

Comparison of Vitamin B12 Levels and Cardiovascular Disease Risk Groups

Vitamin B12 Düzeyleri ile Kardiyovasküler Hastalık Risk Gruplarının Karşılaştırılması

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ABSTRACT

Vitamin B12 is a water-soluble vitamin involved in various metabolic processes. It plays a crucial role in the prevention of anemia, the maintenance of healthy neuronal function, and DNA synthesis. Moreover, vitamin B12 acts as a cofactor in homocysteine metabolism. Elevated homocysteine levels have been associated with a range of endothelial alterations and have been linked to cardiovascular diseases (CVD). In Turkey, CVD-related mortality remains the leading cause of death. However, the relationship between vitamin B12 deficiency and the risk of CVD has not yet been clearly established in the academic literature. In this retrospective cross-sectional study, we aimed to compare individuals' vitamin B12 levels across different CVD risk groups. A total of 128 participants were included, with 32 individuals in each risk group. Using the SCORE (Systemic Coronary Risk Evaluation) risk model, we compared vitamin B12 levels across four CVD risk categories. The differences in vitamin B12 levels among the CVD risk groups were not statistically significant ($p=0.209$), and no correlation was found ($p>0.05$). Given that cardiovascular diseases continue to pose a major public health concern, further studies are needed to identify additional parameters that may assist in the identification of high-risk individuals.

Keywords: Heart diseases, Risk assessment, Vitamin B12

ÖZET

Vitamin B12 suda çözünen ve çeşitli metabolik süreçlerin yürütülmesinde kullanılan bir vitamindir. Aneminin önlenmesinde, sinir hücrelerinin fonksiyonlarını sağlıklı sürdürmesinde ve DNA sentezinde önemli görevleri vardır. Ayrıca B12 vitamini homosistein metabolizmasında kofaktör olarak bulunur. Homosistein endotelde bir dizi değişikliklere sebep olup; kardiyovasküler hastalıklarla (KVH) ilişkilendirilmiştir. KVH'lara bağlı ölümler ülkemizde hala tüm ölümler içinde birinci sırada bulunmaktadır. Vitamin B12 eksikliği ile kardiyovasküler hastalık riski arasındaki ilişki akademik alanda henüz netlik kazanmamıştır. Bu retrospektif kesitsel çalışmada kişilerin vitamin B12 düzeyleri ile KVH risk gruplarını karşılaştırmayı amaçladık. Çalışmaya her risk grubunda 32 kişi olacak şekilde toplam 128 kişi alındı. SCORE (Sistemic Coronary Risk Evaluation) risk modeli kullanılarak dört risk grubunun vitamin B12 düzeylerini karşılaştırdık. KVH risk grupları ile vitamin B12 düzeyleri karşılaştırıldığında aradaki fark istatistiksel olarak anlamlı bulunmadı ($p=0.209$) ve aralarında korelasyon saptanmadı ($p>0.05$). Kardiyovasküler hastalıklar günümüzde hala önemli bir halk sağlığı sorunu olduğundan, yüksek riskli bireylerin belirlenmesinde kolaylık ve fayda sağlayacak ek parametreler üzerinde çalışılması gerekmektedir.

Anahtar kelimeler: Kalp hastalıkları, Risk değerlendirmesi, Vitamin B12

INTRODUCTION

Cardiovascular disease-related deaths rank first among all causes of death in our country as well as in the world.¹ Therefore, knowledge of risk factors for cardiovascular diseases and early detection of high-risk individuals are of great importance in preventing mortality and morbidity. Risk factors for cardiovascular diseases can be broadly divided into two groups. These are: non-modifiable and modifiable risk factors. While age, gender, ethnicity and family history are among the non-modifiable risk factors, hypertension, diabetes, obesity, dyslipidemia, smoking and sedentary life are among the modifiable risk factors. Apart from these risk factors, many biomarkers have been reported to increase the risk of cardiovascular disease. High sensitive-CRP, homocysteine, lipoprotein a, myeloperoxidase, phospholipase A2 are just a few of these biomarkers.²⁻⁴ Homocysteine causes a number of changes in the endothelium, which lines blood vessel walls and inner surface.⁵ Some studies suggest homocysteine increases the risk of cardiovascular disease.^{6,7} The fact that vitamin B12 is involved in homocysteine metabolism raises the question of whether there is a relationship between vitamin B12 and cardiovascular disease risk. Whether vitamin B12 deficiency constitutes an independent risk factor for CVD remains a subject of ongoing debate in the scientific community. While there are studies that show that vitamin B12 deficiency is associated with cardiovascular disease,⁸⁻¹⁰ there are studies that say otherwise.^{11,12} Many risk estimation systems have been developed to determine the risk of CVD. The SCORE (Systemic Coronary Risk Evaluation) calculation model is one of the CVD risk estimation systems.¹³ Turkish Society of Endocrine and Metabolism recommends the use of SCORE as a cardiovascular risk calculator.¹⁴ This model estimates the 10-year risk of fatal cardiovascular disease in individuals aged 40 to 70 years who do not have existing heart disease. When determining the risk score, the point at which the necessary data (age, gender, tobacco use status, cholesterol value and systolic blood pressure) are combined is taken as the basis. SCORE has 4 risk groups: Low risk (<1%), Medium risk (1-4%), High risk (5-9%) and Very high risk (≥10%). In this study, it was aimed to compare vitamin B12 levels with CVD risk groups using the SCORE calculation model.

METHODS

Ethical approval

For this research, ethics committee approval was obtained from the Ethics Committee of the Faculty of Medicine, Karadeniz Technical University, dated September 7, 2021, with the reference number 24237859-758.

Data for this cross-sectional study were obtained through retrospective archival research starting from December 31, 2020.

Sample size calculation

The required sample size for this study was calculated using G*Power version 3.1.9.7. Based on a medium effect size ($d=0.3$), an alpha level of 0.05, and a statistical power of 81%, it was determined that a minimum of 32 participants per group would be necessary. Accordingly, the total sample size required was calculated as 128 participants.

Inclusion criteria

- Age between 40 and 70 years (in accordance with the valid age range for the SCORE risk model)
- Documented smoking and alcohol use status in the patient's medical history
- Blood pressure and anthropometric measurements (height and weight) taken during the polyclinic examination
- Laboratory evaluation including complete blood count, lipid panel, vitamin B12, folate, and ferritin levels
- Not currently receiving vitamin B12 treatment

Exclusion criteria

- Individuals with folate deficiency
- Pregnant individuals
- Individuals with a history of cancer or those currently undergoing chemotherapy or radiotherapy for cancer
- Individuals with any known liver disease

SCORE risk calculator

For individuals aged 40 years and older, the SCORE risk calculator estimates the 10-year risk of fatal cardiovascular events. The risk score is determined by locating the value at the intersection of gender, age, smoking status, total cholesterol level, and systolic blood pressure on the SCORE chart. The chart used for risk calculation is presented in Figure 1.

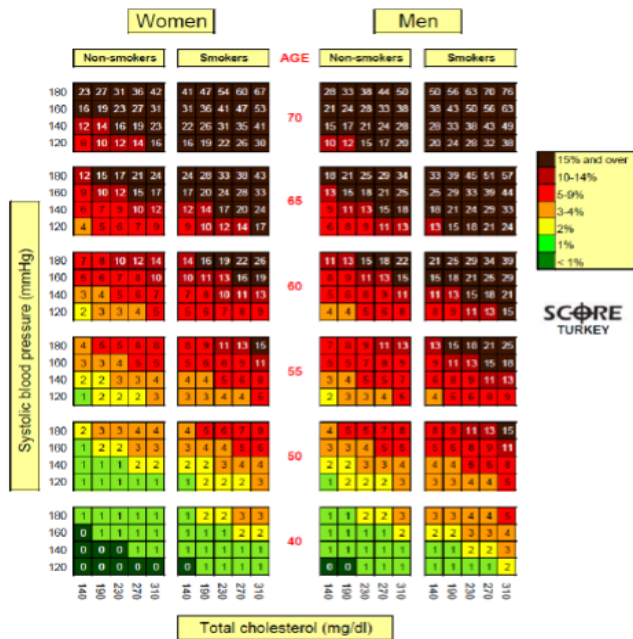


Figure 1. SCORE risk charts for Türkiye showing the 10-year risk of fatal cardiovascular disease based on age, sex, smoking status, systolic blood pressure, and total cholesterol levels

Conducting the study

Hospital records of people aged 40-70 years who met the criteria for admission to the study who applied to our Family Medicine outpatient clinic for any reason were examined retrospectively from 31 December 2020. The values in the first application and first blood analysis of individuals who applied to more than one outpatient clinic and had blood analysis were taken as basis. Individuals with additional illnesses that should be considered high and very high risk in the current situation were placed in the risk group that was suitable for them without the use of the SCORE calculator. SCORE calculator was used for other people. Table 1 shows clinical cases in which individuals are classified as high or very high risk irrespective of the SCORE calculator.

The outcome measure

The main outcome measure of the study is the comparison of vitamin B12 levels with risk groups calculated using SCORE.

Statistical analysis

IBM SPSS for Windows 23.0 statistical package program was used in the analysis of the data. Categorical data are presented as number (n) and percentage (%); quantitative data are presented as mean, standard deviation, median, minimum, maximum and quartile values. Chi-square test was used to compare categorical data.

Table 1. Clinical situations in which individuals would be considered high and very high risk

Very high risk	- Known cardiovascular disease (previous myocardial infarction, acute coronary syndrome etc.) - Presence of cardiovascular disease detected by imaging methods (for example plaque seen on carotid ultrasound) - Patients with diabetes mellitus with complications or diabetes with smoking, hypertension, dyslipidemia - Severe chronic kidney disease
High risk	- Total cholesterol > 310 mg/dl, familial hypercholesterolemia, blood pressure > 180/100 mmHg - Diabetics without complications - Moderate chronic kidney disease

Conformity of the data to the normal distribution was analyzed by Kolmogorov-Smirnov test. The Student t-test or Anova was used to compare data where the distribution was normal. Measurements that did not show normal distribution used either the Mann-Whitney U test or the Kruskal-Wallis test. Bonferroni test was performed in Post Hoc analysis results. Spearman Correlation Analysis was used to assess dependence and relationship. $p < 0.05$ was considered statistically significant.

RESULTS

Of the 128 participants enrolled in the study, 78 (60.9%) were women. The median age (25th–75th percentile) was 50 (45–57) years. A total of 52 participants (40.6%) were obese, and 63 (49.2%) had vitamin B12 deficiency. The distribution of frequency and percentage values according to gender, body mass index, and vitamin B12 classification is presented in Table 2. While 34 (26.6%) of the people in the study smoked, 14 (10.9%) had a history of CVD. 52 (40.6%) of people had a history of dyslipidemia in first-degree family members, while 18 (14.1%) had a history of early CVD in first-degree family members. In addition, 28 (21.9%) of the participants in the study had diabetes and 42 (32.8%) had hypertension. The distribution of factors affecting the cardiovascular disease risk states of people is shown in detail in Table 3.

Table 2. Frequency and percentage values of people according to gender, body mass index and vitamin B12 classification

		n (%)
Gender	Male	50 (39.1)
	Female	78 (60.9)
Body mass index (kg/m²)	Normal (18.5-24.99)	27 (21.1)
	Overweight (25.00-29.99)	49 (38.3)
	Obese (≥ 30.00)	52 (40.6)
Vitamin B12 classification (pg/mL)	Deficiency (<200)	63 (49.2)
	Border (200-300)	43(33.6)
	Normal (>300)	22 (17.2)

Table 4. Comparison of cardiovascular disease risk classes and vitamin B12 classification

	Vitamin B12 classification n (%)			p* Value
CVD risk classes	Deficiency	Border	Normal	
Low and Medium	36 (56.3)	20 (31.3)	8 (12.4)	
High and Very high	27 (42.2)	23 (35.9)	14 (21.9)	0.209

*Pearson Chi-square, n: number, %: percentage, r: Spearman Correlation Analysis

Table 3. Distribution of factors affecting people's CVD risk status

		n (%)
Smoking status	Current smoker	34 (26.6)
	Former smoker	11 (8.6)
	Never smoker	83 (64.8)
Alcohol use status	Current drinker	6 (4.7)
	Former drinker	5 (3.9)
	Never drinker	117 (91.4)
History of CVD	Yes	14 (10.9)
	No	114 (89.1)
Dyslipidemia in the family	Yes	52 (40.6)
	No	76 (59.4)
A family history of CVD	Yes	18 (14.1)
	No	110 (84.9)
Diabetes	Yes	28 (21.9)
	No	100 (78.1)
Hypertension	Yes	42 (32.8)
	No	86 (67.2)
Chronic renal failure	Yes	3 (2.3)
	No	125 (97.7)

Table 5. Comparison of people's age, body mass index and biochemical values according to CVD risk classification

	CVD Risk Classification				P
	Low	Medium	High	Very High	
Age (years)	44 (40-49) ^a	52 (40-60) ^a	55 (40-69) ^a	57 (41-67) ^a	<0.001 ^{c,1}
BMI (kg/m²)	27.5 (20.2-37.5) ^a	29.3 (21.8-46.0) ^a	27.0 (21.7-39.0) ^a	30.3 (22.1-62.6) ^a	0.142 ^c
Vit.B12 (pg/mL)	183 (87-587) ^a	193 (89-370) ^a	241 (123-660) ^a	183 (108-1134) ^a	0.036 ^{c,2}
Folate (ng/mL)	8.82 (5.39-17.49) ^a	7.29 (4.58-17.08) ^a	9.58 (4.62-24.00) ^a	8.16 (4.60-24.00) ^a	0.342 ^c
Ferritin (ml/ng)	18.1 (2.4-138.2) ^a	49.8 (4.7-1021.9) ^a	55.7 (3.7-558.6) ^a	60.5 (0.8-604.2) ^a	0.009 ^{c,3}
T. Cholesterol (mg/dL)	207 $\pm$ 37 ^b	225 $\pm$ 43 ^b	242 $\pm$ 57 ^b	218 $\pm$ 44 ^b	0.021 ^{d,4}
HDL (mg/dL)	53 (27-80) ^a	49 (31-73) ^a	54 (37-87) ^a	47 (32-73) ^a	0.039 ^{c,5}
LDL (mg/dL)	128 $\pm$ 30 ^b	144 $\pm$ 34 ^b	158 $\pm$ 47 ^b	139 $\pm$ 34 ^b	0.014 ^{d,6}
Triglyceride (mg/dL)	103 (47-316) ^a	134 (35-411) ^a	115 (49-514) ^a	122 (53-739) ^a	0.182 ^c
Glucose (mg/dL)	91 (75-144) ^a	94 (61-113) ^a	96 (74-251) ^a	103 (85-561) ^a	0.001 ^{c,7}
Hemoglobin (g/dL)	13.4 (8.4-15.9) ^a	13.9 (9.6-17.3) ^a	13.8 (10.5-17.7) ^a	14.3 (10.9-17.5) ^a	0.055 ^c

^aMedian (minimum-maximum), ^bMean $\pm$ standard deviation, ^cKruskal Wallis H Test, ^dOne-Way ANOVA

1. Significant differences were observed between the low and medium (p<0.001), low and high (p<0.001), and low and very high risk groups (p<0.001).

2. Significant differences were observed between the low and high risk groups (p=0.010), the medium and high risk groups (p=0.029), and the high and very high risk groups (p=0.020).

3. Significant differences were observed between the low and medium (p=0.035) and the low and very high risk groups (p=0.015).

4. A significant difference was observed between the low and high risk groups (p=0.015).

5. Significant differences were observed between the low and medium (p=0.047), the low and very high (p=0.044), the medium and high (p=0.038), and the high and very high risk groups (p=0.035).

6. A significant difference was observed between the low and high risk groups (p=0.009).

7. Significant differences were observed between the low and very high (p=0.001) and the medium and very high risk groups (p=0.005).

While 27 (42.2%) of the people in the group with high and very high risk of cardiovascular disease had vitamin B12 deficiency; Vitamin B12 levels were found to be deficient in 36 (56.3%) of people in the low and medium risk groups in the study. When comparing CVD risk classification with vitamin B12 classification, no statistically dependent relationship was found between them ($r=0.155$ $p>0.05$), (Table 4). With cardiovascular disease risk classification of the people in the study, age ($p<0.001$), vitamin B12 level ($p=0.036$), ferritin ($p=0.009$), cholesterol ($p=0.021$), HDL ($p=0.039$), LDL ($p=0.014$), and blood glucose ($p=0.001$), were found to be statistically significant when the difference was compared; BMI, folate, triglycerides and hemoglobin were compared, the difference was not statistically significant ($p>0.05$), (Table 5).

DISCUSSION

In the study conducted by Mahalle et al. on 300 Indian individuals with coronary artery disease, it was concluded that vitamin B12 was significantly low in patients with diabetes, hypertension and dyslipidemia, and that serum vitamin B12 deficiency and hyperhomocysteinemia were associated with cardiovascular risk factors in these patients.⁸ In a systematic review by Rafnsson et al. in which they examined seven prospective cohort studies, it was concluded that there was insufficient evidence that vitamin B12 deficiency causes cardiovascular diseases in adults.¹¹ Another meta-analysis concluded that there was a positive association between vitamin B12 elevation and cardiovascular mortality rate.¹⁵ A cohort study of 7770 people found that low and high vitamin B12 levels were associated with a higher risk of death from cardiovascular disease in people with type 2 diabetes.¹⁶ In our study, when cardiovascular disease risk groups and vitamin B12 levels were compared, no statistically significant correlation was found between them. When the results of our study are evaluated together with the existing literature, it is evident that the relationship between vitamin B12 levels and cardiovascular disease risk remains inconclusive. In this context, further large-scale and comprehensive studies involving a greater number of patients are needed to clarify this association more definitively. Age is an independent risk factor for CVDs. Advanced age increases the risk of cardiovascular disease.¹⁷ Because age is a predictor of SCORE, as expected in our study, cardiovascular disease risk levels also increased as age increased. 78 (60.9%) of the people in the study were

women. This situation can be explained by the fact that more women apply to our clinic. 52 (40.6%) of the total 128 people were obese. In the 2019 Turkey Diabetes, Hypertension, Obesity and Endocrinological Diseases Prevalence Study (TURDEP-2), the prevalence of obesity in Turkey was found to be 31.2%.¹⁸ The high obesity rate in our study may be due to the fact that we also provide weight management consultancy to obese individuals in our outpatient clinics. In the study conducted by Gebara et al.¹⁹ in 2023, where they compared SCORE and unconventional risk factors, a positive relationship was observed between SCORE and BMI; however, no relationship was observed between SCORE and sociodemographic status and family history. In our study, no significant difference was found between SCORE risk levels and BMI average. In another study investigating whether the addition of other risk markers to the SCORE algorithm improved cardiovascular disease risk stratification, it was found that although 15 of the risk factors were independent risk factors for mortality, none of them had a significant effect on cardiovascular disease risk stratification.²⁰ The pathogenesis of cardiovascular diseases is multifactorial. However, it can be said that the evidence available in the literature is not yet sufficient for the addition of other risk factors to the SCORE risk assessment. Hypertension is one of the most important causes of cardiovascular disease. In a study conducted in our country in 2003, the prevalence of hypertension was found to be 31.8%.²¹ In the 2nd version of the same study conducted in 2012, this rate was found to be 30.3%;²² the number of patients diagnosed with hypertension in our study was 32.8%, which is similar to the literature. In the academic field, hypertension due to vitamin B12 deficiency is at the case level and the level of evidence is low.²³ Today, the main problem with hypertension in our country, as in the world, is still the lack of early detection of hypertension, the level of awareness of patients and the inability to control hypertension. There are conflicting results regarding the role of ferritin in coronary heart disease. It has been suggested that iron can increase the risk of heart diseases through lipid peroxidation by catalyzing the production of free radicals. A retrospective cohort study conducted in 2023 demonstrated that elevated ferritin levels were associated with increased short-term mortality among critically ill patients with heart failure.²⁴ In another study, a negative correlation was found between ferritin level and myocardial

infarction.²⁵ In our study, it was observed that the median value of serum ferritin increased as the cardiovascular disease risk level increased. These findings suggest that the relationship between serum ferritin and cardiovascular risk deserves further investigation in larger prospective studies. As it is known, SCORE2 was published as a new algorithm by the European society of cardiology in January 2021. The study found that SCORE2 facilitated the identification of individuals with high cardiovascular risk in the European population.²⁶ In a study comparing SCORE and SCORE2 in cardiovascular risk assessment, it was found that a significant portion of those in the low risk group with SCORE were in the high risk group with SCORE2.²⁷ In the comparison made in the East Asian population, it was concluded that SCORE 2 showed improvement compared to SCORE, but modifications were also needed.²⁸ Considering that Turkey is located in the European region, it may be more useful to use the new algorithm SCORE2 to identify high-risk individuals. One of the limitations of this study may be considered as why we used the SCORE risk calculator and not the SCORE 2 risk calculator. Since our study was also a thesis study, SCORE 2 was not published during the thesis writing period. In the article period, as authors, we decided to use the SCORE risk calculator to avoid any difference between the thesis and the article. Another limitation of this study is its retrospective design, single-center nature, and limited sample size. Furthermore, the lack of data on potential confounding factors such as dietary intake and homocysteine levels may have influenced the findings.

CONCLUSION

Knowing the risk factors for cardiovascular diseases and early detection of risky individuals are of great importance. For this reason, we think that it would be beneficial to widespread use of the SCORE/SCORE2 risk calculator, which is recommended for use in our country, at all levels of the healthcare system, especially primary care. In our study, venous blood vitamin B12 levels and cardiovascular disease risk groups were compared and no statistically dependent relationship was found between them. Since cardiovascular diseases are still an important public health problem today, additional parameters that will provide convenience and benefit in identifying high-risk individuals need to be studied.

Acknowledgement

We would like to express our sincere gratitude to Prof. Dr. Turan Set, Head of the Department at Karadeniz Technical University, for his invaluable guidance, support, and contributions throughout this study.

Authorship contribution statement

Consept and desing: EA, MG.

Acquisition of data: MG.

Analysis and interpretation of data: MG.

Drafting of the manuscript: MG.

Critical revision of the manuscript for important intellectual content: EA, MG.

Statistical analysis: MG.

Declaration of competing interest: None of the authors have potential conflicts of interest to be disclosed.

Ethical approval: For this research, ethics committee approval was obtained from the Ethics Committee of the Faculty of Medicine, Karadeniz Technical University, dated September 7, 2021, with the reference number 24237859-758.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Funding: No financial support was received for this research.

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- To Cite:** Guner M, Ates E. Comparison of Vitamin B12 Levels and Cardiovascular Disease Risk Groups. *Farabi Med J.* 2025;4(3):73-79.