the development of diabetic foot ulcer. Serum homocysteine differences between the groups according to gender and folic acid values were adjusted and covariance analysis was used. The effect of homocysteine levels on the development of diabetic foot was found to be rather meaningful (p= 0.004).

Hemoglobin A1c (HBA1c) is one of the most important risk factors for the development of complications. In our study, the HBA1c levels in the non-ulcer patients were statistically higher than the ulcer patients. Lower HbA1c levels in patients with foot ulcer were due to the intensive therapy in this group, which was regarded as a temporary characteristic of our study group. On the other hand, the homocystein effect could have been far more meaningful if both groups had similar HbA1c levels. This situation was a flaw of our study.

Hyperhomocysteinemia is not uncommon in diabetic patients and it can aggravate cardiovascular diseases. The mechanism of this increased hyperhomocysteinemia prevalence is vague but it is suggested that, insulin plays a role in the regulation of plasma homocysteine and, insulin resistance causes hyperhomocysteinemia (18). Besides, the mechanisms that cause peripheral and/or autonomic neuropathy are complex and are not yet fully understood. Hypothetically, homocysteine can also contribute to the neuropathy development through neurovascular disruption or through direct toxic effect (8). In some studies in this field, it has been shown that there could be a link between hyperhomocysteinemia and autonomic or peripheral neuropathy (8,24). According to recent data, hyperhomocysteinemia can be a significant risk factor for cardiovascular diseases in the diabetic population (18,25). The relationship between hyperhomocysteinemia and cardiovascular diseases in patients with type 2 diabetics is 1.6 times stronger than in non-diabetic patients (18). Another important factor is the link between nitric oxide (NO) and homocysteine. Nitric oxide (NO) is a free radical gas which has a critical role in wound healing. Homocysteine hinders the NO production in various ways, such as the inhibition of arginine transport, the inhibition of the destruction of NO inhibitor asymmetric dimethylarginine (ADMA), and pro-oxidant behaviours (9). We think that, all the above mentioned mechanisms, combined with the presence of hyperhomocysteinemia, cause the development of foot ulcer in diabetic patients.

As a result, serum homocysteine values are associated with diabetic foot ulcer. It is safe and simple to measure homocysteine levels. In the cases of hyperhomocysteinemia, a cheap treatment could lower the homocysteine levels in most patients. Therefore, in practice, homocysteine levels as well as other efficient parameters (metabolic control such as HBA1c) should be measured routinely before the development of diabetic foot ulcer. We think it will be cost-effective to measure homocysteine levels. However, further studies are needed on this subject.

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