HİTİT MEDICAL JOURNAL
HİTİT ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

e-ISSN: 2687-4717 Cilt|Volume: 6 • Sayı|Issue: 2 - Haziran|June 2024

The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamin D, Lipid Profile, Insulin Resistance and Uric Acid Levels

Safra Taşı Hastalığının Serum RBP4 Düzeyi, D Vitamini, Lipid Profili, İnsülin Direnci ve Ürik Asit Düzeyleri ile İlişkisi

Bediz Kurt İnci[1] | Kamil İnci[1] | Özge Tuğçe Paşaoğlu[2] | Hatice Paşaoğlu[2] Gıyasettin Şükrü Dumlu[1]

[1]Gazi University Faculty of Medicine, Department of Internal Medicine, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department of Biochemistry, Ankara, Türkiye

Sorumlu Yazar | Correspondence Author
Bediz Kurt Inci
bedizkurt@gmail.com
Address for Correspondence: Dr. Abdurrahman Yurtaslan Ankara Onkoloji Hastanesi, Demetevler /Ankara.

Makale Bilgisi | Article Information
Makale Türü | Article Type: Araştırma Makalesi | Research Article
Doi: https://doi.org/10.52827/hititmedj.1424453
Geliş Tarihi | Received: 24.01.2024
Kabul Tarihi | Accepted: 01.05.2024
Yayım Tarihi | Published: 30.06.2024

Atıf | Cite As
İnci BK, İnci K, Paşaoğlu ÖT, Paşaoğlu H, Dumlu GŞ. The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamın D, Lipid Profile, Insulin Resistance and Uric Acid Levels. Hitit Medical Journal 2024;6(2):161-169 https://doi.org/10.52827/hititmedj.1424453

Hakem Değerlendirmesi: Alan editörü tarafından atanan en az iki farklı kurumda çalışan bağımsız hakemler tarafından değerlendirilmiştir.
Etik Beyanı: Çalışma Gazi Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 26/05/2014 tarihinde onaylanmıştır (Karar No:278).
İntihal Kontrolleri: Evet - (Intihal.net)
Çıkar Çatışması: Yazarlar çalışma ile ilgili çıkar çatışması beyan etmemiştir.
Şikayetler: hmj@hitit.edu.tr
Katkı Beyanı: Fikir/Hipotez: BKİ, GŞD Tasarım: BKİ Veri Toplama/Veri İşleme: BKİ, Kİ, ÖTP Veri Analizi: ÖTP, HP, BKİ Makalenin Hazırlanması: BKİ, Kİ.
Hasta Onamı: Çalışmada kullanılan hasta verileri için hastalardan bilgilendirilmiş onam alınmıştır.
Finansal Destek: Finansal destek alınmamıştır.
Telif Hakı & Lisans: Dergi ile yayın yapan yazarlar, CC BY-NC 4.0 kapsamında lisanslanan çalışmalarının telif hakkını elinde tutar.

Peer Review: Evaluated by independent reviewers working in the at least two different institutions appointed by the field editor.
Ethical Statement: Ethical approval was received from Gazi University Clinical Research Ethics Committee on 25/5/2014 (No: 278).
Plagiarism Check: Yes - (Intihal.net)
Conflict of Interest: The authors declared that, there are no conflicts in interest
Complaints: hmj@hitit.edu.tr
Authorship Contribution: Idea/Hypothesis: BKİ, GŞD Design: BKİ Data Collection/Data Processing: BKİ, Kİ, ÖTP Data Analysis: ÖTP, HP, BKİ Article Preparation: BKİ, Kİ.
Informed Consent: Informed consent was obtained from the patients for the patient data used in the study.
Financial Disclosure: There are no financial funds for this article.

The Relationship of Gallstone Disease with Serum RBP4 Level, Vitamin D, Lipid Profile, Insulin Resistance, and Uric Acid Levels

ABSTRACT

Objective: The metabolic parameters associated with gallstones are the subject of numerous studies. RBP4, an adipokine, has been linked to various metabolic diseases; however, no study in the literature establishes its relationship with gallstone disease. Therefore, our study aimed to evaluate the biochemical parameters associated with gallstone disease, primarily serum RBP4.

Material and Method: Between 2015 and 2016, abdominal ultrasound, serum biochemical tests, lipid profiles, uric acid, insulin, and fasting glucose values were available for 80 patients who presented to our hospital's gastroenterology clinic, were included in the study. RBP4 levels were analyzed in the serum samples obtained from the patients.

Results: Out of the 80 participants in the study, 42 had gallstones, while 38 did not. Among the biochemical parameters, no significant difference was found between the groups in terms of total cholesterol values *(p= 0.483)*, LDL values *(p=0.224)*, and TG values *(p=0.764)*. A significant difference was observed between the two groups regarding HDL values(p=0.017). No significant difference was found between the two groups in terms of serum uric acid *(p=0.411)*, fasting glucose *(p=0.214)*, fasting insulin, HOMA-IR score *(p=0.157)*, and vitamin D levels *(p=0.340)*. The mean±SD values of the studied serum RBP-4 levels in the participants were determined as 40.24±7.12 in the control group and 39.75±8.55 in the patient group. No statistically significant difference was found between the two groups *(p=0.776)*. In correlation analyses, a significant positive correlation was found between RBP4 and vitamin D levels *(r: 0.277, p= 0.013)*, total cholesterol *(r: 0.268, p=0.016)*, triglycerides *(r: 0.387, p<0.001)*, GGT *(r: 0.294, p=0.008)*, AST *(r: 0.299, p=0.007)*, and uric acid *(r: 0.255, p=0.022)*.

Conclusion: In conclusion, our study did not find a statistically significant relationship between gallstones and RBP4, vitamin D, LDL, TG, total cholesterol, uric acid, and HOMA-IR. However, our study found a positive correlation between vitamin D levels and RBP4. This has guided future research.

Keywords: Cholelithiasis, Gallstone Disease, RBP4.

ÖZET

Amaç: Safra taşları ile ilişkilendirilen metabolik parametreler birçok çalışmanın konusunu oluşturmaktadır. RBP4, bir adipokin olup metabolik hastalıklarla ilişkilendirilmiştir; ancak bildiğimiz dahilinde, safra taşı hastalığı ile ilişkisini belirleyen bir çalışma bulunmamaktadır. Çalışmamızda, öncelikle serum RBP4 olmak üzere safra taşı ile ilişkilendirilen biyokimyasal parametreleri değerlendirmeyi amaçladık.

Gereç ve Yöntem: 2015-2016 yılları arasında gastroenteroloji kliniğine başvuran 80 hastanın abdominal ultrason, serum biyokimyasal testler, lipid profilleri, ürik asit, insulin ve açlık glukoz değerleri mevcuttu ve çalışmaya dahil edildi. RBP4 seviyeleri, alınan serum örneklerinde analiz edildi.

Bulgular: Seksen hastanın 42'sinde safra taşı bulunurken, 38'inde yoktu. Biyokimyasal parametreler arasında, toplam kolesterol değerleri *(p= 0.483)*, LDL değerleri *(p=0.224)* ve trigliserit değerleri *(p=0.764)* açısından gruplar arasında anlamlı fark bulunmamıştır. İki grup arasında HDL değerleri açısından anlamlı fark gözlemlenmiştir *(p=0.017)*. Serum ürik asit *(p=0.411)*, açlık glukozu *(p=0.214)*, açlık insulin ve HOMA-IR skoru *(p=0.157)*, vitamin D seviyeleri *(p=0.340)* açısından iki grup arasında anlamlı fark bulunmamıştır. Çalışmaya katılan kişilerin incelenen serum RBP4 seviyelerinin ortalama±SD değerleri kontrol grubunda 40,24±7,12, hasta grubunda ise 39,75±8,55 olarak belirlenmiştir. İki grup arasında anlamlı fark bulunmamıştır *(p=0.776)*. Korelasyon analizlerinde; RBP4 ile vitamin D seviyeleri *(r: 0.277, p= 0.013)*, toplam kolesterol *(r: 0.268, p=0.016)*, trigliserit *(r: 0.387, p<0.001)*, GGT *(r: 0.294, p=0.008)*, AST *(r: 0.299, p=0.007)* ve ürik asit *(r: 0.255, p=0.022)* arasında anlamlı pozitif korelasyon bulunmuştur.

Sonuç: Sonuç olarak, çalışmamızda safra kesesi taşları ile RBP4, vitamin D, LDL, TG, toplam kolesterol, ürik asit ve HOMA-IR arasında anlamlı ilişki bulunamamıştır. Ancak çalışmamızda saptanan D vitamin ve RBP4 düzeyi arasındaki pozitif korelasyonun gelecekteki çalışmalar için yol gösterici olduğu düşünülmüştür.

Anahtar Sözcükler: Kolelitiazis, RBP4, Safra Taşı Hastalığı.

Introduction

The oldest information about gallstones is based on the Egyptians and Babylonians. Four thousand years ago, the Babylonians introduced some concepts and definitions of the gallbladder into their daily medical use. However, Alexander Trallius showed in the 5th century AD that stones can form in the gallbladder. Gallstones are a common disease; in the United States, 6 percent of men and 9 percent of women have gallstones (1). For this reason, pathogenesis and the factors that cause a disease with such an old history still attract the scientific world's attention today.

The increase in obesity and accompanying diseases all worldwide have raised the interest in adipose tissue. Studies have revealed that adipose tissue is not only a simple storage site for lipids but also an important tissue that plays a key role in regulatin gendocrine, metabolic, and inflammatory processes (2). Adipose tissue cells have been shown to secrete various bioactive proteins into the circulation. These secretory proteins called adipocytokines are leptin, tumor necrosis factor-α (TNF-α), plasminogen-activator inhibitor type 1 (PAI-1), adipsin, resistin, visfatin, adiponectin, retinol binding protein-4 (RBP4) and lipocalin-2 (2).

Adipocytokines released from adipose tissue cells and adipose tissue infiltrating macrophages cause chronic inflammation, leading to insulin resistance and type 2 diabetes (3). Studies have been conducted that the level of RBP4, an adipocytokine, increases insulin resistance and is associated with metabolic syndrome, type 2 diabetes mellitus, and cardiovascular diseases (4, 5). These diseases are also risk factors for the development of cholelithiasis. Considering this cause-effect relationship, higher RBP4, chemerin, and fibroblast growth factor 21 (FGF-21) levels have been observed in children with cholelithiasis (6). Some studies found a relationship between RBP4 level (increase-decrease) and cholelithiasis, but the literature data on this subject needs to be more comprehensive, sufficient, and contradictory. Thus, we aimed to determine the relationship between RBP4 level and cholelithiasis.

Metabolic syndrome is a disease complex by elevated fasting blood glucose, blood pressure, triglycerides, low high-density lipoprotein (HDL) cholesterol, and the presence of central obesity (7). Clustering these metabolic factors increases the risk of cardiovascular disease and type 2 diabetes (8). Previous studies have shown a significant association between cholelithiasis and metabolic syndrome (9,10). It was thought that insulin resistance and hyperlipidemia, which are the components of metabolic syndrome, may be associated with cholelithiasis is independent of diabetes and obesity. Therefore, as one of the aims of the study, it was planned to examine the relationship between cholelithiasis insulin resistance and hyperlipidemia.

Vitamin D inhibits lipogenesis, induces insulin synthesis, preserves islet cells, decreases insulin resistance, and reduces appetite, favoring obesity and T2DM control (11). Vitamin D deficiency is associated with stasis in the gallbladder, and a role for vitamin D supplementation is thought to have the potential to prevent gallstones, especially in pregnant women (12). Considering the effects of vitamin D on metabolism, it aims to reveal whether there is a change in vitamin D levels in patients with cholelithiasis compared to the average population. High uric acid plays a role in inflammation, insulin resistance, diabetes mellitus, hyperlipidemia, and hypertension. Based on this data in the literature, the relationship between uric acid level and cholelithiasis was examined, and conflicting data were obtained (13).

In light of the literature data, we aimed to examine the relationship between gallstone disease and serum RBP4 levels, vitamin D levels, and metabolic parameters in our patients.

Material and Methods

Patients aged 20-75 years who applied to the gastroenterology department of Gazi University between 2015-2016 and had upper abdomen imaging were included in our study. Our significance level (alpha) was set at 0.05. To determine the minimum required sample size, we utilized an effect size of 0.6, derived from a study by Wang et al. (14) investigating the relationship between RBP-4 concentrations and cholesterol gallstone disease. This effect size served as a benchmark for comparing means between the two groups. Following calculations using G Power software (15), we determined that a minimum of

72 subjects would be needed for our study. When the imaging was evaluated, gallstone disease was detected in 42 patients, and 38 patients without gallstone disease were included in the comparison group. Our study's ethics approval was obtained from the Gazi University Faculty of Medicine Clinical Research Ethics Committee with document number 278 dated May 26, 2014. Informed voluntary consent forms have been obtained from all patients. The blood fasting glucose, fasting insulin, liver function tests, kidney function tests, lipid profiles, and vitamin D levels of the patients included in the study were retrospectively screened. Our hospital's biochemistry laboratory studied the serum RBP4 levels of the patients. The informed consent form was obtained from all participants included in the study.

Inclusion criteria

a- The ages between 20-75 years,

b- Abdominal imaging performed within the last two years,

c- Not taking antihyperlipidemic treatment and vitamin D replacement therapy in the last year,

d- No diagnosis of chronic liver disease, stage 3-4-5 renal disease, diabetes mellitus, or an additional systemic disease

Exclusion criteria:

a- Pregnancy, lactation, obesity,

b- Those who do not fill out an informed consent form

Biochemical tests

Approximately 10 ml of peripheral blood sample from the forearm of all individuals included in the study was taken into separator tubes that did not contain any anticoagulant or other additives and centrifuged at 1000xg for 20 minutes after waiting for 30 minutes at room temperature to ensure coagulation. After centrifugation, approximately 1.5 ml samples from the separated serum samples were transferred to Eppendorf tubes and stored at -80^{0}C until the study day. Laboratory investigations of the research were carried out in the Biochemistry Laboratory of Gazi University Hospital Central Laboratory. RBP4 levels in serum samples were determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method using the Human RBP4 ELISA Kit (BioCER). Each serum sample was detected twice.

Statistical analysis:

SPSS version 22.0 was used to analyze the variables (IBM Corporation, Armonk, New York, United States). The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk test, homogeneity of variance was evaluated with the Levene test, and the Independent-Samples T-test, one of the parametric methods, was used together with the Bootstrap results, while the Mann-Whitney U test, which is one of the nonparametric methods, was used with the Monte Carlo simulation technique in the comparison of the two independent groups according to the quantitative data. Spearman's rho test was used to examine the correlations of the variables with each other. In a comparison of categorical variables with each other, Pearson Chi-Square and Fisher Exact tests were tested with the Monte Carlo Simulation technique. While quantitative variables are shown as mean ± std. (Standard deviation) and median Range (Maximum-Minimum) and categorical variables as n (%) in the tables; the variables were analyzed at a 95% confidence level, and a p-value less than 0.05 was considered significant.

Results

Out of the 80 participants in the study, 42 had gallstones, while 38 did not. Among the 38 patients without gallstones, 26 (68.4%) were female and 12 (31.6%) were male. Of the 42 patients with gallstones, 29 (69%) were female, and 13 (31%) were male. No significant difference in terms of gender was observed between the two groups (*p=1*). The average age in the group without gallstones was 41.82±14.10, ranging from 20-64. In the group with gallstones, the average age was 49.00±14.32, with a range of 24-75. A significant age difference was found between the two groups, with the group with gallstones having a higher average age (*p=0.033*) (Table I).

There was no significant difference between the groups among the biochemical parameters in terms of total cholesterol values (*p= 0.483*), LDL values (*p=0.224*), and TG values (*p=0.764*) (Table II). However, a significant difference was observed between the two groups regarding HDL values, with lower HDL values in the cholelithiasis group (*p=0.017*) (Table II).

Liver function tests were evaluated, and significant differences were observed between the groups in

ALT (*p=0.021*), GGT (*p=0.018*), and ALP (*p=0.033*) values, with these parameters being higher in patients with cholelithiasis (Table II).

Table I. Age and gender characteristics of the participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
Gender				
Female	26 (68.4%)	29 (69%)	55 (68.8%)	*1.0*
Male	12 (31.6%)	13 (31%)	25 (31.3%)	
Age				
Mean±STD (Min-max)	41.82 ± 14.10 (20-64)	49.00 ± 14.32 (24-75)	45.59 ± 14.58 (20-75)	*0.033*

Serum uric acid levels did not significantly differ between the two groups (*p=0.411*) (Table II). HOMA-IR scores were calculated by examining the participants' fasting glucose and simultaneous fasting insulin levels. No significant differences were found between the two groups in terms of fasting glucose (*p=0.214*), fasting insulin, and HOMA-IR score (*p=0.157*) (Table II).

Table II. Biochemical analysis of participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
	Median (max-min)	Median (max-min)	Median (max-min)	
Total cholesterol	183.5 (123-391)	190 (101-337)	186.5 (101-391)	*0.483*
LDL	103.5 (35-250)	116 (64-222)	110.5 (35-250)	*0.224*
Triglyceride	111.5 (30-265)	106 (36-499)	109 (30-499)	*0.764*
HDL	49.5 (30-143)	44.5 (5.5-71)	46 (5.5-143)	*0.017**
ALT	18 (1-87)	21.5 (12-588)	20 (1-588)	*0.021**
AST	19 (5-111)	21 (1-471)	20 (1-471)	*0.512*
GGT	19 (7-99)	28 (9-369)	21 (7-369)	*0.018**
ALP	71 (39-118)	81.5 (34-337)	74 (39-337)	*0.033**
Total Bilirubin	0.455 (0.18-2)	0.545 (0.22-4.4)	0.148 (0.18-4.4)	*0.139*
Direct Bilirubin	0.165 (0.04-0.66)	0.2 (0.09-3.2)	0.185 (0.04-3.2)	*0.143*
Uric acid	4.35 (2.2-8.7)	4.65 (2-9.2)	4.45 (2-9.2)	*0.411*
HOMA-IR	1.445 (0.58-6.8)	1.725 (0.33-12.93)	1.56 (0.33-12.93)	*0.157*

* statistically significant, HDL (High-Density Lipoprotein), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-Glutamyl Transferase), ALP (Alkaline Phosphatase).

No significant difference was found between the two groups regarding vitamin D levels (*p=0.340*) (Table III).

Table III. Vitamin D and Serum RBP4 analysis of participants

	Gallstone Disease (-)	Gallstone Disease (+)	Total	*p value*
Vitamin D Median (max-min)	19.95 (5-44)	15.85 (4-71)	17.75 (4-71)	*0.340*
RBP4 Mean ± STD	40.24 ± 7.12	39.75 ± 8.55	39.98 ± 7.86	*0.776*

The mean±SD values of the studied serum RBP4 levels in the participants were determined as 40.24±7.12 in the control group and 39.75±8.55 in the patient group. No statistically significant difference was found between the two groups (*p=0.776*) (Table III). In correlation analyses, a significant positive correlation was found between vitamin D levels and RBP4 (*r: 0.277, p= 0.013*). Significant positive correlations were also observed between RBP4 levels and total cholesterol (*r: 0.268, p=0.016*), triglycerides (*r: 0.387, p<0.001*), GGT (*r: 0.294, p=0.008*), AST (*r: 0.299, p=0.007*), and uric acid (*r: 0.255, p=0.022*) (Table IV).

Table IV. Correlation Analysis

	VITD		RBP4	
	r	*p value*	*r*	*p value*
Age	*-0.085*	*0,454*	*0,049*	*0,665*
Total cholesterol	*0.124*	*0,272*	*0,268**	*0,016**
LDL	*0.042*	*0,713*	*0,183*	*0,105*
Triglyceride	*0.169*	*0,133*	*0,387**	*<0,001**
HDL	*0.025*	*0.829*	*-0.081*	*0.476*
ALT	*-0.128*	*0.258*	*0.070*	*0.539*
AST	*0.038*	*0.740*	*0.299**	*0.007**
GGT	*0.207*	*0.065*	*0.294**	*0.008**
ALP	*0.018*	*0.874*	*0.152*	*0.178*
Total bilirubin	*-0.088*	*0.440*	*0.045*	*0.692*
Direct bilirubin	*-0.136*	*0.229*	*0.081*	*0.475*
Uric acid	*0.210*	*0.062*	*0.255**	*0.022**
Insulin	*-0.128*	*0.258*	*-0.083*	*0.465*
Fasting Glucose	*0.076*	*0.502*	*0.110*	*0.330*
HOMA-IR	*-0.099*	*0.380*	*-0.070*	*0.536*
RBP4	*0.277**	*0,013**		

*statistically significant, HDL (High-Density Lipoprotein), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-Glutamyl Transferase), ALP (Alkaline Phosphatase).

Discussion

The diagnosis and treatment of gallbladder stone disease are crucial due to the potential complications and symptoms that can significantly impact the quality of life and lead to morbidity. Gallbladder Stones have both preventable and non-preventable etiological factors. Ethnic origin, advanced age, female gender, family history, and genetics are considered non-preventable etiological factors, whereas preventable factors include obesity, rapid weight loss, and a sedentary lifestyle (16). Understanding the relationship between gallbladder Stones and other diseases and illuminating relevant laboratory parameters is essential for identifying preventable factors.

Gender and age are among the most significant non-preventable risk factors for gallbladder stone disease. The female gender, especially in the pre-menopausal age group, has a two-fold higher risk compared to males. The underlying mechanisms for this include factors such as fertility, hormones, oral contraceptive treatments, and estrogen replacement therapies (16). For instance, in a study conducted in Germany 2005 involving 4,202 individuals aged between 20 and 74, the prevalence of gallbladder stones was twice as high in women compared to men (17). Similarly, in a study conducted in Nepal 2012, the female-to-male ratio was determined to be 7 to 1 (18).

In selecting our study participants, we aimed for no significant gender difference between the two groups, particularly considering that levels of vitamin D, RBP4, and lipid profiles may vary between female and male individuals. The study includes 80 patients, with 55 being female and 25 males. No significant difference was observed in gender distribution between patients with gallbladder stones and those without ($p=1.0$).

The frequency of gallbladder stones increases with age, particularly showing a 4-10-fold increase after age 40 (19). In a study conducted in the United States, it was demonstrated that the prevalence of gallbladder stones with age increased in both Mexican American men and women, reaching 44.1% in women aged 60-74 (20). In a study by Katsuroni Sekine and colleagues published in August 2015 in the Journal of Gastroenterology and Hepatology, a total of 717 patients were prospectively examined, and the group with gallbladder stones had a significantly higher mean age (21). In a study by Tazumo and colleagues in May 2015, the expected age range for 612 gallbladder stone patients was 60-70, with a mean age of 63 (22). In our study, the mean age of individuals with gallbladder stones was significantly higher than those without stones. Although the data obtained in our study is similar to literature data, the lower mean age values in our study are thought to be due to the exclusion criterion of patients with chronic diseases in the design of our study.

Dyslipidemia, insulin resistance, and obesity are significant components of metabolic syndrome, and the level of RBP4 is associated with these components. In light of this data, there might be variability in the RBP4 levels in gallbladder stone disease, which includes components of metabolic syndrome in its etiology (23,24). Our study was designed with this purpose in mind. The first study related to our hypothesis was conducted in 2009 by Shen-Nien Wang and colleagues, where a negative correlation between gallbladder stones and RBP4 was identified (14). Another study in 2013 by Han T. and colleagues found that elevated RBP4 levels were associated with metabolic syndrome and cholesterol gallstone formation (25). In another study conducted in 2021 on a pediatric patient group, higher serum RBP4 levels were found in children and adolescents with high body weight and gallbladder stones (6). Our study found no significant difference in RBP4 levels between the two groups with and without gallbladder stones. The limited number of studies on this topic with a small number of patients in the literature and conflicting results emphasize the need for more extensive and diverse studies with a larger number of patients.

Cholesterol-associated gallstones are the most common type of gallbladder stone. The high prevalence of cholesterol-associated gallstones has raised the possibility of a relationship between lipid profiles and gallstones in patients, leading to numerous studies on this topic (26-28). While hypertriglyceridemia and low HDL levels are associated with gallstone formation, the relationship with hypercholesterolemia has not been clearly defined (29, 30). Some studies have shown elevated triglyceride levels to reduce gallbladder motility (31). Our study, consistent with

the literature, did not find a significant relationship between total cholesterol levels, LDL, and gallbladder stones. However, HDL levels were significantly lower in the group with gallstones, supporting the existing literature. The literature contains conflicting data regarding the impact of triglyceride levels on gallstone formation. Our study observed no significant difference in triglyceride levels between the included patient and control groups. However, to enhance their liability of such a study, controlling and equalizing other parameters associated with hypertriglyceridemia between the control and patient groups would be necessary to eliminate differences. Since our study excluded obese patients, a one-to-one match in terms of BMI could not be achieved between the patient and control groups. This could contribute to our study's statistically insignificant association between triglyceride levels and gallbladder stones.

Insulin resistance, another diagnostic criterion for metabolic syndrome, has been the subject of many studies in relation to gallbladder stones. A study conducted by Atamer and colleagues in 2013 and a study published in 2011 shows the relationship between insulin resistance and gallbladder stones (32,33). However, some studies published in the same years with very large patient groups did not find a significant relationship between HOMA-IR and gallbladder stones. The variability in the results among studies highlights the need for further research with diverse patient populations to understand better the relationship between HOMA-IR and gallbladder stones (34). Our study examined serum fasting insulin and fasting glucose levels in non-diabetic patients with gallbladder stones or in the control group. Using these parameters, HOMA-IR levels were calculated in patients. No statistically significant differences were found between the two groups regarding fasting glucose, insulin, and HOMA-IR.

Uric acid is influential in developing many diseases associated with inflammation and accumulation. Among the most significant are components of metabolic syndrome, including insulin resistance, diabetes mellitus, hyperlipidemia, and hypertension (13), as well as non-alcoholic fatty liver disease (35). An epidemiological study in Taiwan investigated the relationship between uric acid levels and gallbladder stones, but no significant association was found. However, in some studies, a relationship between high uric acid levels and gallbladders tones has been identified in women (36). In light of these data, we planned to include patients' serum uric acid levels in our study. However, in our study, no significant difference was found in serum uric acid levels between the groups with and without gallbladder stones. Variability in results among studies regarding uric acid levels has been attributed to the difficulty in effectively controlling both modifiable and non-modifiable metabolic factors, making standardization challenging.

The effects of vitamin D on hormone secretion, regulation of immune functions, and the modulation of cell proliferation and differentiation are now well-known (37). The association between vitamin D deficiency and obesity, insulin resistance, and metabolic syndrome continues to be the subject of numerous studies (38). Our study also investigated the relationship between vitamin D and gallbladder stones based on its metabolic effects. No statistically significant difference was found between the patient and control groups in vitamin D levels. However, correlation analyses revealed a positive correlation between vitamin D deficiency and RBP4 values. This result can guide future studies in determining the correlation between vitamin D and RBP4 levels in metabolic syndrome, a complex endocrine issue with multiple parameters.

The strength of our study is that it is one of the few studies on this subject in the literature and increases the need for new studies due to conflicting results with these studies. Limitations of our study are that it was performed on a heterogeneous and small number of patients.

In conclusion, our study did not find a statistically significant relationship between gallbladder stones and RBP4, vitamin D, LDL, TG, total cholesterol, uric acid, and HOMA-IR. However, a negative association was observed between gallstones and HDL levels, and an increase in the frequency of gallstones with age was demonstrated. The conflicting and limited literature on the relationship between gallstones and RBP4 adds value to our study and guides future research. Nevertheless, the limited number of participants poses a disadvantage in determining correlations between parameters. The absence of

other literature publications on the relationship between gallstones and vitamin D suggests that our study could Pioneer new research. Despite various studies on lipid levels, uric acid, and HOMA-IR in the literature, the existence of conflicting data emphasizes the need for more extensive retrospective or prospective studies involving many patients.

References

1. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. Gastroenterology. 1999;117(3):632-639.

2. Polson DA, Thompson MP. Macronutrient composition of the diet differentially affects leptin and adiponutrin mRNA expression in response to meal feeding. The Journal of Nutritional Biochemistry 2004;15(4):242-246.

3. Antuna-Puente B, Feve B, Fellahi S, Bastard J-P. Adipokines: the missing link between insulin resistance and obesity. Diabetes & metabolism 2008;34(1):2-11.

4. Perumalsamy S, Ahmad WAW, Huri HZ. Retinol-Binding Protein-4A Predictor of Insulin Resistance and the Severity of Coronary Artery Disease in Type 2 Diabetes Patients with Coronary Artery Disease. Biology 2021;10(9):858.

5. Olsen T, Blomhoff R. Retinol, retinoic acid, and retinol-binding protein 4 are differentially associated with cardiovascular disease, type 2 diabetes, and obesity: an overview of human studies. Advances in Nutrition 2020;11(3):644-666.

6. Zdanowicz K, Ryzko J, Bobrus-Chociej A, Wojtkowska M, Lebensztejn DM. The role of chemerin in the pathogenesis of cholelithiasis in children and adolescents. Journal of Paediatrics and Child Health 2021;57(3):371-375.

7. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. Circulation 2009;120(16):1640-1645.

8. Kaur J. A comprehensive review on metabolic syndrome. Cardiology research and practice 2014;2014: 943162.

9. Zhu Q, Sun X, Ji X, et al. The association between gallstones and metabolic syndrome in urban Han Chinese: a longitudinal cohort study. Scientific reports 2016;6(1):29937.

10. Lin I, Yang YW, Wu MF, et al. The association of metabolic syndrome and its factors with gallstone disease. BMC family practice 2014;15(1):1-6.

11. Cândido FG, Bressan J. Vitamin D: link between osteoporosis, obesity, and diabetes? International journal of molecular sciences 2014;15(4):6569-6591.

12. Onal ED, Berker D, Guler S. Vitamin D deficiency and gallbladder stasis. Digestive diseases and sciences 2015;60:3823-3824.

13. Loria P, Lonardo A, Lombardini S, et al. Gallstone disease in non-alcoholic fatty liver: prevalence and associated factors. Journal of gastroenterology and hepatology 2005;20(8):1176-1184.

14. Wang SN, Yeh YT, Wang ST, et al. Decreased retinol binding protein 4 concentrations are associated with cholesterol gallstone disease. Journal of the Formosan Medical Association 2010;109(6):422-429.

15. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods 2007;39(2):175-191.

16. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut and liver 2012;6(2):172.

17. Völzke H, Baumeister SE, Alte D, et al. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. Digestion 2005;71(2):97-105.

18. Shrestha K, Dahal P, Shah L, Singh R. Relation of lipid profile, BMI and cholelithiasis in Nepalese population. Postgraduate medical journal of National Academy of Medical Sciences 2012;12:40-44.

19. Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. New England Journal of Medicine 1985;313(5):277-282.

20. Maurer KR, Everhart JE, Ezzati TM, et al. Prevalence of gallstone disease in Hispanic populations in the United States. Gastroenterology 1989;96(2):487-492.

21. Sekine K, Nagata N, Sakamoto K, et al. Abdominal visceral fat accumulation measured by computed tomography associated with an increased risk of gallstone disease. Journal of gastroenterology and hepatology 2015;30(8):1325-1331.

22. Tazuma S, Kanno K, Kubota K, et al. Report on the 2013 national cholelithiasis survey in Japan. Journal of Hepato-Biliary-Pancreatic Sciences 2015;22(5):392-395.

23. Chen LY, Qiao QH, Zhang SC, Chen YH, Chao GQ, Fang LZ. Metabolic syndrome and gallstone disease. World journal of gastroenterology 2012;18(31):4215.

24. McTernan PG, Kumar S. Retinol binding protein 4 and pathogenesis of diabetes. Oxford University Press; 2007. p. 2430-2432.

25. Han T, Zhang D, Fu Z, Sun Y, Yang W, Yuan C. Retinol-binding protein 4 as a risk factor for cholesterol gallstone formation.

Molecular and cellular biochemistry 2013;377:219-227.
26. Atamanalp SS, Keles MS, Atamanalp RS, Acemoglu H, Laloglu E. The effects of serum cholesterol, LDL, and HDL levels on gallstone cholesterol concentration. Pakistan journal of medical sciences 2013;29(1):187.
27. Olokoba A, Bojuwoye B, Katibi I, et al. Relationship between gallstone disease and serum lipids in normal adult Nigerians. African Scientist 2021;7(3):113-116.
28. Song ST, Shi J, Wang XH, et al. Prevalence and risk factors for gallstone disease: A population-based cross-sectional study. Journal of digestive diseases 2020;21(4):237-245.
29. Stender S, Frikke-Schmidt R, Benn M, Nordestgaard BG, Tybjærg-Hansen A. Low-density lipoprotein cholesterol and risk of gallstone disease: a Mendelian randomization study and meta-analyses. Journal of hepatology 2013;58(1):126-133.
30. Atamer A, Kurdas-Ovunc AO, Yesil A, Atamer Y. Evaluation of paraoxonase, malondialdehyde, and lipoprotein levels in patients with asymptomatic cholelithiasis. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association 2014;20(1):66.
31. Smelt A. Triglycerides and gallstone formation. Clinica chimica acta 2010;411(21-22):1625-1631.
32. Atamer A, Övünç AOK, Yeşil A, Atamer Y. Evaluation of leptin and insulin resistance in patients with cholelithiasis. Indian Journal of Biochemistry and Biophysics 2013; 50(4):266-272
33. Kim SS, Lee JG, Kim DW, et al. Insulin resistance as a risk factor for gallbladder stone formation in Korean postmenopausal women. The Korean journal of internal medicine 2011;26(3):285.
34. Shebl F, Andreotti G, Meyer T, et al. Metabolic syndrome and insulin resistance in relation to biliary tract cancer and stone risks: a population-based study in Shanghai, China. British journal of cancer 2011;105(9):1424-1429.
35. Hung MC, Chen CF, Tsou MT, Lin HH, Hwang LC, Hsu CP. Relationship between gallstone disease and cardiometabolic risk factors in elderly people with non-alcoholic fatty liver disease. Diabetes, Metabolic Syndrome and Obesity 2020;13:3579-3585.
36. Chen CH, Huang MH, Yang JC, et al. Prevalence and risk factors of gallstone disease in an adult population of Taiwan: an epidemiological survey. Journal of gastroenterology and hepatology 2006;21(11):1737-1743.
37. Martens P-J, Gysemans C, Verstuyf A, Mathieu C. Vitamin D's effect on immune function. Nutrients 2020;12(5):1248.
38. Karampela I, Sakelliou A, Vallianou N, Christodoulatos G-S, Magkos F, Dalamaga M. Vitamin D and obesity: current evidence and controversies. Current obesity reports 2021;10:162-180.