***Smyrna Tıp Dergisi*** ***Araştırma Makalesi***

# The Role of Homocystein and Lipoprotein-A in the Formation of Stones in the Gallbladder
# Safra Kesesi Taşı Oluşumunda Homosistein ve Lipoprotein-A'nın Rolü

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**Summary**
**Objective:** Gallstone disease is a major health problem worldwide. Specific dietary contents as potential risk factors for gallstone formation in humans have been examined in several studies. In this study it was aimed to elucidate the relationship between type of gallstones and plasma homocysteine and lipoprotein A levels.
**Material and Method:** A total of 48 patients were included in this study. To compare the clinical and biochemical parameters of these patients, 47 persons were selected to serve as a control group. Age, gender, height, weight, weight change in the past year, number of children,, family history of gallstone disease, smoking and alcohol use, status of physical activity, comorbid conditions and drug use were all investigated. Biochemical analysis of blood samples of the patients in the two groups and chemical analysis of gallstones obtained from the operated patients were performed.
**Results:** Of the 48 patients in the study group, 85.4% were female. Mean age was 45.7±13.0. When, the groups were compared with regard to height, weight, body mass index, weight change and number of children, no statistically significant differences were found. Family history of gallstone disease was more frequent in the study group. Among the gallstones obtained from the patients, 85.4% were cholesterol stones, while the remaining 14.6% were found to be among the "other" stone group. When homocysteine and lipoprotein A levels with cholesterol stones and other stones were compared, no statistically significant difference was observed.
**Conclusion:** We have found no relationship between the formation and type of gallstones and serum homocysteine and lipoprotein A levels.
**Key words:** Gallstone, homocysteine, lipoprotein-A

**Özet**
**Amaç:** Safra taşı hastalığı dünya çapında önemli bir sağlık sorunudur. İnsanlarda safra taşı oluşumu için potansiyel risk faktörleri olarak belirli diyet içerikleri çeşitli çalışmalarda incelenmiştir. Bu çalışmada safra taşı tipi ile plazma homosistein ve lipoprotein A düzeyleri arasındaki ilişkiyi ortaya koymak amaçlanmıştır.
**Gereç ve Yöntem:** Bu çalışmaya toplam 48 hasta dahil edildi. Bu hastaların klinik ve biyokimyasal parametrelerini karşılaştırmak için 47 kişi kontrol grubu olarak seçildi. Yaş, cinsiyet, boy, kilo, son bir yıldaki kilo değişimi, çocuk sayısı, ailede safra taşı hastalığı öyküsü, sigara ve alkol kullanımı, fiziksel aktivite durumu, komorbid durumlar ve ilaç kullanımı araştırıldı. İki gruptaki hastaların kan örneklerinin biyokimyasal analizi ve opere olan hastalardan alınan safra taşlarının kimyasal analizi yapıldı.
**Bulgular:** Çalışma grubundaki 48 hastanın %85,4'ü kadındı. Genel olarak yaş ortalaması 45,7±13,0 idi.. Gruplar boy, kilo, vücut kitle indeksi, kilo değişimi ve çocuk sayısı açısından karşılaştırıldığında istatistiksel olarak anlamlı fark bulunmadı. Ailede safra taşı hastalığı öyküsü çalışma grubunda daha sıktı. Hastalardan elde edilen safra taşlarının %85,4'ünün kolesterol taşları olduğu, kalan %14,6'sının ise "diğer" taş grubundan olduğu tespit edildi. Kolesterol taşı ve diğer taşları olan hastalarda homosistein ve lipoprotein A düzeyleri karşılaştırıldığında istatistiksel olarak anlamlı fark gözlenmedi.
**Sonuç:** Safra taşlarının oluşumu ve tipi ile serum homosistein ve lipoprotein A düzeyleri arasında ilişki bulunamadı.
**Anahtar kelimeler:** Safra taşı, homosistein, lipoprotein-A

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## Introduction

Gallstone disease is an important health problem all over the world. In ultrasonography studies, its prevalence in industrialized countries was found to be 10-15% (1,2). While 60-80% of people with gallstones remain asymptomatic throughout their lives, the probability of developing stone-related symptoms after diagnosis is 2-4% (2,3). There are non-modifiable and modified risk factors for gallstone formation. Non-modifiable risk factors include female gender and age, as well as genetic factors such as ethnicity and family factors. Obesity, rapid weight loss, hypertriglyceridemia, slow intestinal transit, high-calorie diet, fiber content in the diet, smoking and sedentary life can be counted as modifiable or environmental factors. Regardless of the overall prevalence of gallstones, the incidence of gallstones in all populations is almost twice as high in women as in men. Epidemiological studies show that the prevalence of gallstones increases with increasing age (4).

In order to determine the risk factors for gallstones, the pathogenesis of gallstones should be known exactly. Cholesterol stones are formed due to abnormalities in cholesterol metabolism. Three types of abnormalities have been identified that are responsible for cholesterol gallstone formation. The most basic of these is the supersaturation of bile in terms of cholesterol. Cholesterol saturation of bile is evaluated by the ratios of cholesterol, bile acids and phospholipids, the three main lipids in bile. Another factor that plays a role in gallstone formation is an increase in the nucleation of cholesterol crystals. The third factor is stasis of the gallbladder (4).

There are many studies examining whether specific dietary ingredients are a potential risk factor for gallstone formation in humans. The effects of fatty acids, cholesterol, fibers, carbohydrates, alcohol, some vitamins and minerals on gallstone formation have been investigated. However, conflicting results were obtained in these studies. There are factors that limit the standardization of these studies. Gallstones are usually asymptomatic and take several years to form. This characteristic of gallstones makes it difficult to know the time of onset of the disease and therefore to evaluate environmental factors such as diet in gallstone formation. In addition, it is difficult to follow the energy intake and diet practices of the patients during the prospective studies (5).

While there are studies investigating the effects of the main nutrients in the diet on the formation of gallstones, there are also studies investigating the effects of minor nutrients. In a study investigating the correlation of known coronary risk factors; it has been found that the presence of gallstones is associated with total homocysteine level, but not with total cholesterol, triglyceride, gamma glutamyl transferase, glucose or folate (6). However; a disadvantage of this study is that gallstones were not typified. In addition, since the study was conducted only in middle-aged men, it would not be correct to generalize the relationship between homocysteine and gallstones based on the data of this study (6). In a study by Worthington et al. (7), no relationship was found between the total homocysteine level and the presence of gallstones, and it was reported that there was a weak reverse relationship. Considering the studies above (6,7), the relationship between homocysteine and gallstone disease provided conflicting results and gallstones were not classified in these studies.

In this study, it was tried to elucidate whether the type of gallstones and plasma homocysteine and lipoprotein-A levels are associated.

## Material and Method

Forty eight patients who applied to the 4$^{th}$ and 5$^{th}$ general surgery outpatient clinics of Ankara Training and Research Hospital with the diagnosis of gallstone disease were enrolled. The study has been approved by the ethics committee and conducted according to the Guidelines of the Declaration of Helsinki.

A control group of 47 people was also included in the study. The presence of gallstone was excluded by performing abdominal ultrasonography in the patients in the control group. Those with malignant disease and a previous history of cholecystectomy were not included in the study.

Patients' age, gender, height, weight, weight change in the last year, number of children in female patients, family history of stones, smoking and alcohol use, physical activity level, presence of concomitant chronic disease, and drug use were questioned. In evaluating the nutritional

habits of the patients; The amount of weekly vegetable, meat and fat consumption were questioned.

Blood analysis of the patients and control groups, has been routinely conducted. In the study group, blood was drawn before the operation. Blood samples were stored at -80°C until the study day. Triglyceride, cholesterol, HDL cholesterol, ALP and GGT tests have been processed via Olympus AU 2700 analyzer using original Olympus kits; homocysteine in the Agilent 1100 HPLC system with the commercial Chromosystem kit, Apo-A1, Apo-B and lipoprotein-A measurements are on Beckman Coulter Immage Immunochemistry analyzer with original Beckman Coulter kits, Vitamin B12 and folic acid tests were measured on a Unicell DxI 800 analyzer using commercial original Beckman Coulter kits and LDL cholesterol was analyzed by the Friedewald formula by proportioning Cholesterol/HDL to each other.

The stones taken during the surgery were analyzed at the Mineral Research and Exploration (MTA) Institution. The stones were pulverized and XRD analysis was performed between 2-40˚ with the Philips PW 3710/1830 XRD analyzer with Cu X-ray tube.

### *Statistical Analysis*

Data analysis was conducted via SPSS 11.5 package program. Whether the distribution of continuous-measure variables was normal or not was investigated using the Shapiro Wilk test. Descriptive statistics were presented as mean±standard deviation or median for continuous-measure variables, and number of observations and (%) for nominal variables. Student's t-test was used to determine the statistically difference between the groups in terms of means and median values was examined with the Mann Whitney U test. Nominal variables were evaluated with Pearson's Chi-Square or Fisher's Exact Probability test. A value of $p<0.05$, was considered statistically significant.

## Results

Of the 95 people included in the study, 26 (55.3%) of the control group were female and 21 (44.7%) were male; In the control group, 41 (85.4%) were female and 7 (14.6%) were male. The overall mean age was 45.7±13.0; It was 46.2±13.1 in the control group and 45.2±13.0 in the case group.

When the subjects included in the study were compared in terms of height, weight, body mass index, weight change and the number of children; it was observed that there was no statistical difference between the groups. Distribution of patient characteristics in groups were summarised in table.1 (Table.1). The history of gallstones in the family was higher in the study group compared to the control group ($p=0.002$).

Weekly use of vegetables, red meat, margarine and other fats was not statistically different between the groups ($p=0.303$, 0.606, 0.660 and 0.604). The consumption of white meat, olive oil and butter was statistically significantly higher in the study group than in the control group ($p<0.001$, 0.040 and $<0.001$) (Table 2). There was no statistical difference between the groups in terms of smoking, alcohol intake, drug use and chronic disease anamnesis.

When the patients were compared in terms of biochemical analysis; cholesterol, high-density lipoprotein and Apo A levels were found to be statistically significantly higher in the control group compared to the study group ($p=0.007$,$<0.001$ and $<0.001$). There was no statistically significant difference fort he other parameters between the study and control groups (Table 3).

Cases were divided into two groups as “cholesterol stone” and “other stones” according to stone types. While 85.4% (n=41) of the stones taken from the patients were cholesterol stones, 14.6% (n=7) constituted the “other” stone group. There was a statistically significant difference between the groups in terms of mean age ($p=0.016$). The mean age of the group with cholesterol stones (44.0±12.1) was lower than that of the “other” stone group (57.2±11.7). There was no statistically significant difference between the groups in terms of homocysteine and lipoprotein-A levels, gender distribution, mean body mass index, eating habits, smoking, alcohol, drug use, and comorbid diseases ($p>0.05$) (Table 4).

***Table 1.*** *Distribution of patient characteristics in groups*

| Variables | Control Group (n=47) | Study Group (n=48) | P Value |
|---|---|---|---|
| **Height (cm)** | 165.1±8.04 | 162.5±6.57 | 0.089 |
| **Weight )kg)** | 76.0±12.57 | 71.4±13.43 | 0.085 |
| **BMI (kg/m$^2$)** | 28.0±5.0 | 27.1±5.3 | 0.387 |
| **Weight Change (kg)** | 0 (-11 – 10) | 0 (-20 – 15) | 0.247 |
| **Family Stone History** | 11 (%23.4) | 26 (%54.2) | 0.002 |
| **Number of children** | 3±2 (1-9) | 2±2 (0-8) | 0.172 |
| **Systolic Blood Pressure (mmHg)** | 124.7±18.2 | 120.8±11.8 | 0.434 |
| **Diastolic Blood Pressure (mmHg)** | 79.4±9.6 | 72.5±8.1 | <0.001 |

***Table 2.*** *Distribution of dietary habits in groups*

| Variables | Control Group (n=47) | Study Group (n=48) | P Value |
|---|---|---|---|
| **Vegetable** | 4±2 (0-7) | 4±2 (2-7) | 0.303 |
| **Red Meat** | 2±1 (0-5) | 2±1 (0-7) | 0.606 |
| **White Meat** | 1±1 (0-5) | 3±2 (0-7) | <0.001 |
| **Olive Oil** | 3 (0-7) | 4 (0-7) | 0.040 |
| **Margarine** | 2 (0-7) | 2 (0-7) | 0.660 |
| **Butter** | 1 (0-5) | 2 (0-7) | <0.001 |
| **Other** | 4 (0-7) | 4 (0-7) | <0.001 |

***Table 3.*** *Biochemical parameters*

| Variables | Control Group (n=47) | Study Group (n=48) | P Value |
|---|---|---|---|
| Glucose (mg/dl) | 91 (65-173) | 107 (57-234) | 0.089 |
| Triglyceride (mg/dl) | 117 (113-294) | 152 (104-259) | 0.757 |
| ALP (U/L) | 73 (48-242) | 71 (17-308) | 0.620 |
| GGT (U/L) | 22 (11-148) | 26 (7-621) | 0.258 |
| Vitamin B12 (pg/ml) | 171 (80-1500) | 205.5 (76-1175) | 0.090 |
| Folic acid (ng/ml) | 5.7 (3-13) | 7.0 (1-20) | 0.123 |
| Homocysteine (umol/L) | 9.5 (3.7-18.6) | 101. (4.8-24.1) | 0.104 |
| Lipoprotein A (mg/L) | 198 (23-1400) | 157 (23-1910) | 0.732 |
| Apo A/B (mg/L) | 1.9±0.83 | 1.7±0.74 | 0.092 |
| Apo B/A (mg/L) | 0.6±0.23 | 0.7±0.23 | 0.096 |
| **Total Cholesterol** (mg/L) | 180.3±37.9 | 159.4±35.8 | 0.007 |
| **HDL** (mg/L) | 46.7±9.9 | 40.4±7.9 | <0.001 |
| **LDL** (mg/L) | 106.0±26.8 | 95.5±31.0 | 0.086 |
| **Total Cholesterol /HDL** (mg/L) | 3.98±0.98 | 4.07±1.02 | 0.669 |
| **Apo A** (mg/L) | 1799.8±365.87 | 1510.4±246.25 | <0.001 |
| **Apo B** (mg/L) | 1036.0±347.32 | 1013.2±312.80 | 0.738 |

## Discussion

In a study investigating the content of gallstones removed by cholecystectomy and comparing gallstone types with demographic characteristics, female gender and higher body mass index were found to be associated with cholesterol gallstones (8). Gallstones show heterogeneity in terms of their contents and pathogenesis. It is accepted that the increase in risk factors, especially due to lifestyle, increases the formation of cholesterol gallstones (9). However, in the study of Schafmayer et al. (8) no relationship was found between the factors

***Table 4.*** *Distribution of patient characteristics by gallstone type*

| Variables | Control group (n=47) | Study group (n=48) | P-value |
|---|---|---|---|
| **Gender** | | | >0.05 |
| Male | 6 | 1 | |
| Female | 35 | 6 | |
| **BMI (kg/m$^2$)** | 27.2±5.4 | 26.4±5.8 | >0.05 |
| **Smoking** | | | >0.05 |
| No | 26 | 5 | |
| Quit | 5 | 1 | |
| Yes | 10 | 1 | |
| **Alcohol Consumption** | | | >0.05 |
| No | 35 | 5 | |
| Quit | 3 | 1 | |
| Yes | 3 | 1 | |
| **Drug Usage** | | | >0.005 |
| Yes | 16 | 4 | |
| No | 25 | 3 | |
| **Comorbid Disease** | | | >0.05 |
| Yes | 24 | 3 | |
| No | 17 | 4 | |

other than gender and body mass index and the type of gallstone. In the presented study, 85.4% (n=41) of the stones taken from the patients were cholesterol stones, while 14.6% (n=7) constituted the "other" stone group. There was a statistically significant difference between the groups in terms of mean age (p=0.016). The mean age of the group with cholesterol stones (44.0±12.1) was lower than that of the “other” stone group (57.2±11.7).

High-fiber foods in the form of wheat bran reduce the cholesterol saturation of bile. Fibrous foods has a protective effect against cholesterol gallstones by increasing the intestinal transit time and thus reducing the deoxycholate in the bile. An inverse association has been found between a vegetarian diet and the risk of gallstones (9). Although many studies have been published on the relationship between diet and gallstone disease, it has not been determined whether changing the diet content changes the risk of the disease. The two main reasons for the differences in study results are the problems in defining the disease and the difficulties in measuring the dietary content. In studies on gallbladder disease; diagnosing the disease is difficult, as up to two-thirds of cases with gallstones are asymptomatic. Therefore, it is difficult to compare the factors affecting stone development and clinical disease progression (10).

Studies investigating the effects of dietary factors on gallstone formation have focused on energy intake, fatty acids, cholesterol, refined carbohydrates and fiber foods, and calcium and alcohol intake. Although there are clear differences between the results of the studies, and an evaluation is made, in general no strong correlation could be demonstrated between energy intake or fat or cholesterol intake, and gallstone formation. It was shown that there was an inverse relationship between simple sugars, and an inverse relationship between fibrous food and alcohol intake and gallstone formation. There aren’t sufficient number of studies to reach a general conclusion about fish oil, calcium, and antioxidants (10,11).

In a study by Worthington et al. (7), no relationship was found between the total homocysteine level and the presence of gallstones, and it was reported that there was a weak reverse relationship. This inconsistency between the study of Worthington et al. (7) and the study of Sakuta and Suzuki (6) could be explained by the difference between the groups selected for the study. Studies have shown that total homocysteine levels were higher in older age and male gender. Sakuta and Suzuki's study (6) was conducted in middle-aged men, while Worthington et al. (7) included both men and women in a wider age range.

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Accordingly, the relationship between total homocysteine levels and the presence of gallstones was interpreted differently in these two studies. In the study of Worthington et al. (7), homocysteine and metabolic pathways were emphasized. Homocysteine metabolism is a process of transsulfuration or remethylation. Remethylation process requires vitamin B12 as a coenzyme and methyltetrahydrofolate as a cofactor. The transsulfuration of homocysteine involves vitamin B6 dependent reactions. Therefore in cases where folate, vitamin B6 or vitamin B12 are deficient, hyperhomocysteinemia can easily develop (12,13,14). In the study of Worthington et al. (7) folate and vitamin B6 levels were lower than normal, while the homocysteine/methionine ratio was found to be higher than the control group, indicating less remethylation in patients with cholesterol stones. However, homocysteine levels were not found to be high in these individuals. Although there seemed to be a contradiction in these results, the low plasma methionine and cysteine concentrations due to decreased intake supported these findings. Despite these explanations, more research is needed to determine the stage at which antioxidant deficiency plays a role in gallstone formation or whether it is an effective factor in stones other than cholesterol stones (7).

Hyperhomocysteinemia is associated with oxidative stress in the vasculature. This link is thought to be due to impaired glutathione homeostasis. Homocysteine is a sülfür containing amino acid formed during the metabolism of methionine. When this amino acid is synthesized, it undergoes remethylation to be converted into methionine, catalyzed by the enzyme methionine synthetase. The enzyme methionine synthetase uses methyltetrahydrofolate as a methyl donor and cobalamin (vitamin B12) as a cofactor. As an alternaive homocysteine undergoes transsulfuration to form glutathione, and vitamin B6 plays a role in this process (15).

Hyperhomocysteinemia is closely related to lifestyle and especially dietary habits. Although a direct link between such genetic disorders and gallstone disease has not been demonstrated, the homocysteine/methionine ratio was found to be higher in patients with gallstones than in patients without gallstones. However, a cause-effect relationship between hyperhomocysteinemia and gallstone disease has not yet been demonstrated (16).

The underlying mechanism of the relationship between hyperhomocysteinemia and gallstone disease is not fully understood. The main causes of gallstone formation are metabolic abnormalities and physicochemical events. Gallstones cause inflammatory changes in the gallbladder mucosa. However; whether this inflammation is secondary to gallstone formation or whether inflammation in the gallbladder wall leads to the formation of precipitates and crystals, which is the initial step in gallstone formation. A defect in homocysteine metabolism increases oxidative stress on the gallbladder, as homocysteine is metabolized by transsulfuration to the antioxidant glutathione (17). Clinically, the finding that antioxidant intake reduces the prevalence of gallstones is consistent with the hypothesis that oxidative stress is one of the risk factors for gallstone formation (18). In addition, reactive oxygen metabolites have been found to cause the formation of cholesterol crystal nuclei (19). However; Since there is a mutual interaction of pro-nuclear and anti-nuclear factors in the formation of cholesterol stone nuclei in vivo, it would not be correct to generalize in the light of the results of this in vitro study. Oxidative stress occurs due to the balance between oxidants and antioxidants. However, there is still debate about whether hyperhomocysteinemia is due to gallstones or causes gallstone formation. In addition, it has been reported that hyperhomocysteinemia accompanying gallstone disease may be a risk factor for gallbladder cancer (17). Because ongoing oxidative stress can cause carcinogenic effects by causing DNA damage. Although there is no direct data on gallbladder cancer; High homocysteine levels have been reported in some cancer types such as breast, ovarian and pancreatic cancers (20).

## Conclusion

In this study, no relationship between the formation and type of gallstones and serum homocysteine and lipoprotein A levels was found. However, considering the implementation and follow-up difficulties in studies investigating the link between gallstones and diet, it would be appropriate to investigate diet-gallstone relationships in larger series.

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