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A CASE CONTROL STUDY ASSESSING THE RELATIONSHIP BETWEEN CORONARY HEART DISEASE AND SERUM VITAMIN B_{12}, HOMOCYSTEINE AND FOLIC ACID LEVELS*

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Purpose: To test the hypothesis that the coronary heart disease is associated with high serum homocysteine and low vitamin B-12, and folic acid concentrations.

Methods: In this population-based case control study, "304 cases" with ischemic ECG findings and "301 controls" without ischemia in their ECGs were selected as the study population.

Results: The mean value of serum homocysteine levels of the cases (6.52 µmol/L ±6.38, median= 1.90) was not significantly higher than the values of the control group (6.19±5.99µmol/L, median= 1.80) (p= 0.556). The mean value the folic acid levels of the cases (6.76 ng/ml ±3.10, median= 6.10) were higher than the values measured for the control group (6.32 ng/ml ±2.54, median= 6.20). However, this difference was not statistically significant (p= 0.089). Serum vitamin B_{12} levels (mean =282.87 pg/ml ±125.96, median= 230.50), on the other hand, were found to be significantly higher in cases than those in the controls (mean =252.81 pg/ml ±105.59, median= 105.59) (p= 0.005). Multivariate analysis controlling for age and serum homosistein, vitamin B_{12} and folic acid levels simultaneously suggested high vitamin B_{12} as a significant predictor of coronary hearth disease.

Conclusion: These results do not support the hypothesis that coronary heart disease is related to high serum homocysteine concentration. Yet, there is suggestive evidence of a positive association between coronary hearth disease and serum vitamin B_{12} levels. The results are not conclusive due to inability to adequately control for potential confounders.

Key Words: Coronary heart disease, homocysteine, vitamin B_{12}, and folic acid

KORONER ARTER HASTALIĞI VE SERUM VİTAMİN B_{12}, HOMOSİSTEİN VE FOLİK ASİT DÜZEYLERİ ARASINDAKİ İLİŞKİYİ İNCELEYEN BİR VAKA-KONTROL ÇALIŞMASI

Amaç: Koroner arter hastalığının yüksek serum homosistein; düşük vitamin B_{12} ve folik asit konsantrasyonu ile ilişkili olduğu hipotezini test etmek

Gereç ve yöntemler: Toplum tabanlı bu vaka kontrol çalışmasında 304 koroner kalp hastası ("vaka") ve 301 EKG bulgusunda patoloji saptanmamış erişkin ("kontrol") kişi çalışma grubu olarak seçilmiştir.

Bulgular: Vaka grubundaki kişilerin ortalama serum homosistein düzeyi (6.52±6.38, ortanca= 1.90), kontrol grubundakilerin serum ortalama homosistein değerinden (6.19±5.99, ortanca= 1.80) istatistiksel olarak anlamlı düzeyde farklı değildir (p= 0.556). Vaka grubundaki kişilerin ortalama serum folik asit düzeyi (6.76±3.10, ortanca= 6.10), kontrol grubundakilerin folik asit değerinden (6.32±2.54, ortanca= 6.20) yüksektir, ancak, bu ilişki istatistiksel olarak anlamlı değildir (p= 0.089). Diğer taraftan vaka grubundaki kişilerin ortalama serum vitamin B_{12} düzeyi (282.87±125.96, ortanca= 230.50), kontrol grubundakilerin vitamin B_{12} değerinden (252.81±105.59, ortanca= 105.59) istatistiksel olarak anlamlı düzeyde yüksek bulunmuştur (p=0.005). İleri analizlerde, yaş için kontrol edilerek, serum homosistein, vitamin B_{12} ve folik asit düzeyleri ile koroner kalp hastalığı arasındaki ilişki eş zamanlı değerlendirildiğinde, serum vitamin B_{12} düzeyinin koroner arter hastalığı açısından önemli bir belirleyici olduğu saptanmıştır.

Sonuç: Bu sonuçlar koroner arter hastalığı ile yüksek serum homosistein düzeyleri arasındaki ilişkiyi desteklememektedir. Ancak, koroner arter hastalığı ile serum vitamin B_{12} düzeyleri arasında pozitif bir ilişki olasıdır. Çalışmada tüm olası karıştırıcı değişkenler kontrol edilemediği için bu ilişkinin ileri çalışmalarla tekrar incelenmesi uygun olacaktır.

Anahtar Kelimeler: Koroner arter hastalığı, homosistein, vitamin B_{12}, folik asit.

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INTRODUCTION

Cardiovascular diseases particularly coronary or ischemic heart diseases, hypertensive and rheumatic heart diseases are the leading causes of death in western countries (1,2,3,4).

An important number of studies conducted on coronary heart diseases (CHD) and high plasma homocysteine concentration showed that high levels of serum homocysteine cause atherosclerosis due to endothelial dysfunction. Recently, hyperhomocystenemia is accepted as a new preventable and independent risk factor of CHD (5, 6). Folate and vitamin B-12 are essential for the metabolic pathways for decreasing the serum homocysteine concentrations (6). Life style, age, gender, having another chronic disease, vitamin deficiencies and some drugs may increase the levels of homocysteine concentrations. On the contrary, vitamin B fortification and supplementation (especially vitamin B-12) may decrease the serum homocysteine levels (7).

Despite several studies on blood pressure and other risk factors of coronary heart disease, there are not so many studies investigating the relationship between the serum homocysteine concentrations and coronary heart diseases in Turkish population. Tokgözoğlu et al., carried out a study to determine the effects of thermolabile methylene tetrahydrofolate reductase mutation on the presence and extent of coronary atherosclerosis among inpatients with low plasma folate levels. In this study, high plasma homocsyteine concentration was found to be a significant risk factor for coronary artery disease (9).

Majority of the studies about hyperhomocysteinemia and CHDs has been carried out among inpatients at the hospitals. However, results of the population-based studies may give more accurate results about this relationship and would be more generalizable.

This study was carried out in the catchment area of a Primary Health Care Unit in Ankara (the capital city of Turkey) in a selected population representing the adult age group, for assessing the relationship between coronary heart diseases and serum homocysteine, folic acid and vitamin B-12 concentrations.

In this population-based case control study of adults aged 25-64 years we aimed;

1. to determine serum vitamin B-12, homocysteine and folic acid concentrations among individuals with ischemic findings in their ECGs (i.e., cases),
2. to determine serum vitamin B-12, homocysteine and folic acid concentrations among individuals without ischemic findings in their ECGs (i.e., controls),
3. to assess the relationship between the coronary heart diseases and serum vitamin B-12, homocysteine and folic acid concentrations in the study population, comparing cases and controls.

MATERIAL and METHODS

Study population

The study population consisted of 304 cases with ischemic ECG findings and 301 controls without ischemia in their ECGs. Both cases and controls were selected from among participants of a population-based study conducted in the catchment area of a primary health care unit entitled "Risk Factors of Coronary Heart Diseases". In this study, 2400 individuals aged 25-64 years representing the total population living in the catchment area of the primary health care unit were selected in 1998, using a stratified sampling technique where strata were gender (Female/Male) and age groups (25-34, 34-44, 45-54- 55-64). 1672 (69.6%) people were interviewed and the data were collected on socio-demographic characteristics and coronary heart disease risk factors. Electrocardiograms of 1214 people were completed and the blood samples of 1210 participants (72. 4%) were collected. Among the participants whose ECG tests were completed, ischemia was diagnosed in 319 people and all of them were included in the study as "cases". The same professor of cardiology using standard criteria conducted all ECG readings. Out of those without iscemia, 183 people were excluded from the study because of presence of other cardiac pathologies in ECGs. A total of 319 individuals were selected as controls, using simple random sampling technique. Controls were matched to cases for gender.

The study population consisted of 304 out of 319 cases with ischemic ECG findings and 301 out of 319 controls without ischemia in their ECGs. Blood samples of 33 cases and controls could not be analyzed. Biochemical analyses were completed for majority of the participants: assessment rates were (483/605, 80%), (480/605, 80%) and (485/605, 80%), for homocysteine, vitamin B-12 and folic acid, respectively.

Questionnaire

A standardized questionnaire was used for collecting data on socio-demographic characteristics and coronary risk factors like smoking, diabetes mellitus, hypertension, etc. Questionnaires were completed using face-to-face interviews by trained interviewers.

Blood samples and ECG evaluation

Blood samples were drawn in the fasting state in 1998. They were all centrifuged and preserved at –20 °C in the refrigerator until 2002. They were all analyzed for homocysteine, vitamin B-12 and folic acid in the Clinic-pathology Laboratory of a University Hospital. Homocysteine levels were examined by Fluorescence polarization Immunoassay (FPIA) method (IMX, Abbott Laboratories, Germany). Folic acid and vitamin B-12 were detected by chemiluminescent immunoassay system (Immulite 2000, United Kingdom). The reference values for homocysteine, folic acid and vitamin B-12 are shown below.

The same radiology technician took all electrocardiograms of the participants and a cardiology specialist evaluated the ECGs of study participants for assessing ischemia.

Reference categories

	Reference value
Homocysteine	5-15 μmol/L
Folic acid	3-17 ng/ml
Vitamine B-12	160-900 pg/ml

Data analysis

Statistical Package for Social Sciences (SPSS) program, Version 10.0 was used for data entry and analysis. Analyses included frequency and percent distributions, calculations of means, standard deviations, medians, and percentiles. Multivariate analysis included analysis of covariance and logistic regression modeling. All variables found to be potentially associated with coronary heart diseases in univariate analyses (at p=0.10) are included in the model.

Study included all "cases" arising from the study population. In retrospective power analyses 304 cases and 301 controls, correspond to a study power of 80% at α=0.05 for detecting an association(OR) of 2.2 for a population prevalence of 7% or more (for hyperhomocystenemia) (10).

RESULTS

In this study, 304 "cases" and "301" controls were evaluated. There were no statistically significant differences between cases and controls for sex (p= 0.803), family type (p= 0.147), health insurance (p= 0.181), possession of the house lived in (p= 0.138), ownership of a real estate (p= 0.371), and ownership of a car (p= 0.090). However, the mean age of the cases (47.0±11.3 years) was significantly lower than the mean age of the controls (41.8±10.6 years)(p< 0.001). Similarly, the educational status of the cases was statistically significantly lower than that of the controls (p= 0.005). In both controls and cases, the majority of the people were married (89.1%) (p= 0.966). Cases stating that they had a job outside home were higher than that in the control group and this difference was statistically significant (p= 0.033) (Table 1).

Some of the coronary risk factors were evaluated by case status. Current smoking was more common in cases than in controls. Cases were also more likely than controls to share their houses with at least one smoker. Neither of these differences was statistically significant (Table 2).

After adjusted by gender, no statistically significant association was detected between coronary heart disease and body mass index (kg/m2), or waist/hip ratio (Table 3).

The mean value of serum homocysteine levels of the cases (6.52 μmol/L ±6.38, median= 1.90) was slightly but not significantly higher than those of the control group (6.19 μmol/L ±5.99, median= 1.80) (p= 0.556). The mean value of folic acid levels was higher in cases (6.76 ng/mlt ±3.10, median= 6.10) than that in controls (6.32ng/mlt ±2.54, median= 6.20) but this difference was not statistically significant (p= 0.089). Serum vitamin B12 levels, on the other hand, were statistically sig-

Table 1. Socio-Demographic Characteristics Of The Participants By Case Status

CHARACTERISTICS	CASES Number	%	CONTROLS Number	%	OR (95% CI)	p-value *
Gender						
Male	109	35.9	105	34.9	1.04 (0.74-1.48)	Not significant
Female (ref)	195	64.1	196	65.1	1.00	(matched)
Age group						
25-34 (ref)	94	30.9	58	19.3	1.00	**< 0.001**
35-44	100	32.9	64	21.3	0.96(0.60-1.56)	
45-54	60	19.7	80	26.6	0.46(0.28-0.76)	
55-64	50	16.5	99	32.8	0.31(0.19-0.51)	
Family type						
Nuclear (ref)	220	72.4	196	65.1	1.00	0.147
Extended	78	25.7	96	31.9	0.72(0.50-1.05)	
Separated	1	1.9	1	3.0	0.59(0.18-1.86)	
Educational attainment						
None (ref)	74	24.3	110	36.6	1.00	**0.005**
Primary school graduate	178	58.6	36	12.0	7.35(4.51-12.02)	
Secondary school education and above	52	17.1	28	9.3	2.76(1.55-4.95)	
Marital status						
Married (ref)	271	89.1	268	89.0	1.00	**< 0.001**
Other**	33	10.9	33	11.0	0.99(0.58-1.70)	
Health insurance						
Private (ref)	96	31.6	76	25.2	1.00	**0.181**
Pension fund	31	10.2	49	16.3	0.50(0.28-0.89)	
SIO	113	37.2	118	39.2	0.76(0.50-1.15)	
Bağ-Kur	45	14.8	43	14.3	0.83(0.48-1.43)	
Green card	17	5.6	12	4.0	1.12(0.47-2.68)	
Other	2	0.6	3	1.0	0.53(0.06-4.01)	
Employment status						
Yes	93	30.6	69	22.9	1.48(1.01-2.17)	**0.033**
No (ref)	211	69.4	232	77.1	1.00	
TOTAL	**304**	**100.0**	**301**	**100.0**		

* Chi square test p-value
**Widowed, spouse died

nificantly higher in cases (mean =282.87pg/mlt ±125.96, median= 230.50) than in controls (mean =252.81pg/mlt ±105.59, median= 105.59) (p= 0.005) (Table 4).

Mean values of serum homocysteine, vitamin B-12 and folic acid levels were compared for cases and controls, controlling for age, using covariance analysis. Controlling for age, none of the differences between cases and controls for serum homocyteine, vitamin B-12, and folic acid levels was statistically significant. In logistic regression modeling of age and serum levels of homocysteine, vitamin B-12, and folic acid, no statistically significant association was detected between coronary heart disease and plasma homocysteine, or folic acid levels. However, a positive and significant association was detected between coronary heart disease and vitamin B-12.

DISCUSSION

The disease profile started to change worldwide since the beginning of the 1900s. This trend is more obvious in developed countries compared to developing countries. At the beginning of the last century, the most prevalent mortality and morbidity causes were infectious diseases, but replaced by chronic diseases, coronary heart diseases and degenerative diseases today (1). As the life expectancy at birth increased, the burden of chronic and degenerative diseases increased.

Since 1950, various studies have been conducted about the risk factors of coronary heart diseases. Recently, scientists have been studying on the new independent risk factors. In the Framingham study (Massachusetts, ABD), the relationship between the carotid artery stenosis and the plasma levels of some new risk factors such as folic acid, vitamin B-12, homocysteine were evaluated. This study indicated a negative relationship between blood homocysteine levels and serum folic acid concentrations but no association was found between vitamin B-12 intake and blood homocsyteine levels (11). Scientists continue to conduct various kinds of studies including genetic studies about coronary heart diseases (9, 12, 13). The environmental and genetic factors leading to coronary heart disease are well defined. Studies have shown that hyperhomocysteinemia causes coronary heart diseases due to atheroscle-

Table 2. Selected Personal Characteristics And Some Risk Factors Of The Participants By Case Status

CHARACTERISTICS	CASES (N=304) [a] Number	%	CONTROLS (N=301)[a] Number	%	OR (95% CI)	p-value *
Possession of the house lived in						
Owner (ref)	175	57.6	191	63.5	1.00	0.138
Tenant	129	42.4	110	36.5	1.28(0.91-1.80)	
Owner of any real estate						
Yes (ref)	24	7.9	30	10.0	1.00	0.371
No	280	92.1	271	90.0	0.96(0.83-1.75)	
Has a private car						
Yes (ref)	79	26.0	61	20.3	1.00	0.09
No	225	74.0	241	79.7	0.72(0.48-1.07)	
Employment status						
Yes (ref)	93	30.6	69	22.9	1.00	**0.033**
No	211	69.4	232	77.1	0.68(0.46-0.99)	
Ever smoked						
Yes	139	45.7	125	41.5	1.19(0.85-1.66)	0.298
No (ref)	165	54.3	176	58.5	1.00	
Current smoking status						
Regular smoker	94	67.6	65	52.0	5.30(3.08-9.16)	**0.008**
Smokes occasionally	15	10.8	11	8.8	5.00(1.92-13.17)	
Non-smoker (ref)	30	21.6	110	39.2	1.00	
Any smoker at home						
At least one	193	63.5	166	55.1	1.16(0.84-1.68)	**0.037**
None (ref)	111	36.5	113	44.9	1.00	
Any smoker at home and/or at the office						
At least one	224	73.7	190	63.1	0.43(0.26-0.70)	**0.010**
None (ref)	80	26.3	29	36.9	1.00	
Alcohol consumption						
Currently consuming	27	8.9	14	4.6	2.06(1.01-4.25)	0.078
Consumed in the past	42	13.8	36	12.0	1.25(0.75-2.07)	
Never consumed (ref)	235	77.3	251	83.4	1.00	

[a] Information about some characteristics of cases and/or controls were missing in some analyses. Thus, the total numbers varied.
* Chi square test p-value

rosis and thrombosis by endothelial damage, smooth muscle proliferation and oxidation in the low-density lipoproteins (5, 9). The low plasma levels of vitamin B-12 and folic acid increases the homocsyteine concentration, which in turn might cause arteriosclerosis (6, 12).

Our study has some limitations. One of the most important limitations of this study is about the diagnosis criterion. We determined the CVS diseases according to the ECG findings. However, similar ECG changes might have been due to hypertension, pericardial infections and digital usage as well as myocardial infarction. In a study conducted by Greenland et al., it was reported that minor ST-T abnormalities are common on the resting electrocardiogram of otherwise healthy persons. ST-T segment depression and/or T-wave abnormalities are common in the clinical settings and population studies. Although ST and T-wave findings were routinely considered benign and non-specific, Burch reported in the 1950s that ST-T abnormalities could reflect early signs of coronary heart diseases (14). This should be taken into consideration while interpreting the results. On the other hand, in such a population-based study, ECG seems to be a cost effective method for it is a practical, economic and easy measurement tool. In our study, plasma vitamin B-12 levels of the cases (people with ischemic findings in their ECGs) were statistically significantly higher than the serum levels of the control groups. This unexpected result might be because of the age difference between cases and controls. The mean age of the controls was higher that the mean age of the cases and this difference was statistically significant. Physiologically, serum concentrations of vitamin B12 are lower (due to intrinsic factor) in older ages than in younger ages (Table 5). Age correction in analysis aimed to control for potential confounding due to age difference in cases and controls.

Absence of any ischemic changes in ECG was considered in the study as “absence of coronary disease”. Yet, atherosclerotic process increases with age, even in the absence of clinical coronary heart disease. Inequalities in age between cases and controls could make it more likely that some of the controls

Table 3. Body Mass Index (Bmi) And Waist To Hip Ratio (Whr) Values By Case Status

CHARACTERISTICS	CASES Number	%	CONTROLS Number	%	p-value *
BMI					
Male					
< 25.0	45	43.3	50	46.3	0.573
25.0-29.9	34	32.7	39	36.1	
≥ 30.0	25	24.0	19	17.6	
mean±sd	26.17±4.08		25.81±4.50		0.537†
median (min, max)	25.44 (16.05-38.75)		25.69 (18.56-36.51)		
Female					
< 25.0	23	11.8	31	16.9	0.129
25.0-29.9	56	28.9	58	29.7	
≥ 30.0	113	59.3	84	63.4	
mean±sd	31.10±5.01		30.63±5.52		0.388†
median (min, max)	30.26 (17.83-48.22)		30.84 (17.31-48.05)		
WHR					
Male					
mean±sd	0.91±0.06		0.91±0.05		0.909†
median (min, max)	0.92 (0.78-1.08)		0.92 (0.77-1.05)		
Female					
mean±sd	0.82±0.07		0.81±0.06		0.121†
median (min, max)	0.82 (0.46-0.99)		0.81 (0.66-1.07)		
TOTAL	**304**	**100.0**	**301**	**100.0**	

* Chi square test p-value
† p value for independent samples' t-test

Table 4. Homocystein, Vitamin B12 And Folic Acid Concentrations

CHARACTERISTICS	CASES (N= 304)	CONTROLS (N= 301)	p-value * (%95 G.A.)
Homocysteine			
Number (percent)	247 (% 81.3)	236 (% 78.4)	p= 0.560
mean ± SD	6.52 ± 6.38	6.19 ± 5.99	difference= 0.33
(min, max) median (µmol/L)	(1.10-28.70) 1.80	(1.10-23.72) 1.80	(-0.78-1.44)
Vitamin B_{12}			
number(percent)	244 (% 80.3)	236 (% 78.4)	**p= 0.005**
mean ± SD	282.87 ± 125.96	252.81 ± 105.59	difference= 30.06 (9.18-
(min, max) median (pg/mlt)	(102.00-756.00) 230.50	(100.00-980.00) 253.00	50.95)
Folic Acid			
number(percent)	249 (% 81.9)	236 (% 78.4)	p= 0.089
mean ± SD	6.76 ± 3.10	6.32 ± 2.54	difference= 0.44
(min, max) median (ng/mlt)	(2.20-25.00) 6.10	(0.82-21.00) 6.20	(-0.0067-0.95)

*p value for independent samples' t-test

Table 5. Logistic Regression Model Of Risk Factors Associated With Coronary Heart Diseases

Variables	Beta	Standard error	Wald test value	Degree of freedom	p-value	Relative ratio (95% confidence interval)
Constant	-2.456	0.422	33.931	1	< 0.001	
Homocysteine	-0.001	0.001	3.683	1	0.055	0.999 (0.999-1.000)
Vitamine B-12	0.002	0.001	7.745	1	0.005	1.002 (1.001-1.003)
Folic acid	-0.001	0.001	1.193	1	0.275	0.999 (0.998-1.001)
Age	0.46	0.08	29.870	1	< 0.001	1.047 (1.030-1.064)

might have subclinical coronary heart disease and this might have underestimated the association between coronary heart disease and serum levels of homocsyteine, vitamin B-12, and folic acid (if any).

Cases and controls were selected among the participants of a population-based study. Premature deaths of individuals with coronary heart disease might have prevented inclusion of all CHD cases in the study. A prospective cohort study is a more appropriate design to prevent this kind of bias due to "premature deaths" and "selective survival". Yet they are very expensive and require long terms of follow-up (15).

In Turkey, elderly individuals with certain chronic diseases, in particular, usually prefer to refer to a cardiologist or internal medicine specialist, rather than a general practitioner and they are usually prescribed supplementary drugs as well as disease-specific drugs. The possibility of using some kind of vitamin supplementation (especially vitamin B-12) could be higher in cases than in controls. If this assumption were true, cases would be more likely to have high vitamin B12, and folic acid levels and low homocsyteine concentration. Unfortunately, data were not collected on vitamin supplementation and this issue could not be investigated in the study. We highly recommend collecting information about the vitamin supplementation status of the patients in future studies.

Serum folic acid level was not associated with coronary heart disease whereas, a statistically significant positive association was detected serum vitamin B-12 level and coronary heart disease. Study results suggested a negative association between coronary heart disease and serum homocysteine levels but this association was not statistically significant at alpha= 0.05. A type II error could not be excluded in the study. Also, inability to control for all potential confounders might have concealed the association between serum homocysteine levels and coronary heart disease (if any).

Conclusion: These results do not support the hypothesis that coronary heart disease is related to high serum homocysteine concentration. Yet, there is suggestive evidence of a positive association between coronary hearth disease and serum vitamin B-12 levels. The results are not conclusive due to inability to adequately control for potential confounders. Cohort studies in larger populations are strongly recommended for conclusive results.

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