Sağlıklı Bir Türk Populasyon Örneğinde Homosistein ile Folik Asit, B_{12} Düzeyleri

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Özet

Amaç: Yüksek plazma total Hcy düzeyleri kardiovasküler hastalıklar için önemli bir risk faktörüdür. Bu çalışmanın amacı, sağlıklı bir Türk populasyonunda cinsiyet ve yaş grupları arasındaki plazma Hcy, vitamin B_{12} ve folat konsantrasyonlarındaki farkları belirlemekti**.**

Metot: Çalışmaya Elazığ / Türkiye'de yaşayan ve yaşları 1-70 arasında değişen sağlıklı 210 kadın ve erkek seçildi. Plazma Hcy düzeyleri, enzim immunoassay (EIA), vitamin B_{12} ve folat düzeyleri ise kemiluminesens yöntemlerle ölçüldü.

Bulgular: Hem kadınlarda hem de erkeklerde yaşla birlikte plasma Hcy düzeylerinde gözlenen artış, 1-20 yaş grubunda 21-70 yaş grubuna göre daha belirgin bulundu (p=0.000). Ortalama plasma Hcy düzeyleri, yaşları 31-70 arasında olan 150 yetişkin birey için (26%) 15 µmol/L iken 31-40 yaş grubunda ise erkeklerde kadınlardan daha yüksek olarak belirlendi (p=0.001). Serum folat ve vitamin B_{12} düzeyleri, 1-20 yaş grubunda 21- 70 yaş grubundan önemli derecede yüksekken (p=0.0001), cinsiyetler arasında bu vitaminlerin düzeylerinde anlamlı bir farklılık bulunamadı (p>0.05). Plazma Hcy ve serum vitamin B_{12} düzeyleri ile plazma Hcy ve serum folat düzeyleri arasındaki ilişki incelendiğinde sırasıyla r= -0.229; p=0.001 ve r=-0.346; p=0.000 bulundu.

Sonuç: Sağlıklı Türk populasyonunda yaş ve cinsiyetin plazma Hcy düzeylerini etkilediği belirlendi. Böylece bu bulguların, aynı ülkedeki diğer populasyonlar için de önemli referans değerler olabileceği düşünülmektedir.

Anahtar Kelimeler: Homosistein, vitamin B_{12}, folat, yaş, cinsiyet

Abstract
Homocysteine and Folic Acid, B_{12} Levels in a Healthy Turkish Population Sample

Objective: Elevated plasma total homocysteine levels are an important risk factor for cardiovascular disease. The aim of this study was to determine the differences in plasma homocysteine (Hcy), vitamin B_{12} and folate concentrations among gender and age groups in a healthy Turkish population.

Method: Two hundred and ten healthy males and females between the ages of 1 to 70 living in the Elazig province of Turkey were randomly selected. Plasma Hcy levels were measured with enzyme immunoassay (EIA); vitamin B_{12} and folate levels were measured with chemiluminesence methods.

Results: Plasma Hcy levels elevated with age in both males and females, and the increase was significantly higher in the age group 21-70 than the 1-20 age group (p=0.000). The average plasma Hcy levels were over 15 µmol/L for 150 adults (26%) of the 31-70 age group, and were significantly higher in males than in females for the age group 31-40 (p=0.001). The serum folate and vitamin B_{12} levels in the age group 1-20 were significantly higher than in the age group 21-70 (p=0.0001), but there were no significant changes in vitamin levels between genders (p>0.05). The association between plasma Hcy and serum vitamin B_{12} was compared to that of homocysteine and serum folate levels, r= -0.229; p=0.001 and r=-0.346; p=0.000, respectively.

Conclusions: Age and gender appear to influence plasma Hcy levels in a healthy Turkish population. Therefore, this may be considered as an important reference value for other populations living in the same country.

Key Words: Homocysteine, vitamin B_{12}, folate, age, gender.

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Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine and is derived from dietary proteins (Boushey, Beresford, Omena & Motulsky, 1995). Intracellular Hcy is metabolized through the trans-sulfuration or remethylation pathway. Hcy is converted in the trans- sulfuration pathway to cysteine by the enzymes cystathionine beta-synthase and gamma cystathionase. Vitamin B_6 is the co-enzyme of these enzymes. In the remethylation pathway, a methyl group donor, N5-methyltetrahydrofolate, is used, and the reaction is catalyzed by the enzyme methionine synthase (homocysteine methyltransferase). The formation of this methyl donor is related to N5, N10-methylenetetrahydrofolate, which comes from dietary folate. The methyl cobalamin, a derivative of vitamin B_{12}, is the essential cofactor of methionine synthase (Finkelstein, 1998). Methylenetetrahydrofolate reductase converts N5, N10-methylenetetrahydrofolate to N5- methyltetrahydrofolate (Kang vd., 1996). In this remethylation pathway folic acid is used as a coenzyme and substrate (Stryer, 1988). It has been well documented that Hcy levels become elevated when the vitamins used in these metabolic pathways are lacking (Halifeoglu, Gur, Aydin & Ozturk, 2004; Konechy, 1997; Selhub, Jacques, Wilson, Rush & Rosenberg, 1993; Welch, Upchurch & Loscalzo, 1997).

High Hcy levels (hyperhomocysteinaemia), of over 5 μmol/L, have been shown to be risk factors for cardiovascular disease, though its molecular basis is not well established (Welch & Loscalzo, 1998). Increased Hcy levels are a major risk factor for the development of atherosclerosis and may be associated with the race, ethnicity (Alfthan, Anthi & Gey, 1997; Dierkes, 2001), gender, and age (Boushey vd., 1995). In addition, previous studies have argued that the rate of an increase of 0.5 μmol/L in plasma total cholesterol level resulting from an increase of 5 μmol/L in plasma Hcy causing cardiovascular diseases is about 1.8% in females and 1.6% in males. Therefore, reference values of Hcy in various populations were determined to prevent the high prevalence of

cardiovascular diseases. The reference values of homocysteine levels in the Turkish population was not yet investigated in detail. In a study of the Turkish population by Taskin vd., (2 0 0 6) Hcy levels were established in healthy individuals in the age range between 20 and 61, but levels of vitamin B_{12} and folate, which have a part in Hcy metabolism, were not measured. Still another study of the Turkish population determined plasma Hcy, folate and vitamin B_{12} levels in individuals in the age range between 31 and 73 **(**Aksoy, Geyikli & Saygili, 2006).

The aim of this study to re-determine the reference values of Hcy, vitamin B_{12} and folate levels in healthy individuals aged 1-70 years in Turkey and to compare them with other studies from different populations.

Material and Methods

Two hundred and ten individuals (105 male and 105 female) aged 1-70 years were performed. The subjects included individuals of different socio-economic status with no systemic diseases that affect plasma Hcy levels, taking no hormone replacement therapy or medication that interferes with the vitamins responsible in Hcy metabolism, and non-alcohol and tobacco users. In addition, the body mass index of the individuals was determined and the obese individuals were not included. The subjects were asked to fast at least 10 hours before venous blood samples were taken. The study was approved by the Firat University Ethical Committee and written consent was obtained from the individuals or their legal guardians before sampling. Both male and female subjects were divided into 7 different age groups of 10-year age difference with 15 individuals in each age group.

Venous blood for Hcy measurement was collected in tubes containing EDTA. All samples were taken in the morning, collected on ice, and transported to the laboratory within 60 min. After centrifugation of the EDTA blood at 3.000 rpm for 10 min,

plasma was separated from blood cells and frozen at -20°C, and repeated thawing were avoided. The plasma Hcy concentrations were measured as μmol/L by enzyme immunoassay (EIA) method using Axis (Biochemicals ASA, Norway) kits at 450 nm in Immulite 2000 analyzer (Diagnostic Products Co., Los Angeles, CA, USA). Vitamin B_{12} and folate levels from the serum samples without anticoagulant were measured by the chemiluminesence method by Roche Elecsys analyzer (F. Hoffmann-La Roche Ltd. Basel, Switzerland). Serum lipid profile was measured by using trade mark Olympus AU 600 autoanalyser by commercial Randox kits.

Statistical analysis was done by SPSS for the Windows 10.0 software program. One-way ANOVA was used for the comparison of the parameters between age groups. To determine the statistical mean between the groups the Tukey-HSD test was used as a Post Hoc test. The comparison between the two groups (according to gender) was determined by the Mann-Whitney U test. The correlation between parameters was determined by Spearman correlation analysis. The results were stated as both mean/standard deviation and median/percentile**,** and p values smaller than 0.05 were accepted as significant.

Results

Mean ± SD values of plasma Hcy, serum vitamin B_{12} and folate levels in both genders in different age groups are shown in Table 1.

Mean plasma Hcy, serum vitamin B_{12} and folate levels in the age groups 1-20 and 21-70 were shown in Table 2.

In older adults (both males and females), plasma Hcy levels were shown to be elevated. This increase was more significant in the age group 21-70 than the age group 1-20 (p=0.000), (figure 1). All young individuals, regardless of gender, were found to have Hcy concentrations of 12 μmol/L or lower. 26% of individuals in age group 31-70 showed Hcy levels greater than 15 μmol/L. Hcy concentrations in the 1-20 age group are

consistent with the published pediatric values of 3.7-10,3 μmol/L [12]. Plasma Hcy levels were significantly higher in men than women in the age group 31-40 ($p<0.005$), but not very different for ages 41-50.

Table 1. Mean plasma Hcy, serum vitamin B_{12} and folate levels according to gender in different age groups

	Age Groups*	Mean Age (year)	Hcy (μmol/L)	Vit B_{12} (pg/mL)	Folate (ng/mL)
Female	1-10	6.80±2.70	7.09±1.04	316.0±39.4	7.19±1.12
	11-20	17.60±2.02	7.63±1.02	278.2±67.6	5.46±1.78
	21-30	26.46±3.11	9.82±2.15	218.7±76.6	4.42±1.25
	31-40	34.80±3.16	11.90±3.07	229.5±58.5	4.69±1.96
	41-50	43.86±2.82	12.93±2.91	239.0±66.4	4.19±1.32
	51-60	54.93±3.47	15.09±3.73	272.5±100.1	4.24±2.26
	61-70	64.40±2.92	14.32±2.67	278.6±100.1	4.46±1.44
	(N=105)	**35.91±19.49**	**11.26±3.85**	**261.79±79.8**	**4.95±1.88**
Male	1-10	8.26±2.08	7.57±1.18	419.0±157.1	6.25±1.74
	11-20	15.73±3.01	7.71±1.09	250.0±62.1	6.55±1.35
	21-30	26.40±2.82	10.31±2.62	307.7±132.2	3.70±1.15
	31-40	35.13±3.31	13.66±2.69	285.3±106.5	5.36±1.34
	41-50	45.80±3.07	12.39±2.26	236.5±67.3	4.15±1.61
	51-60	56.73±3.17	13.95±3.86	245.0±94.0	4.58±1.95
	61-70	67.00±4.54	16.90±4.15	253.7±45.4	4.40±1.65
	(N=105)	**36.17± 19.85**	**1178± 4.19**	**268.9± 87.17**	**5.00± 1.82**

***15 individuals in each age group.*

Table 2. Plasma Hcy, serum vitamin B_{12} and folate levels of age groups 1-20 (30 young males, 30 young females) and 21-70 (75 adult males, 75 adult females)

	Young Females	Adult Females	Young Males	Adult Males
Age (year)	1-20	21-70	1-20	21-70
Mean age	12 ±6	45 ±14	12±5	46±15
Hcy (μmol/L) [median, (90th percentile)]	7.1 (9.0)	12.8 (16.7)	7.3 (8.9)	13.3 (17.5)
Vit B_{12} (pg/mL) [median, (90th percentile)]	298 (372)	229 (290)	298 (351)	251 (400)
Folate (ng/mL) [median, (90th percentile)]	6.7 (8.0)	4.2 (6.9)	6.5 (8.3)	4.4 (6.5)

However, much higher Hcy levels were detected in women than men in age group 51-60 ($p<0.005$). In both males and females, the Hcy levels in age group 51-70 were significantly higher than in the age group 1-20 ($p=0.0001$), (Table 1).

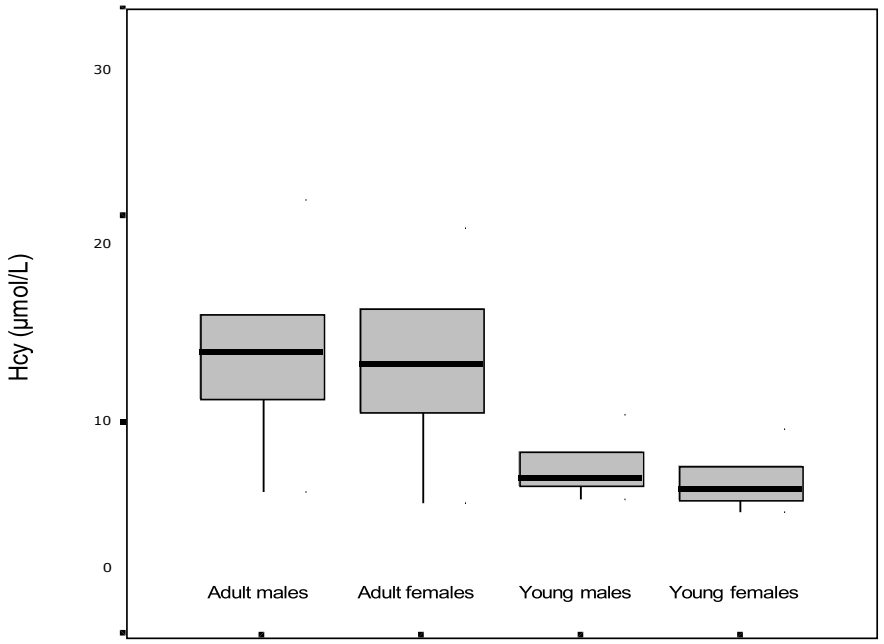

Figure 1. Plasma Hcy levels of age groups 1-20 (30 young males, 30 young females) and 21-70 (75 adult males, 75adult females)

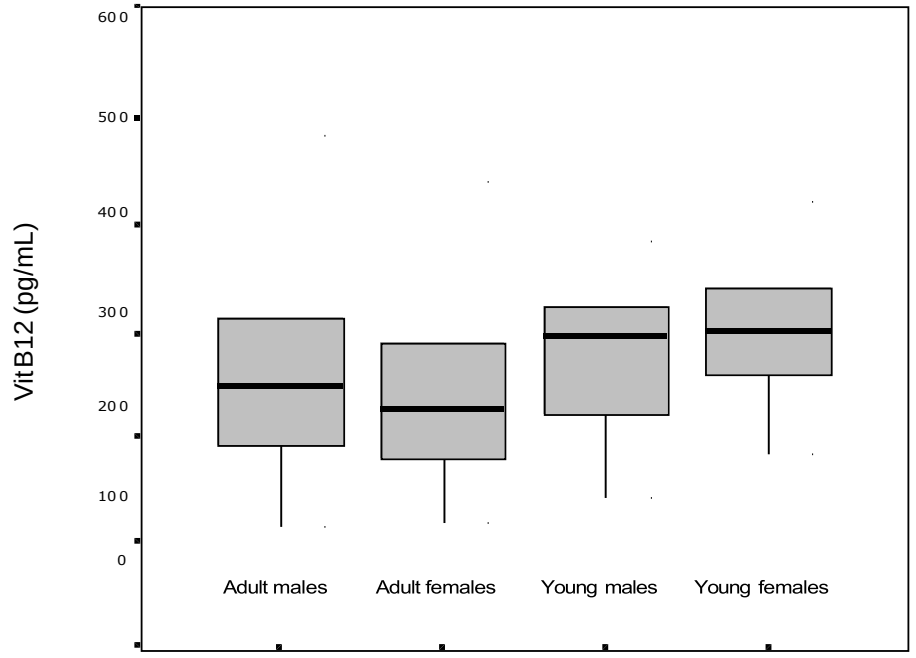

Figure 2. Serum vitamin B_{12} levels of age groups 1-20 (30 young males, 30 young females) and 21-70 (75 adult males, 75adult females)

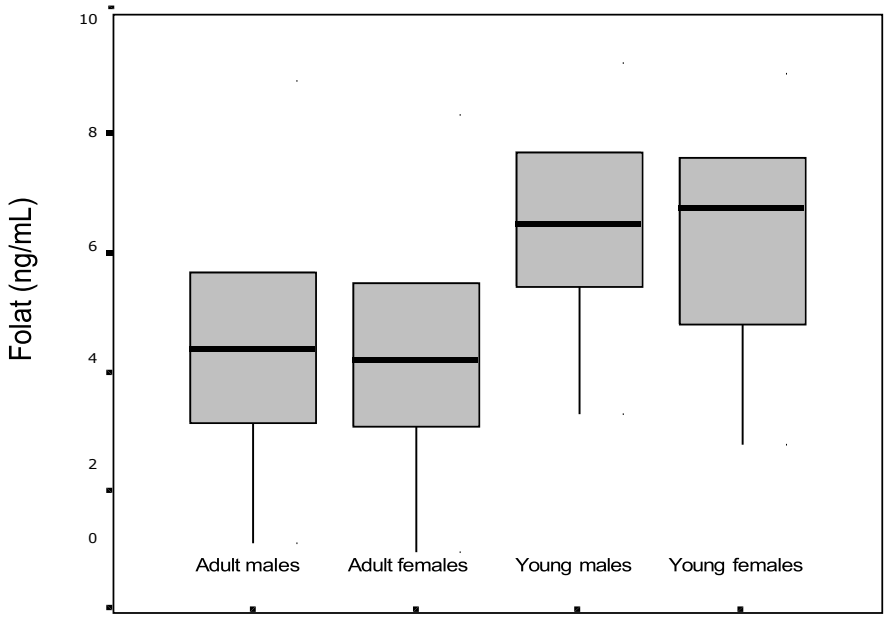

Figure 3. Serum folate levels of age groups 1-20 (30 young males, 30 young females) and 21-70 (75 adult males, 75 adult females)

The serum folate and vitamin B_{12} levels were significantly higher for the 1-20 age group than for 21-70 (p=0.0001). Among all age groups, there was a weakly correlation between Hcy and serum vitamin B_{12} levels **(**r = -0.229; p= 0.001), whereas a significant association was observed between Hcy and serum folate levels (r = -0.346; p=0.000).

Mean ± SD values of serum lipid levels in both genders in different age groups are shown in Table 3.

An examination of the correlation between plasma homocysteine and BMI levels in all individuals has revealed that the increase observed in BMI as a result of the elevation of plasma Hcy levels is significant (r = 0.495 ; p = 0.001). Similarly, when the correlation between plasma homocysteine levels of these individuals and their total cholesterol, triglyceride and LDL cholesterol levels was examined, elevated plasma Hcy levels were associated with a significant increase in the levels of these parameters (r = 0.513, p = 0.001 ; r = 0.349; p = 0.001; r = 0.473 ; p = 0.001, respectively). As for the correlation between plasma homocysteine and HDL cholesterol levels, a negative, but

weak correlation was found between these two (r = - 0.252 ; p = 0.001).

Table 3. Mean serum lipid levels according to gender in different age groups

	Age Groups*	Mean Age (year)	BMI (kg/m^2)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)
Female	0-10 yaş	6.80±2.70	16.75±1.75	145.30±41.90	89.87±21.45	91.63±10.62	4680±5.42
	11-20 yaş	17.60±2.02	21.78±3.24	155.47±41.87	101.73±22.52	87.33±18.58	46.20±9.00
	21-30 yaş	26.46±3.11	25.55±4.29	163.80±20.71	102.80±38.22	99.20±15.92	44.40±5.90
	31-40 yaş	34.80±3.16	26.65±2.43	182.53±34.46	157.67±63.20	114.67±38.15	43.00±5.63
	41-50 yaş	43.86±2.82	26.89±3.99	186.67±27.35	122.33±52.60	114.80±19.30	47.40±4.88
	51-60 yaş	54.93±3.47	27.72±3.42	205.80±26.91	116.20±38.60	135.13±28.55	45.00±8.09
	61-70 yaş	64.40±2.92	27.47±3.23	229.53±38.52	189.33±64.80	142.07±37.62	49.53±5.69
	(N=105)	35.91±19.49	24.69±4.93	181.29±40.67	125.70±55.62	114.56±32.69	42.71±6.34
Male	0-10 yaş	8.26±2.08	15.60±1.33	160.27±16.82	98.73±33.33	92.93±14.54	47.60±6.77
	11-20 yaş	15.73±3.01	20.72±4.40	153.07±15.70	99.07±28.86	94.60±16.69	43.07±6.30
	21-30 yaş	26.40±2.82	23.19±3.07	172.07±26.24	111.93±38.32	110.60±26.66	40.27±6.13
	31-40 yaş	35.13±3.31	26.88±3.02	207.07±32.47	152.13±60.84	106.53±17.10	41.26±6.32
	41-50 yaş	45.80±3.07	27.24±3.06	188.40±22.50	191.27±62.40	121.00±33.87	38.47±4.94
	51-60 yaş	56.73±3.17	27.20±2.79	206.33±47.84	177.07±68.52	130.73±37.58	39.40±4.05
	61-70 yaş	67.00±4.54	26.26±2.88	200.07±25.57	165.60±58.90	119.53±20.00	40.47±2.50
	(N=105)	36.17±19.85	23.87±5.05	183.89±34.62	142.26±62.21	110.85±27.73	41.50±6.03

15 individuals in each age group

Table 4. Fasting plasma homocysteine levels on healthy subjects from different populations

Population	Age range	Hcy /male (μmol/L)	Hcy / famele (μmol/L)
Chili	22-78	11.50	8.5
Finnish population	25-74	11.3	9.2
Japa	53-73	12.6	9.8
Saudi Arabia	20-69	9.91	8.08
Non-Hispanic black	>12	9.8	8.2
Non-Hispanic white	>12	9.6	7.9
Norway	40-42	10.8	9.1
Thailand	20-65	11.5	8.5
Turkey	>20	9.51	7.38

Discussion

The correlation between high plasma Hcy levels and coronary heart disease, cerebral, and atherosclerotic cardiovascular diseases have been well established (Kalra, 2004; Temple, Luzier & Kazierad, 2004; (Dikmen, 2004). In our study, a significant increase in Hcy levels attributed to aging was determined ($p<0.005$).

Average Hcy levels were about 7.5 μmol/L in the age group 1-10, and no significant increases occurred throughout puberty. The results are consistent with the results of similar studies (Tonstad vd., 1996; De Laet vd., 1999).

Although the Hcy levels of males between the ages of 21-40 were shown to be higher than that of females in the same age group ($p<0.005$), the Hcy levels of both sexes between the ages of 41-70 were shown to be very similar in our study. It was also determined that 26% of adults between the ages of 31-70 showed Hcy levels of 15 μmol/L ($p<0.005$). In post-menopausal women, an inverse relation between plasma Hcy and eustrediol levels was reported. However, with hormone replacement therapy a significant reduction of high Hcy levels has also been observed (Jousilahti, Tuomilahto, Vartiainen, Pekkanen & Puska, 1996).

We, however, were not able to observe any significant difference in the Hcy levels of our subjects. This might be due to a lack of hormone replacement therapy in women included in this study.

In our study, Hcy levels in the age group 21-70 were around 12.81 μmol/L in females and 13.44 μmol/L in males. Our results were found to be significantly higher than that of individuals between the ages of 20-65 from Thailand (Leowattana, Bhuripanyo, Mahanonda & Pokum, 2001). between the ages of 22-78 from Chili (Bunout, 1998), between the ages of 22-78 from Finland (Alfthan, Laurinen, Valsta Pastinen & Aro, 2003). and between the ages of 20-69 from Saudi Arabia (Ardawi, Rouzi, Qari, Dahlawi & Al-

Raddadi, 2002). Although the Hcy levels in females between the ages of 40-42 from Norway (Nygard, 1995) were similar to our findings, the Hcy levels of males in this age group as well as the 65-67 age group were higher than our results. The increase in Hcy levels that accompanied with age might be due to a decrease in glomerular filtration rates (Calabrese, 1984; Rea vd., 2000). In a study which registered healthy individuals in the age range between 20 and 61 in the Turkish population, Hcy levels were established to be 9.51 μmol/L in males and 7.38 μmol/L in females, but levels of vitamin B12 and folate were not quantified (Taskin vd., 2006). Still another study of the Turkish population determined plasma Hcy, folate and vitamin B_{12} levels in individuals in the age range between 31 and 73 (Aksoy vd., 2006).

Plasma Hcy levels found in this study which covered a more extensive age range are significantly higher than the results of both of the previously mentioned studies. The studies have demonstrated that vitamin B_{12} and folate are also important factors in determining plasma Hcy levels. Therefore, increases of Hcy levels in healthy adults might be the result of insufficient absorption of these vitamins, in addition to diminished renal functions (Brattström, 1996).

In general populations, vitamin B_{12} concentrations have less influence than folate in high Hcy levels due to low levels of vitamin B_{12} (Selhub vd., 1999).

In our study, the serum folate levels in the age group 1-20 were shown to be significantly higher than the adults in the age group 21-70 ($p=0.000$). The correlation between Hcy and vitamin B_{12} ($r= -0.229$) was also found to be significant compared to that between the Hcy and folate ($r= -0.346$). In both age groups vitamin B_{12} and folate levels were not significantly different among genders ($p>0.05$). Previous published results of low folate levels among Turkish populations support the findings of our study (Tokgozoglu vd., 1999).

The plasma Hcy levels in boys between the ages of 12-15 in Taiwan showed a inverse correlation with both vitamins (Chang vd., 2003). However, there was no significant difference between the Hcy levels and both vitamin levels among the same age group of boys and girls ($p>0.05$). In another study, the plasma Hcy levels were negatively correlated with folate in children between the ages of 5-19 in Belgium (De Laet vd., 1999). Ardawi vd., (2002) also reported a significant inverse correlation between plasma Hcy levels and both vitamins in individuals of 20 to 69 years.

In our study, there was no significant difference between plasma Hcy, folate and vitamin B_{12} in the same age group of both sexes ($p>0.05$). In Britain, a negative correlation between plasma Hcy and vitamins was seen in populations of both sexes over 65 years (Bates vd., 1997). However, our results showed no difference between Hcy and both vitamins in similar age groups of both sexes ($p>0.05$).

In the present study, an examination of the correlation between plasma Hcy levels and total cholesterol, triglyceride and LDL cholesterol levels demonstrated that an increase in plasma Hcy levels corresponded to a significant elevation of the said parameters. It is known from previous studies that an increase in LDL cholesterol is in direct proportion with an increase in cardiovascular diseases (Barbir, Wile, Trayner, Aber & Thomson, 1988). Elevated plasma Hcy levels cause aggragation of LDL, which carries about 70% of the plasma cholesterol, with the reactive homocysteine thiolactone. Aggregates of LDL-homocysteine thiolactone, which are released from the liver to the blood, are captured by arterial wall macrophages and converted into foam cells. These foam cells ruin LDL-homocysteine thiolactone aggregates, releasing fat and cholesterol into develoing plaques and homocysteine thiolactone into the cells surrounding the arterial wall. This results in formation of highly reactive oxygen radicals in cells, which cause injury to endothelial cells, increase clotting and enhance arterial muscle cells which lead to the formation of fibrous tissues, mucoid matrix and degenerative elastic tissues (McCully, 1996).

In conclusion, Hcy levels were established to be 7 μmol in the age range between 1 and 20 and 13 μmol in the age range between 21 and 70. In consideration of the fact that Hcy levels higher than 5 μmol pose a significant risk factor for cardiovascular diseases (Welch & Loscalzo, 1998). These results seem to be quite elevated.

Because the prevalence of these diseases are very common in Turkey, monitoring Hcy, vitamin B_{12} and folate levels among different age groups and genders provides not only invaluable information for prevention of disease, but also serves as a reference for both ethnic and general Turkish populations. Further studies are needed for the genetic polymorphism in the regulatory enzymes of homocysteine metabolism, and the correlation between the genotype and homocysteine and folate levels in the Turkish population.

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