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ORIGINAL ARTICLE

Atherosclerosis in male patients with ankylosing spondylitis: the relation with methylenetetrahydrofolate reductase (C677T) gene polymorphism and plasma homocysteine levels

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Abstract The aim of this study was to determine the intima-media thickness (IMT) in carotid arteries and to assess the relation of these values with plasma homocysteine (pHcy) levels and methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism in patients with Ankylosing spondylitis (AS). Serum lipids, vitamin B12, folic acid, pHcy and acute phase protein levels were measured in all cases. MTHFR C677T gene polymorphisms were determined, and IMT of main carotid artery were evaluated ultrasonographically in all subjects. Bath Ankylosing Spondylitis Disease Activity Index, Ankylosing Spondylitis Disease Activity score and Bath Ankylosing Spondylitis Metrology Index were used to assess disease activity and spinal mobility. Fifty AS patients (mean age of 36.6 ± 4.79 years) and 50 control subjects (36.34 ± 4.72 years) were included in the study. Plasma homocysteine levels of AS patients and control group were also similar (14.26 ± 9.96 vs. 11.81 ± 5.53 μmol/L). Hyperhomocysteinemia was present in 11 subjects in patient group (22.0 %), while it was seen in 5 subjects in the control group (10.0 %). The MTHFR C677T genotype distribution was as follows: CC 31 (62 %), CT 14 (28 %), TT 5 (10 %) in AS patients. The mean carotid IMT values were also found to be similar between the groups. The most important factor influencing pHcy level was found as MTHFR 677TT genotype. We indicated no difference of atherosclerosis indices revealed by IMT values and pHcy levels AS patients and control subjects. But an association between MTHFR 677 gene polymorphism and pHcy levels was concluded, which may suggest that MTHFR 677 TT polymorphism may be a potential prognostic factor for cardiovascular disease in patients with AS.

Keywords Ankylosing spondylitis · Atherosclerosis · Homocysteine levels · MTHFR C677T gene polymorphism · Doppler ultrasonography · Intima-media thickness

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease that is characterized by bilateral sacroiliitis, inflammatory axial joint involvement and various involvements of other organs, such as the gastrointestinal tract, skin, eyes and cardiovascular system [1]. Some studies have noted the high prevalence of cardiovascular disease (CVD) including conduction disturbances, aortitis or aortic insufficiency, and coronary arteries involvement in AS patients [1, 2]. The results of some imaging studies showed impaired vascular functions of the coronary artery and common carotid artery (CCA) in AS patients [1]. The causes underlying this increased incidence of CVD were not entirely illuminated [3].

Potential mechanisms for cardiovascular complications include chronic inflammatory condition with increased levels of circulating cytokines and acute phase reactants and atherogenic lipid profile including high levels of LDL cholesterol and low levels of HDL cholesterol [3, 4]. Age, smoking and HLA B27 status were potential parameters related to increased risk of cardiovascular events in AS patients [1]. Previous studies indicated that prevalence ratios for atherosclerosis were higher in AS patients than in

controls [5]. Plasma total homocysteine level (pHcy) is an independent risk of vascular disease, similar to that of smoking or hyperlipidemia [6]. Some studies [6, 7] have noted an increased pHcy level in AS patients compared with the control group.

MTHFR is an enzyme involved in the folate pathway. An association of MTHFR C677T polymorphism with CVD was described by former studies [8, 9]. MTHFR C677T polymorphisms were reported to result in raised pHcy level and lower MTHFR enzyme concentrations in patients with peripheral arterial disease [8]. In our knowledge, there was no study that has examined MTHFR C677T gene polymorphism with AS patients in the English literature.

High-resolution B-mode ultrasonography has been used frequently in early detection of atherosclerosis or endothelial dysfunction, and indices related to atherosclerosis including intima-media thickness (IMT) can be determined by this method. The data of Choe et al. [1] study showed that carotid IMT in young AS patients were not different from healthy controls.

The aims of our study were to determine the IMT of CCA using high-resolution ultrasonography for atherosclerotic progression and also to investigate the association between IMT values and the pHcy, the MTHFR C677T mutation in patients with AS.

Materials and methods

Sixty-two patients with AS, who met the modified New York criteria [10] for AS, were recruited from the outpatient clinics of Ankara Training and Research Hospital, Rheumatology unit of Physical Medicine and Rehabilitation Clinic, between October 2010 and February 2011. We excluded twelve AS patients due to comorbid condition. Fifty age-, sex- and body mass index (BMI)-matched subjects, who had non-inflammatory low back pain and volunteered to participate in the study, were included as a control group.

We obtained informed consent from all the patients and controls, and the study was approved by the Ethics Committee of the Ankara Training and Research Hospital. Only male patients were included in the study as gender was thought to influence the pHcy concentrations. We excluded subjects with hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or taking current antihypertensive drugs), diabetes mellitus, renal failure, hyperlipidemia (total cholesterol >200 mg/dl), increased BMI (>30), thyroid disease, hepatic insufficiency and previous history of ischemic heart disease.

Demographic characteristics including age, sex, body mass index of all subjects, and drug intake and disease duration of AS patients were recorded. All patients underwent a clinical interview and physical examination.

In order to determine disease activity in patients with AS, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [11] and Ankylosing Spondylitis Disease Activity score (ASDAS) [12] were used. Inactive patients were rated by a score less than 3 in BASDAI. In the evaluation of spinal mobility, Bath Ankylosing Spondylitis Metrology Index (BASMI) [13] was used.

All patients were asked to fast overnight and refrain from smoking and alcohol for 24 h prior to the test. Fasting pHcy levels were determined by Agilent 1100 high-pressure liquid chromatography (HPLC) kits. A pHcy level of >17 μmol/L was considered as hyperhomocysteinemia. Serum lipids, vitamin B12, folic acid, homocysteine and acute phase protein levels were measured in all cases.

A peripheral blood sample in EDTA was obtained from enrolled patients for genotype determination. DNA was extracted using the High Pure PCR Template Preparation Kit. MTHFR 677 was determined by Light Cycler real-time polymerase chain reaction (RT-PCR). The IMT values of the right and left CCA were evaluated by High-Resolution B-mode Doppler Ultrasonography with 6–7.5 MHz linear array transducer (SA-660A, Toshiba, Japan) [1]. All measurements were performed by the same radiologist who was unaware of the clinical characteristics of the patients, after the subject had rested in supine position.

The IMT measurements of both right and left CCAs were performed at three points in each CCA from 1 cm proximal to the bifurcation of the CCA at the posterior wall. The distance between the leading edge of the first bright line (the lumen-intima interface) of the far wall and the leading edge of the second bright (collagen-containing upper layer of tunica adventitia) indicates the IMT as previously reported IMT values of each CCA were calculated as the average of the three points. Plaque was defined as prominent protrusion of more than 1.3 mm into the vessel lumen [1, 3, 14].

Statistical analysis

Data analysis was performed using the SPSS statistical package program version 15.0 for Windows.

Data for variables were described using mean ± SD. The intergroup comparisons for continuous variables between the two groups were performed by Mann–Whitney *U* test. Kruskal–Wallis test was performed for the comparison of more than two groups. Correlation analysis for continuous variables was performed using the Spearman's correlation test. Linear and logistic regression analysis was performed to identify the parameters accounting for IMT and Hcy level in AS. Odds ratios are expressed with 95 % confidence intervals (CIs). The value of $p < 0.05$ was considered significant.

Results

The demographic characteristics and cardiovascular risk factors for AS patients and the controls are shown in Tables 1 and 2. The results showed no differences between the two groups, except ESR and CRP values. Acute phase proteins were significantly higher in patient group than in control group ($p = 0.000$).

According to the classification regarding the medications received by patients, 15 patients (30 %) were on NSAII therapy, 2 patients (4 %) were on sulfasalazine (SSZ) therapy, 24 patients (48 %) were using SSZ + NSAII, 1 patient (2 %) etanercept, 4 patients (8 %) etanercept + NSAII, 2 patients (4 %) SSZ + etanercept, 1 patient (2 %) adalimumab + NSAII, 1 patient (2 %) adalimumab. The anti-TNF-alpha therapy was initiated in 9 patients. Seven patients were started on etanercept (50 mgr once weekly), 2 on adalimumab (40 mgr twice weekly).

Plasma homocysteine levels of AS patients were 14.26 ± 9.96 μmol/L, while those of control group were 11.81 ± 5.53 μmol/L with no significant difference between groups ($p = 0.321$).

C677T SNP was present in 31 (62 %) AS patients as resulted homozygote (CC); 14 (28 %), patients heterozygote (CT); and 5 patients (10 %), homozygote (TT). The prevalence of CC, CT and TT genotypes was 52, 44 and 4 %, respectively, in controls (Table 3). There was no statistically significant difference according to frequency of polymorphism between AS patients and controls ($p = 0.174$).

Carotid IMT was compared between AS patients and controls. The results again showed no significant difference in IMT values (Table 4).

Also, no statistically difference was detected between Hcy level and consumption coffee, SSZ user, smoking, MTHFR genotype in AS patients and controls (Table 5).

Patients with AS were divided into two groups according to the disease activity. While the patients with BASDAI >3 and ESR >mm/h were classified as active AS group, those with values lower than that were considered as inactive AS group. According to this classification, during this study, 17 patients were in active period, whereas 33 were in inactive period. There was no statistically significant difference between two groups in regard to pHcy level and IMT values (Table 6).

There was a statistically significant correlation between folate levels and pHcy levels in AS patients ($p < 0.05$). According to vitamin B12 levels (≤190 and >190) of plasma, Hcy level was found to be different between patients with vitamin B12 depletion and without it ($p < 0.05$).

Logistic regression analyses showed that pHcy level could only be explained MTHFR 677 TT genotype ($p = 0.028$, odds ratio: 11.308, 95 % CI (1.301–98.3099) in AS patients. As revealed by logistic regression analysis, the adjusted odds ratio for Hcy >17 μmol/L for TT compared with CC and CT subjects was 11 (1.3–98.3). Approximately, a 11-fold increased risk of hyperhomocysteinemia was found in our AS patients within the MTHFR 677 TT genotype.

Linear regression analyses showed that IMT, the most related factors, were age, ESR and BASMI (mean IMT = 0.114 + 0.013 × age + 0.013 × BASMI + −0.003 × ESR).

Discussion

Our study is designed to determine IMT values by Doppler Ultrasonography, which is an indicator of subclinical atherosclerosis in carotid arteries, in male AS patients compared to male control group and to investigate the relation of this value with pHcy levels and MTHFR C677T gene polymorphism.

This is, to our knowledge, the first study that considers the association between the MTHFR genotype and Hcy levels in AS patients. In our study, we did not find any association between MTHFR genotype and Hcy level and IMT values.

Epidemiological studies have shown that increased mortality (20–40 %) in AS patients is largely attributable to CVD. An increased carotid IMT reflects the atherosclerotic burden and predicts the development of CVD in

Table 1 Demographic and clinical characteristics of patients and controls

	Patients with AS $n = 50$ (male)	Control Group $n = 50$ (male)	p
Age (years)	36.66 ± 4.79	36.34 ± 4.72	0.882
BMI (kg/m^2)	24.92 ± 3.09	25.04 ± 3.04	0.871
Disease duration (years)	9.8 ± 4.87	–	
Smoker:non-smoker (n)	25:25	25:25	0.133
BASDAI	2.39 ± 1.66	–	
BASFI	2.56 ± 2.06	–	
BASMI	4.02 ± 2.22	–	
ASDAS-CRP	1.50 ± 0.74	–	

Data are mean ± SD

Table 2 Comparison of clinical characteristics in AS and controls

	AS Patients $n = 50$	Control group $n = 50$	p
Vitamin B_{12} (pg/ml)	262.54 ± 97.59	289.18 ± 112.86	0.959
Folic Acid (ng/ml)	7.57 ± 3.03	8.11 ± 2.43	0.181
ESH (mm/h)	26.10 ± 17.70	10.72 ± 8.03	**0.000***
CRP (mg/dl)	1.29 ± 1.48	0.36 ± 0.34	**0.000***
TG (mg/dl)	112.24 ± 47.39	129.52 ± 49.80	0.073
TC (mg/dl)	171.10 ± 28.04	179.92 ± 25.81	0.079
LDL (mg/dl)	103.80 ± 19.57	108.46 ± 21.06	0.185
HDL (mg/dl)	43.32 ± 9.57	43.14 ± 9.31	0.836

Data are mean ± SD

TG triglyceride, *TC* total cholestrol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *Hcy* homocysteine

* $p < 0.05$ statistically significant

Table 3 MTHFR genotype comparison between AS patients and healthy control population (Chi-square test)

	AS group $n = 50$	Control group $n = 50$	p
677CC	31 (62 %)	26 (52 %)	0.154
677CT	14 (28 %)	22 (44 %)	0.174
677TT	5 (10 %)	2 (4 %)	0.241

Table 4 Common carotid artery IMT in ankylosing spondylitis and controls

	AS patients $n = 50$	Controls $n = 50$	p
Right CCA IMT (mm)	0.56 ± 0.14	0.53 ± 0.12	0.508
Left CCA IMT (mm)	0.59 ± 0.13	0.55 ± 0.10	0.152
Mean IMT (mm)	0.57 ± 0.13	0.54 ± 0.10	0.298
Plaque			
Yes	2 (4 %)	5 (10 %)	0.436
No	48 (96 %)	45 (90 %)	

Data are mean ± SD

CCA common carotid arteries, *IMT* intima-media thickness

Table 5 Comparison of plasma homocysteine level and clinical parameters

	Homocysteine level			
	AS	p	Control	p
Consumption coffee				
Yes	15.27 ± 10.70		12.16 ± 5.95	
No	9.68 ± 2.47	0.057	10.01 ± 1.46	0.233
Smoking				
Yes	13.67 ± 7.75		10.73 ± 3.15	
No	14.86 ± 11.90	0.404	12.89 ± 7.08	0.426
SZN				
Yes	15.36 ± 12.07		–	
No	12.87 ± 6.37	0.837	–	–
MTHFR genotype				
CC	11.80 ± 4.65		10.44 ± 3.14	
CT	14.78 ± 7.444	0.187	13.42 ± 7.39	0.191
TT	28.09 ± 24.21		12.03 ± 0.04	

Data are mean ± SD

SZN sulfasalazine, *MTHFR* methylenetetrahydrofolate reductase

Table 6 Comparison of IMT, homocysteine level and physical activity parameters according to disease activity

	Disease activity		
	Inactive AS $n = 33$	Active AS $n = 17$	p
Right IMT (mm)	0.56 ± 0.14	0.55 ± 0.16	0.672
Left IMT (mm)	0.60 ± 0.14	0.55 ± 0.12	0.207
Mean IMT (mm)	0.58 ± 0.13	0.55 ± 0.13	0.369
Hcy (μmol/L)	14.79 ± 11.74	13.25 ± 5.15	0.660

Data are mean ± SD

IMT intima-media thickness, *Hcy* homocysteine

the general population [15–19]. But the question whether AS patients are really at risk of accelerated atherosclerosis compared to the general population is still unresolved.

Recently, relationships between chronic inflammation in AS and endothelial dysfunctions or impaired microvascular function have been proposed [1, 3]. Proinflammatory cytokines associated with the pathogenesis of AS such as interleukin-6 and tumor necrosis factor-alpha have been suggested as potential risk factors for cardiovascular problems as well as disease activity markers [1]. Several studies [1, 18, 20] showed no differences of subclinic atherosclerotic indices including IMT parameters, between AS patients and controls while a few studies [3] showed significantly increased IMT values in the AS patients compared with healthy controls. Our study showed that carotid IMT values in AS patients were not different from those of sex- and age-matched healthy controls. The reasons of the similarity in regard to carotid IMT values between patients and controls may be due to the relatively young age and having short disease duration and lower disease activity of our AS patients and the exclusion patients with comorbid disease that would increase the risk of atherosclerosis.

MTHFR is an enzyme that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, with nicotinamide adenine dinucleotide phosphate (NADPH) and the vitamin riboflavin as cofactors. Two common SNPs have been described for MTHFR: C677T

(cytosine to thymine substitution in nucleotide 677) that leads to a MTHFR variant with reduced activity and A1298C (adenosine to cytosine substitution) that produces a milder reduction [21].

There is still uncertainty as to the association between the C677T mutation and CVD. Several studies (have suggested a significant association while others did not [9]. The data of Cho et al. [9] supported those that suggested an association between the C677T mutation and CVD in patients attending the emergency department in Korea. In our analysis of the genotypes of 100 patients (AS and control), we found that the MTHFR C677T allele were not different between both groups.

The prevalence of the MTHFR C677T genotypes in our AS patients was 62 % for 677CC, 28 % for C677CT, 10 % for 677 TT groups. We could not compare our results with previous studies as MTHFR gene analysis has not been performed previously in AS patients. In our study, genotype distribution was found different from that reported in rheumatoid arthritis patients from Turkey (45, 43 and 10 %, respectively) [22]. Van Ede et al. [23] found that genotype frequency in 236 RA patients was 52 % CC, 40 % CT, 8 % TT. The discrepancy between our data and previous results may be due to ethnical differences or disease characteristics.

Increased Hcy level has direct and indirect toxic effects on vascular endothelial cells, and therefore, it is considered as an independent risk factor for atherosclerotic CVD [7, 9]. Hyperhomocysteinemia was found in 5 % of general population and 13–47 % in patients with atherosclerosis [6, 24]. Wei et al. and Başkan et al. found that pHcy was increased in patients with AS compared to normal controls [6, 7]. In our study, we found that pHcy level was increased in patients with AS compared to controls but difference was not statistically significant. DMARDs are known to interfere with the metabolism of vitamin B and Hcy levels. But in our study, there was no statistically significant difference between the SSZ group (56 % patients) with 15.36 μmol/L mean Hcy level and NSAII and anti-TNF groups (44 % patients) with 12.87 μmol/L.

An association between the C677T mutation and plasma Hcy levels without nutritional influence has not been clearly established. In one study, no association was found between C677T polymorphism and Hcy levels. In others, the C677T mutation was related to hyperhomocysteinemia only for low serum folate concentrations in Korea population [9]. An association among plasma Hcy levels and folate levels and vitamin B12 was shown in our study in accordance with same previous data [6].

Our results suggest that the Hcy concentrations are closely associated that folate and vitamin B12 levels cannot compensate for the reduced activity of the MTHFR enzyme and hyperhomocysteinemia. Hcy is eliminated through vitamin B-dependent pathways. The decrease in serum folate and vitamin B12 levels resulted in an increase in serum Hcy levels [6, 7]. An association between plasma Hcy level and MTHFR 677TT gene polymorphism was shown in our AS patients.

CRP was a predictor of future CVD events in patients with inflammatory polyarthritis [3]. In our study, the mean CRP level was higher in AS patients. In contrast, we found no correlation between IMT and CRP, or BASDAI values [3] but age, ESH and BASMI were found to be correlated with IMT values.

Age is one of the important risk factors for carotid atherosclerotic disease. Ghilardi et al. [25] showed that 90 % of subjects with significant carotid arterial diseases were more than 55 years of age. In our study, they also showed that IMT of CCA is closely related age, ESH and BASMI values.

Arterial stiffness is a marker for vascular dysfunction and an independent risk factor for CVD. Measurement of IMT of the far wall of the CCA high-resolution ultrasonography has been established as a clinically useful index for identifying early-stage atherosclerosis. The CCA is shown strongly to be correlated with the presence of coronary artery disease [3].

The major limitation of our study may be limited the statistical power due to the small sample size but our study groups reliable as they did not have any conventional or classic risk factors such as high blood pressure, advanced age, dyslipidemia or diabetes mellitus.

Conclusion

We indicated no difference of atherosclerosis indices revealed by IMT values and pHcy levels AS patients and control subjects. But an association between MTHFR C677T gene polymorphism and pHcy levels was concluded, which may suggest that MTHFR 677 TT polymorphism may be a potential prognostic factor for CVD in patients with AS. Further studies are needed to illuminate the descriptive factors underlying this relationship in patients suffering from this chronic condition.

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