PLASMA TOTAL HOMOCYSTEINE, LIPOPROTEIN (A) AND VON WILLEBRAND FACTOR LEVELS IN ASSESSMENT OF METABOLIC CONTROLS OF CHILDREN WITH TYPE I DIABETES MELLITUS

Sabahattin Ertuğrul,[1] Yaşar Şen,[2] Erdal Yılmaz,[3] Mustafa Aydın,[4] Bilal Üstündağ[5]

[1] Dicle Üniversitesi Tıp Fakültesi, Neonatoloji Bilim Dalı, Diyarbakır

[2] Selçuklu Üniversitesi Tıp Fakültesi, Çocuk Endokrinolojisi Bilim Dalı, Konya

[3] Fırat Üniversitesi Tıp Fakültesi, Çocuk Kardiyolojisi Bilim Dalı, Elazığ

[4] Eğitim ve Araştırma Hastanesi, Yenidoğan Kliniği, Elazığ

[5] Fırat Üniversitesi Tıp Fakültesi, Biyokimya ve Klinik Biyokimya Anabilim Dalı, Elazığ

ABSTRACT

Objective: *Diabetes is a significant risk factor for early onset of atherosclerosis. In this study, it was aimed to determine whether total homocysteine (tHcy), lipoprotein(a) [Lp(a)] and von Willebrand factor (vWF) levels have an early predictive value like glycolysated hemoglobin (HbA1c) levels in assessment of metabolic condition of children with type I diabetes.*

Material and Method: *Study group consisted of 65 children and adolescents who had type I diabetes but not clinical findings of microvascular and macrovascular complications and 20 healthy children and adolecents with the same age group and gender. Blood samples were obtained from diabetic patients, treatments were arranged and patients were followed up (Group I diabetic patients). Patients were re-evaluated at the end of 1 year and blood samples were obtained again (Group II diabetic patients).*

Results: *A statistically significant difference was found between the first (11.1±3.3 µmol/L) and the second tHcy levels (10.2±1.7 µmol/L) of diabetic patients and tHcy levels of control group (8.9±2 µmol/L) (p<0.05 and <0.01, respectively). A statistically significant difference was found between the first (10.4±5.4 mg/dl) and the second Lp(a) levels (9.0±4.3 mg/dl) of diabetic patients and Lp(a) levels of control group (5.3±2.8 mg/dl) (p<0.01 and <0.001, respectively). A statistically significant difference was not found when vWF levels of diabetic groups I and II and control group were compared (p>0.05). A statistically significant positive correlation was found between tHcy and HbA1c, microalbumin in Group I diabetic patients (r=0,389, p=0,02; r=0,286, p=0,034, respectively). A statistically significant positive correlation was found between HbA1c and homocysteine, vWF, microalbumin (r=0,428, p=0,001; r=0,328, p=0,024; r=0,742, p=0,001, respectively) and vWF and microalbumin levels (r=0,560, p=0,001) in Group II diabetic patients.*

Conclusion: *In conclusion, that tHcy and Lp(a) could be beneficial for assessment of metabolic control of diabetes, treatments toward reducing the complications of diabetes or the outcomes of additions to diet.*

Keywords: *Diabetes mellitus, homocysteine, lipoprotein (a), von Willebrand factor* ***Nobel Med 2014; 10(3): 75-80***

TİP 1 DİABETES MELLİTUSLU ÇOCUKLARIN METABOLİK KONTROLLERİNİN DEĞERLENDİRİLMESİNDE PLAZMA TOTAL HOMOSİSTEİN, LİPOPROTEİN (A) VE VON WİLLEBRAND FAKTÖR DÜZEYLERİ

ÖZET

Amaç: Diyabet erken başlangıçlı ateroskleroz için önemli bir risk faktörüdür. Bu çalışmada tip I diyabetli çocukların metabolik durumunun değerlendirilmesinde total homosistein (tHcy), lipoprotein (a) [Lp (a)] ve von Willebrand faktör (vWF) düzeylerinin glikolize hemoglobin (HbA1c) düzeyleri gibi erken belirleyici değerinin olup olmadığının gösterilmesi amaçlandı.

Materyal ve Metod: Çalışma grubu mikrovasküler ve makrovasküler komplikasyonların klinik bulguları olmayan tip 1 diyabetli 65 çocuk ve adölesan hasta grubu ile uygun yaş ve cinsiyette 20 sağlıklı çocuk ve adölesandan oluştu. Diyabetli hastalardan gerekli parametreler için kan örnekleri alındıktan sonra uygun şekilde tedavileri ayarlanıp izleme alındı (diyabetli I. hasta grubu). Bir yıl sonunda hastalar tekrar değerlendirilip aynı şekilde veriler için kan örnekleri alındı (diyabetli II. hasta grubu).

Bulgular: Diyabetli hastaların ilk (11,1±3,3 µmol/L) ve ikinci tHcy düzeyleri (10,2±1,7 µmol/L), kontrol grubu tHcy düzeyleri (8,9±2 µmol/L) arasında istatistiksel olarak anlamlı farklılık saptandı (sırasıyla, p<0,05 ve <0,01). Diyabetli hastaların ilk Lp(a) (10,4±5,4 mg/dl) ve ikinci Lp(a) (9,0±4,3 mg/dl) düzeyleriyle, kontrol grubu Lp(a) (5,3±2,8 mg/dl) düzeyleri arasında istatistiksel olarak anlamlı farklılık saptandı (sırasıyla p<0,01 ve <0,001). Diyabetli I., II. hasta grupları ve kontrol grubunun vWF düzeyleri karşılaştırıldığında istatistiksel olarak anlamlı farklılık saptanmadı (p>0,05). Diyabetli hasta I. grubunda tHcy ile HbA1c, mikrolabümin arasında istatistiksel olarak anlamlı pozitif bağıntı saptandı (sırasıyla, r=0,389, p=0,02; r=0,286, p=0,034). Diyabetli hasta II. grubunda HbA1c ile homosistein, vWF, mikroalbümin (sırasıyla, r=0,428, p=0,001; r=0,328, p=0,024; r=0,742, p=0,001) ve vWF ile mikroalbümin düzeyleri arasında (r=0,560, p=0,001) istatistiksel olarak anlamlı pozitif bağıntı saptandı.

Sonuç: Homosisteinin ve Lp(a)'nın diyabetin metabolik kontrolünün ve diyabetin komplikasyonlarını azaltma amaçlı yapılacak tedavilerin veya diyete yönelik eklemelerin sonuçlarını değerlendirmede yararlı olabileceği sonucuna varıldı.

Anahtar Kelimeler: Diabetes mellitus, homosistein, lipoprotein (a), von Willebrand faktörü **Nobel Med 2014; 10(3): 75-80**

INTRODUCTION

Metabolic changes developing in patients with diabetes mellitus (DM) are accountable for clinical findings and the course of the disease.[1] Glycolysated hemoglobin (HbA1c) is used for follow up of long term metabolic control of diabetes and the effectiveness of treatment.[2] Increased total homocyteine (tHcy), lipoprotein (a) [Lp (a)] and von Willebrand factor (vWF) levels are stated to be perceived as a sign of developing vascular complications in diabetic patients with poor metabolic control.[3-5]

Diabetes is an endocrine disease characterized with hyperglycemia and related complications. Chronic complications involve micro and macrovascular system. Atherosclerosis is seen 6 fold greater in diabetic patients compared to non-diabetic ones.[6-8] Good plasma glucose regulation may reduce the frequency of severe complications in heart, kidneys and blood vessels.[9] As most of diabetic patients die due to atherosclerosis-related complications, preventive measures which were proven to reduce cardiovascular risk must be applied to them.[8] Children with type I diabetes continue their lives as diabetic adults, therefore we must enable them to sustain their lives as healthy adults through appropriate treatments.

Hyperhomocysteinemia is an independent risk factor for atherosclerosis and atherosclerosis risk significantly increases when they coexist.[10,11] In literature, there are different results about diabetes and tHcy levels.[12-14] While hyperhomocysteinemia is found high only in patients with diabetic nephropathy, Agardh et al. showed that it was also related with proliferative retinopathy and microangiopathy.[15] In the study of Agullo-Ortuno et al., they detected a relationship between high tHcy levels and microangiopathy, retinopathy and nephropathy prevalence in adult type diabetic patients.[16] They found tHcy levels of diabetic patients with nephropathy higher than those of patients without nephropathy. These researchers suggested that plasma tHcy levels must be accepted as an indicator of complication and used together with →

biochemical parameters for assessment of the disease. In another study, hyperhomocystinemia was shown in diabetic patients who did not have renal insufficiency but had only nephropathy and macroangiopathy (proteinuria/ microalbuminuria).[17]

In this study, it was aimed to determine whether plasma tHcy, Lp(a) and vWF levels could be used as a marker like HbA1c in assessment of metabolic condition and complications of children with type I DM.

MATERIAL and METHOD

Sixty five children and adolescents with type I DM (34 boys, 31 girls) who did not have the clinical evidence of micro and macrovascular complications and 20 healthy children and adolescents (10 boys, 10 girls) who were being followed up at Pediatrics Clinic of Fırat Medical Center between June 2003 and December 2005 were included in this prospective study. Patients who had hepatic, renal, thyroid diseases, who were using vitamins, cigarette or alcohol, who had the family history of premature coronary artery disease and/or familial hyperlipidemia were not included in the study. Diagnosis of type I DM was made according to the criteria of World Health Organization.[18] Duration of diabetes varied between 12 and 96 months (mean 25.4±31.8 months). No subjects had ketosis, hypoglycemia or infection when samples were obtained. The study was approved by the Clinical Investigations Ethics Committee. Informed consents were obtained from the parents.

After basic tests (blood glucose, HbA1c, tHcy, Lp(a), vWF, lipid profile and urine microalbumin) had been obtained, appropriate medical treatments were arranged and patients were called for follow up with certain intervals (there was no difference between cases in terms of insulin type used for treatment). Initial tests were accepted as the data of group I (data I). All cases were re-evaluated at the end of the first year and similar tests were done, these were accepted as the data of group II (data II). Blood and urine samples were taken once from control group.

All samples were obtained from venous blood after one night fasting and before insulin administration in patients who were using insulin. Citrated tube was used for homocysteine and vWF; EDTA tube was used for HbA1c, normal tube was used for Lp(a) and lipids. Cobas Integra (Roche) commercial kits were used for HbA1c measurement and results were given as %. Total homocysteine levels were measured with high performance liquid chromatography (HPLC) fluorescence detector method using ClinRep (Recipe, Germany) commercial kits and values were given as µmol/L. Von Willebrand factor levels were measured using BC vonWillebrand Reagent (Dade Behring) commercial kits and results were given as %. Lipoprotein (a) was determined with Space protein analyser and results were given as mg/dL. Microalbuminemia was determined with nephelometric method in 24-hour urine. Routine biochemical methods were used for other tests. Serum Vitamin B_{12} and folate concentrations were measured by immunoassay method. Plasma vitamin B_6 concentration was measured by High performance liquid chromatography method.

Table 1: Characteristics of diabetic groups and control group

Case number (n)	Control (20)	Data I (65)	Data II (65)	p
Age (year)	9 ± 3.8	10.4 ± 4.3		NS
Gender (boy/girl)	10/10	34/31		NS
Body mass index (kg/m^2)	17.1 ± 2.1	16.5 ± 3.4		NS
Glucose (mg/dL)	91.7 ± 12.1	165.1 ± 48.2*	154.4 ± 32.5*	
HbA1c (%)	4.9 ± 0.5**	11.1 ± 3.1***	8.9 ± 1.9***	
tHcy (µmol/L)	8.9 ± 2.0	11.1 ± 3.3†	10.2 ± 1.7†	
Lp(a) (mg/dL)	5.3 ± 2.8	10.4 ± 5.4‡	9.0 ± 4.3 ‡	
VWF (%)	118.3 ± 21.2	110.7 ± 30.3	110.8 ± 26.3	NS
Microalbumin (mg/L)	7.1 ± 1.4	7.1 ± 1.6	7.9 ± 2	NS

NS: Not significant, ***:** I and II mean glucose levels of diabetic were statistically significantly higher than control group ($p<0.05$). ****:** I and II HbA1c levels of diabetic patients were significantly higher than those of control group ($p<0.05$). *****:** A significant difference was found between the initial and the final HbA1c levels of diabetic patients ($p<0.05$). **†:** A statistically significant difference was detected between the first and the second tHcy levels of diabetic patients and tHcy levels of control ($p<0.05$). **‡:** A statistically significant difference was detected between the first and the second Lp(a) levels of diabetic patients and Lp(a) levels of control group ($p<0.05$).

Statistical analyses were done using SPSS software (version 10.0, SPSS, Inc, Chicago IL 60606, www.spss.com). Data were given as mean ± standard deviation. One way variance analysis (ANOVA) and postANOVA tests, Tukey B and Scheffe tests were used for assessment of differences between groups in terms of parameters. Relationship of data between groups was evaluated with Pearson-Spearman correlation tests and a p level of <0.05 was taken as statistically significant.

RESULTS

Of the 65 patients in diabetes group, 34 (53%) were boys and 31 (48%) were girls; of 20 children in control group, 10 (50%) were boys and 10 (50%) were girls. Mean age of study and control groups was 10.4±4.3 and 9±3.8 years, respectively (Table 1).

The initial and the final mean glucose levels of diabetic patients (165.1±48.2, 154.4±32.5 mg/dL, respectively) were statistically significantly higher →

Table 2: Vitamin, apolipoprotein and lipid values of diabetes and control groups			
Case number (n)	**Control (20)**	**Diabetes (65)**	**p**
Folic acid (ng/mL) (normal:3.5-15 ng/ml)	9.9 ± 2.4	10.1 ± 3.9	NS
Vit B_6 (µg/L) (normal:8.6-27.2 µg/L)	16.1 ± 3.9	18.9 ±5.8 *	
Vit B_{12} (pg/mL) (normal:174-878 pg/ml)	545.2 ± 91,4	459,4 ± 107,7 *	
Apolipoprotein(A) (mg/dL)	105.6 ± 20.4	132.5 ± 23.7 *	
Apolipoprotein(B) (mg/dL)	103.6 ± 22.3	111,5 ± 26.8	NS
Lipid levels:			
Total cholesterol (mg/dL)	113,7 ± 20,3	117,5 ± 31,9	NS
Triglyceride (mg/dL)	106,7 ± 23,0	114,1 ± 32,9	NS
LDL-C (mg/dL)	82,7 ± 21,7	88,0 ± 23,9	NS
HDL-C (mg/dL)	51,0 ± 11,9	49,4 ± 7,8	NS
NS: Not significant, ***:** p<0.05			

than those of healthy control group (91.7±12.1 mg/dL) as expected (p<0.001). I and II HbA1c levels of diabetic patients (11.1±3.1% and 8.9±1.9%) were significantly higher than those of control group (4.9±0.5%) (p<0.001). A significant difference was also found between the initial (11.1±3.1%) and the final (8.9±1.9%) HbA1c levels of diabetic patients.

A statistically significant difference was detected between the first (11.1±3.3 µmol/L) and the second (10.2±1.7 µmol/L) tHcy levels of diabetic patients and tHcy levels of control group (8.9±2 µmol/L) (p<0.05 and <0.01, respectively) (Table 1).

A statistically significant difference was detected between the first (10.4±5.4 mg/dL) and the second (9.0±4.3 mg/dL) Lp(a) levels of diabetic patients and Lp(a) levels of control group (5.3±2.8 mg/dL) (p<0.01 and <0.001, respectively).

A statistically significant difference was not detected between the first and the second vWF levels of diabetic patients (110.7±30.3%, 110.8±26.3%, respectively) and vWF levels of control group (118.3±21.2%) (p>0.05).

There was no statistically significant difference between microalbumin levels of diabetic patients (7.1±1.6 mg/L, 7.9±2 mg/L, respectively) and microalbumin levels of control group (7.1±1.4 mg/L) (p>0.05).

A significant relationship was observed between the first HbA1c levels and microalbumin levels of diabetic patients (r=0.387, p=0.01). A positive correlation was also detected between tHcy and HbA1c (r=0.389, p=0.02) and microalbumin levels (r=0.286, p=0.034).

A positive correlation was found between the last HbA1c levels of diabetic patients and tHcy (r=0.428, p=0.001), vWF (r=0.328, p=0.024) and microalbumin levels (r=0.742, p=0.001). In addition, there was a positive correlation between vWF and microalbumin levels (r=0.560, p=0.001).

Apolipoprotein (A) levels of diabetic patient group (132.6±23.7 mg/dL) were greater than those of control group (105.6±20.4 mg/dL) and this difference was statistically significant (p<0.001). A similar relationship was not detected between apolipoprotein (B) levels (p>0.05).

There was no statistically significant difference between folate levels of diabetic patient group (10.1±3.9 ng/mL) and folate levels of control group (9.9±2.4 ng/mL) (p>0.05).

Vitamin B_6 levels of diabetic group (18.9±5.8 µg/L) were greater than vitamin B_6 levels of control group (16.1±3.9 µg/L) and there was a statistically significant difference (p<0.05).

Vitamin B_{12} levels of diabetic group (459.4±107.7 pg/mL) were greater than vitamin B_{12} levels of control group (545.2±91.4 pg/mL) and there was a statistically significant difference (p<0.01).

Plasma lipid profiles of diabetic and control groups were normal (Table 2).

DISCUSSION

Since diabetes is in high risk group in terms of cardiovascular disease development, effective treatments are needed. Trying to reduce tHcy levels may alter the natural course of newly developing renal disease and retinopathy in insulin-dependent diabetic patients.[19] In the study of Cronin et al. conducted with 119 young adults with type I diabetes, they found tHcy levels significantly higher compared to control group.[12] Chiarelli et al. showed that tHcy levels of type I diabetic young adults and adolescents with microvascular complications (persistent microalbuminemia and/or retinopathy) increased and there was a positive correlation between plasma tHcy and HbA1c.[12]

Our results are consistent with those of the two studies above. In our study, initial tHcy levels of diabetic patients were higher compared to control group. We consider that this condition resulted from →

poor metabolic control of diabetic cases (high HbA1c levels). Finding a significant difference between tHcy level at the end of the first year and tHcy level of control group indicated that tHcy did not return to normal despite treatment and stayed high just as HbA1c.

Initial HbA1c and tHcy levels of diabetic patients being high compared to control group and their reduction at the end of the first year supports the opinion that tHcy levels could give us information about metabolic controls of diabetic patients.

Wiltshire et al. could not find a significant relationship between tHcy, HbA1c and duration of diabetes.[20] Although study design is similar to that of ours, hyperhomocysteinemia was not detected in type I diabetic patients 14 years and above in the study of Cotelessa et al. and a gender-related difference was not found in plasma tHcy concentrations.[21] Similarly, Pavia et al. did not find a difference in tHcy levels between adolescents with type I diabetes and healthy adolescents.[13] Microalbuminemia developed in none of the patients and folate level, an index of nutritional condition was found normal in all patients. Metabolic controls being better in that study may be the reason for this difference.

Vitamin B_6, vitamin B_{12} and particularly folate, as a substrate, play important roles in homocysteine metabolism.[13] Hyperhomocysteinemia may develop as the result of inadequate dietary intake of folate, B_6 and/or B_{12} vitamin. Inadequate absorption and transport of these vitamins may also lead to hyperhomocysteinemia.[22] Although Pavia et al. found a negative correlation between tHcy and serum folate and vitamin B_{12}, they did not find a significant relationship between plasma tHcy and vitamin B_6 levels.[13] Wiltshire et al. showed a negative correlation between tHcy level and serum vitamin B_{12}, serum and erythrocyte folate levels.[20] In our study, there was no significant difference between diabetic patient group and control group in terms of folic acid levels. Vitamin B_6 level was higher in diabetic patient group compared to control group. On the contrary, vitamin B_{12} level of control group was significantly higher than that of diabetic patient group (Table 2). However we did not find a statistically significant correlation between vitamin levels and tHcy.

Plasma Lp(a) levels are not affected by plasma lipid levels and is metabolized through a different mechanism.[23] Structural properties of lipoprotein (a) give it a potential power for atherogenic and thrombogenic activities. Increased serum Lp(a) levels play a role both in progression of atherosclerosis and in premature coronary heart disease.[4] Lp(a) levels of poorly controlled diabetic patients were reported to be high and this was a significant risk factor for atherosclerosis development.[24] Recent studies show that diabetic patients have high Lp(a) levels.[24] Erem et al. showed that plasma Lp(a) levels significantly increased in adult non-insulin dependent diabetes mellitus (NIDDM) patients who have vascular complications.[25] Wollesen et al. suggested that serum Lp(a) levels must be measured as a risk factor predictor for peripheral atherosclerosis in diabetic patients.[26] Chiarelli et al. showed a positive correlation between serum cholesterol, LDL and Lp(a) levels and tHcy concentration.[19] Nakata et al. reported that plasma Lp(a) levels were significantly higher in uncomplicated NIDDM patients compared to healthy control group.[27] In the studies, it was concluded that high Lp(a) levels is a risk factor for angiopathy in NIDDM patients and it was suggested that patients with high plasma Lp(a) concentration must be under strict glycemic control.[24] In our study, there was no significant difference between I and II Lp(a) levels of the cases however there was statistically significant difference between study and control groups. Initial Lp(a) levels (together with HbA1c) being significantly higher in diabetic patient group compared to control group is similar to literature data. Lp(a) levels being reduced together with HbA1c at the end of the first year in diabetic group supports that metabolic control is going well and this indicates that Lp(a) levels may be used for assessment of metabolic control.

In previous studies, serum vWF concentration was shown to be a predictor of widespread endothelial injury and contribute to platelet aggregation in vascular endothelium, the first stage in thrombosis.[28,29] Janger et al. showed the evidence of an independent association between cardiovascular mortality and vWF in general population and concluded that vWF is a predictor of widespread endothelial dysfunction which is a characteristic of atherothrombotic diseases.[30] Becker et al. found the relationship between tHcy and vWF significant both in non-diabetics and type II diabetics.[31] In our study, a statistically significant difference was not found between vWF levels of diabetic and control groups and thus vWF levels were considered not to be a reliable indicator for assessment of glycemic control.

Microalbuminuria is known to be a predictor of increased cardiovascular risk and early mortality in diabetic patients.[32] Chen et al. showed a progressive increase in vWF levels together with increased urinary albumin excretion in NIDDM patients and found high vWF levels in all diabetics compared with healthy control group.[28] In addition, an association was shown between cardiovascular disease and high vWF levels in nonalbuminuric NIDDM patints however they could not find a relationship between retinopathy and vWF. In our study, a statistically significant →

difference was not found between the initial and the final urinary microalbumin levels of diabetic group and microalbumin levels of control group. This result was considered to be related with short follow up period and regular medical therapy.

Small number of patients and short follow up period is the limitation of our study. Therefore longer follow up of these patients together with complications was considered to give more information about the benefit of these parameters.

CONCLUSION

Particularly tHcy and Lp(a) levels were found to be correlated with HbA1c levels in our study. It was concluded that homocysteine and Lp(a) could be beneficial for assessment of metabolic control of diabetes, treatments toward reducing the complications of diabetes or the outcomes of additions to diet.

* The authors declare that there are no conflicts of interest.

CORRESPONDING AUTHOR: Sabahattin Ertuğrul Peyas Mah 272. Sok. Güneş Yapı Koop Sitesi C blok No:7, 21070, Kayapınar, Diyarbakır, Türkiye sertugrul68@yahoo.com

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