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Physical, Chemical and Mineralogical Properties of Gallstones obtained from Antalya Province of Turkey

Türkiye'nin Antalya İlinde Safra Kesesi Taşlarının Fiziksel, Kimyasal ve Mineralojik Özelliklerinin Analizi

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Abstract: Gallbladder stone disease is the most common disease with a high prevalence in many societies in the word. In the literature, the primary risk factors discussed for this disease have been evaluated as gaining and losing weight rapidly, obesity, age, gender, genetic factors, having given birth too many children, life style and medications. The aim of this investigation is to find out the chemical and physical properties of gallstones in patients living in the province of Antalya, Turkey. For this purpose, the chemical and mineralogical properties of 1243 gallstone samples from 69 patients were analyzed using X-ray diffractometer (XRD), scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR) applications.

In accordance with the outcome of SEM and FTIR analysis, cholesterol, calcium carbonate, calcium bilirubinate, calcium phosphate, carbonate apatite, and protein contents were observed. Cholesterol was detected in 95% of 69 patients; with 44 samples obtained from female patients. X-Ray Diffractometer (XRD) analysis showed newberyite, struvite, and aragonite minerals were found in the composition of gallstones. Newberyite was present in 59% of the samples. The physical, chemical and mineralogical characteristics of these gallstones are very important for the understanding of gallstone formation. The results of the study are consistent with the 5F Rule (Female, Forty, Fatty, Fair, Fertile). The relationship between bilirubinate and the presence of bacteria was determined. The presence of barium acetate and aluminum silicate in gallstones revealed the relationship with environmental pollutants.

Keywords: Aragonite, Gallstone properties, medical geology, newberyite, struvite.

Öz: *Safra taşı hastalığı, dünya çapında birçok toplumda yüksek prevalansa sahip en yaygın hastalıktır. Literatürde bu hastalık için tartışılan başlıca risk faktörleri hızlı kilo alımı, obezite, yaş, cinsiyet, genetik faktörler, çok çocuk doğurma, yaşam tarzı ve ilaçlardır. Bu çalışmanın amacı, Antalya'da yaşayan hastalarda safra kesesi taşlarının kimyasal ve fiziksel özelliklerini belirlemektir. Bu amaçla, 69 hastadan alınan 1243 safra taşı örneğinin kimyasal ve mineralojik özellikleri X-ışını difraktometresi (XRD), taramalı elektron mikroskobu (SEM) ve Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR) uygulamaları kullanılarak analiz edilmiştir.*

SEM ve FTIR analiz sonuçlarına göre kolesterol, kalsiyum karbonat, kalsiyum bilirubinat, kalsiyum fosfat, karbonat apatit ve protein içerikleri gözlenmiştir. Kolesterol 69 hastanın %95'inde tespit edilmiştir; 44 örnek kadın

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hastalardan elde edilmiştir. X-Işını Difraktometre (XRD) analizi safra taşlarının bileşiminin nevberyit, struvit ve aragonit minerallerini içerdiğini göstermiştir. Nevberyit örneklerin %59'unda mevcuttur. Bu safra taşlarının fiziksel, kimyasal ve mineralojik özellikleri safra taşı oluşumunu anlamak için çok önemlidir. Çalışmanın sonuçları 5F Kuralı (Female, Forty, Fatty, Fair, Fertile) ile uyumludur. Bilirubinat ile bakteri varlığı arasındaki ilişki belirlenmiştir. Safra taşlarında baryum asetat ve alüminyum silikat varlığı çevresel kirleticilerle bir ilişki olduğunu ortaya koymuştur..

Anahtar Kelimeler: *Aragonit, nevberyit, safra taşı özellikleri, struvit, tıbbi jeoloji.*

INTRODUCTION

Soil and water, which is an important aspect of our environment, is exposed to pollutants due to natural processes and human activities. In this phenomena, natural and anthropogenic contamination such as contaminated soils, contaminated potable water, harmful waste of agricultural products, pests in the food chain, waste waters from industrial production and heavy metal production or the toxic effects of heavy metal production or toxic effects of heavy metal production can cause serious health problems (McBride, 1995; Cohen et al., 1984; Damalas et al., 2008). In addition, external environmental influences can exacerbate or worsen people's existing health problems. One of the health problems that external factors can worsen is gallbladder stone (gallstone) formation (Zuo et al., 2016; Cen et al., 2018; Molinero et al., 2019). Some scientific studies have determined the influence of gut microbiota and Desulfovibrion Ales on gallstone formation. In particular, its effect on bile acid and cholesterol metabolism has been inferred. Its role as an environmental regulator was explained. In addition, it was stated metabolic conditions such as obesity and diabetes predispose to gallstone disease (Hu et al., 2022). The importance of obesity for gallstone disease has been emphasized (Grigor'eva, 2020). Personal factors such as female gender, increasing age, ethnicity, genetic make-up and family history or genetic make-up especially cholesterol, can cause gallstones in individuals. These characteristics are risk factors that cannot be modified in patients. Pregnancy, medications such as octreotide, thiazide and cefthiazone diuretics, feeding a person with liquid nutritional products (parenteral nutrition) and starvation are important for bill sludge formation. Obesity and certain risk factors (metabolic syndrome) that increase the risk of heart attack stroke and diabetes mellitus, cholesterol gallstones have been described to increase the number (incidence) of new cases of disease or diseases. Therefore, an active life style, avoiding obesity and rapid weight loss, can prevent to formation of cholesterol gallstones (Stinton and Shaffer, 2012). Capsaicin is known as the pungent alkaloid or red pepper (Capsicum annuum). Capsaicins lowers blood cholesterol levels below normal (hypocholesterolemic) and prevent cholesterol gallstones. It also maintains the structural integrity of erythrocytes under conditions of hypercholesterolemia. Capsaicin is also known as a cardio-protector (Srinivasan, 2016). Type 2 diabetes, obesity and smoking negatively affect gallstone disease (Yuan et al., 2022). Caffeine and especially caffeine-storing coffee negatively increase gallstone disease (van Dam et al., 2020). Various studies have revealed the relationship between gallstones and factors such as obesity, age, fertility, race, and nutrition, concluding that natural conditions and anthropogenic activities are influential in the formation of gallstones. Gallbladder stones have been classified as "cholesterol stones, pigment stones, calcium carbonate stones, phosphate stones, calcium stearate stones, protein stones, cystine stones and mixed stones" based on their morphological characteristics and contents (Qiao et al., 2013). Gallstones in the biliary tract are important in the treatment of the patient. This

condition takes the first and then the composition of the gallstones becomes important (Lammert et al., 2016). While investigating the causes of gallstones, environmental factors and health problems of the individual should be examined. Understanding the mechanisms of gallstone formation and their possible treatment may be achievable by understanding the interrelationship between the basic elements such as bilirubin and protein contained in gallstones, as well as determining their physical, chemical and mineralogical properties. (Kleiner et al., 2002; Liu et al., 2002; Wang et al., 2009; Altinkaya Altınkaya et al., 2011; Yu et al., 2013; Usai et al., 2017; Dorvash et al., 2018; Gazali et al., 2019). As mentioned above, the study of content of kidney Stones and salivary stones, as well as gallstone, is crucial for understanding the processes involved gallstone formation.

In physical, chemical, and mineralogical studies on gallstones, several analysis methods among " nuclear magnetic resonance spectroscopy, fourier-transform infrared spectroscopy (FTIR), x-Ray diffraction(XRD), energy dispersive spectroscopy, fluorescence microscopy, synchrotron radiation (SRXRF) and laser microRaman spectroscopy'' were selected and used in accordance with the purpose of the study. (Liu et al., 2002; Athanasiadou et al., 2013; Iordanidis et al., 2013; Soodan et al., 2014; Sharma et al., 2015; Weerakoon et al., 2015; Kabakci Kabakçı et al., 2016; Parviainen et al., 2016; Ramya et al., 2017; Pichugina et al., 2018). In a study conducted in Nigde province in Turkey, FTIR analysis revealed wavellite, strengite, whitlockite, newberyite, struvite, aragonite, calcite, and apatite minerals in gallstone samples obtained from the study area (Yalcin, 2013; Yalcin at al., 2011). On the other hand, several studies have suggested that many environmental factors have an impact on the formation of gallstones, but there was no direct relationship found between metal concentration and gallstones (Parviainen et al., 2016; 2018). However, Ca-bilirubinate-rich pigment stones have been reported to have a high metal content, and metals have been claimed to play a role in the formation of pigment stones (Suzuki et al., 1975; Zhou et al., 2018). It has been reported that the risk of gallbladder disease is high in patients consuming water contaminated with heavy metals in India (Unisa et al., 2011) In other studies, the content of Ca, Cu, Fe, Mg, Mn, Pb, and Zn tended to increase in pigment stones (Suhara et al., 1998; Ashok et al., 2003; Rautray et al., 2007; Palchik and Moroz, 2005; Omer, 2011; Sharma et al., 2015; Weerakoon et al., 2015).

The study aims to describe the physical, chemical, and mineralogical properties of gallstones obtained from in the Turkish province of Antalya. For this purpose, the mechanisms of gallstone formation and the destruction mechanisms of gallstones were tried to be investigated and explained in the study. In support of this aim, we attempt to investigate and explain the mechanisms of gallstone formation as well as the destruction mechanisms of gallbladder stones. Furthermore, it is thought that an understanding of the natural and anthropogenic sources contributing to the formation of these minerals in the body can provide helpful information needed for the treatment of the diseases occurring due to the existence of gallstones.

MATERIAL and METHODS

Gallstones from patient operated upon due to gallstone between October 2014 and July 2015 were included in the study. A total of 69 gallbladder samples were obtained from twenty-five male and forty-four female gallstone patients. The gallstone of these gallbladder were analyzed. One large gallstone was removed from gallbladder sludge were taken together at the same time; sampling was performed under full-fledged hospital conditions with the consent of the patient. After enumerating each gallbladder sample, the

gallstones were separated from their gallbladders then washed over a No. 200 sieve and disinfected. The gallstones were left to dry naturally and their photographs were taken on a millimeter scale. Following that, the physical properties such as color, appearance, hardness, shape and size were determined as well as the number of gallstone samples in each patient's gallbladder were determined; SEM, XRD, FTIR analyses were performed and the gallstones were grouped based on the data obtained.

XRD and SEM analyses were performed to determine the mineralogical properties of the gallstones. 16 samples which could be finely cross-sectioned were sent to Erciyes University KOSGEB laboratory to be coated with Au/Pd; each sample was examined at two angles in four scales (Mag 500, 1000, 2000, 5000) using the LEO 440 Computer Controlled Digital SEM system and the images of these areas were taken.

The 69 samples used in the study were pulverized using an agate mortar and sent to Erciyes University KOSGEB Laboratory for XRD analysis. Their XRD analyses were performed using the Bruker AXS D8 Advance Model system.

In order to determine the chemical properties of gallstones FTIR (Fourier transform infrared) spectroscopy analysis was performed to understand the chemical bonds between gallstone, to determine the bonding area of the stones with each other and whether the structure is aromatic or aliphatic (Yalcin et al., 2011; Huner et al., 2017). Infrared (IR) absorption spectroscopy is a type of vibrational spectroscopy; IR rays are absorbed by the vibrational motions of the molecule. The radiation intensity is taken measured as a function of time in the Fourier transformed spectra. High-resolution spectra can be obtained quickly without scanning at each wavelength.

The chemical content is determined by comparing the spectra of the samples to the reference tables with wavelength ranges of infrared bands that define the most chemically active parts of a molecule. FTIR is also used to determine the structural analysis of carbohydrates, proteins, phospholipids, and amino acids. The samples were analyzed using the "Perkin Elmer 400 FTIR/FTFIR Spectrometer Spotlight 400 Imaging" system.

RESULTS and DISCUSSION

The physical properties of the samples were examined and they were classified into groups. When the physical structures of some gallstone samples were examined, it was understood that they showed different hardness. Some samples were soft and some samples were moderately hard strength. The gallstone samples of both harnesses are distributed in yellow, white, black, brown and green colors and it is understood that they do not show a homogeneous structure. When the diameters of the gallstones were examined, it was seen that they showed different sizes and showed sizes between 0.1 - 4.4 cm. When the physical shapes of the gallstone samples were examined, it was observed that they varied between oval, morular, cubic, anhedral, dendritic surface, prismatic and fragmented forms. According to these characteristics, the gallstone samples were identified and classified as cholesterol stone, mixed cholesterol stone and pigment stone (Figure 1 & 2).

In the SEM and FTIR analysis of the samples, we observed cholesterol, carbonate apatite, calcium carbonate, calcium phosphate carbonate, calcium bilirubinate, and protein (Figure 3 & 4). Also, bacteria were detected in the pigment stone (Figure 4a).

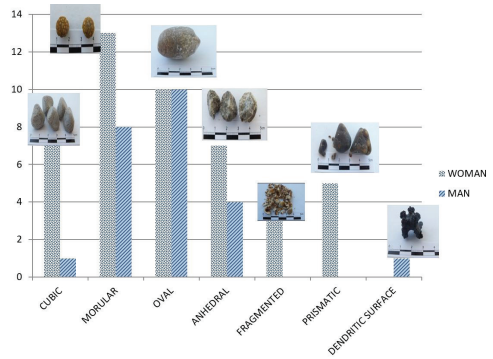

Figure 1. Chart of the numbers of samples by gender by their physical/formal structure.

Şekil 1. *Cinsiyete göre örneklem sayılarının fiziksel/ biçimsel yapılarına göre grafiği.*

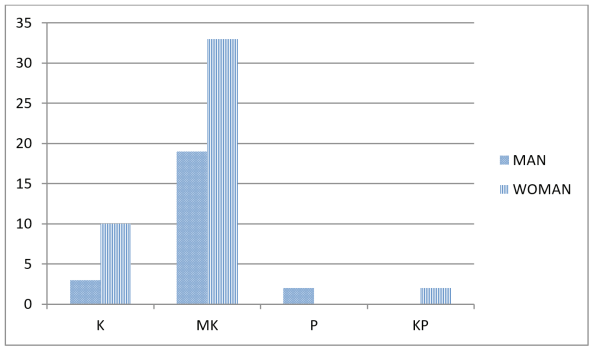

Figure 2. Chart of the number of cholesterol stones, mixed cholesterol stones, pigment stones, mix pigment stones by gender.

Şekil 2. *Cinsiyete göre kolesterol taşı, karışık kolesterol taşı, pigment taşı, karışık pigment taşı sayısı grafiği.*

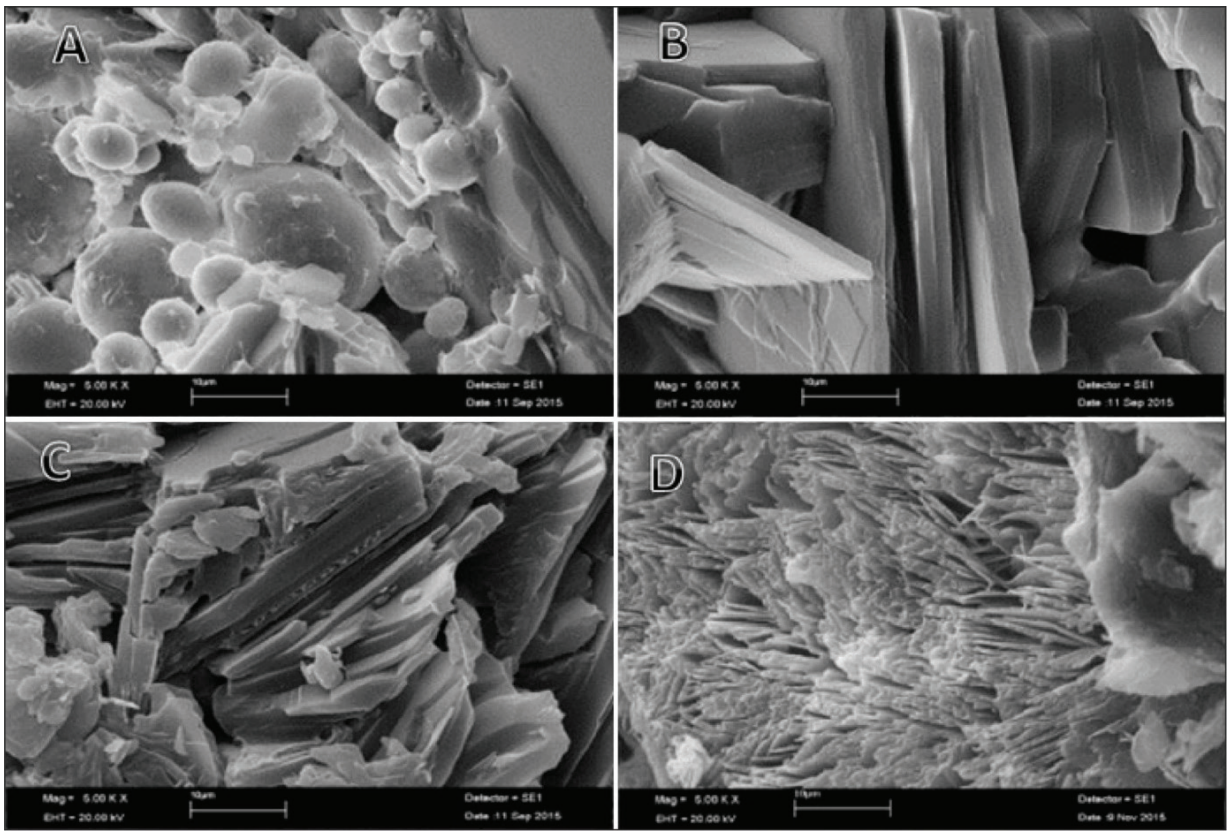

Figure 3. Interpretation of SEM images obtained from different samples; **A)** Cholesterol, Calcium Carbonate; **B)** Cholesterol; **C**) Cholesterol, Carbonate apatite, Calcium phosphate carbonate; **D)** Calcium bilirubinate, Cholesterol, Protein.

Şekil 3. *Farklı örneklerden elde edilen SEM görüntülerinin yorumlanması;* ***A)*** *Kolesterol, Kalsiyum Karbonat;* ***B)*** *Kolesterol;* ***C)*** *Kolesterol, Karbonat apatit, Kalsiyum fosfat karbonat;* ***D:*** *Kalsiyum bilirubinat, Kolesterol, Protein.*

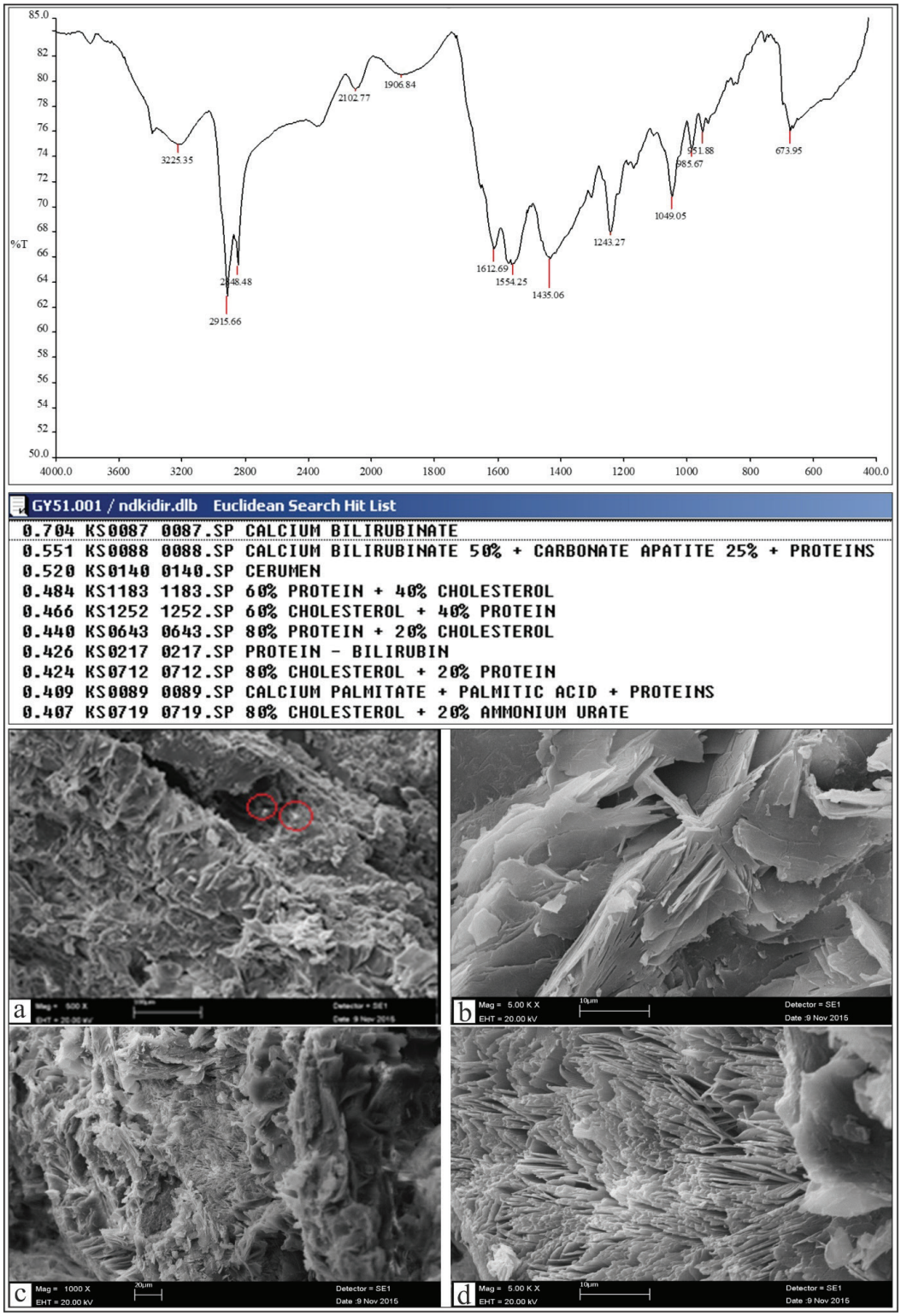

Figure 4. Comparison of FTIR analysis and SEM images of the Sample 51.

Şekil 4. *Örnek 51'in FTIR analizi ve SEM görüntülerinin karşılaştırılması.*

According to the general results of XRD analysis, it was calculated that 59 samples contained newberyite ($MgHPO_4(H_2O)_3$), 3 samples contained struvite ($MgNH_4PO_4(H2O)_6$), 1 sample contained Aragonite ($Ca(CO_3)$), 9 samples contained calcium hydrogen phosphate urea ($C_4H_{16}N_8O_4Ca(H_2PO_4)_2$ / $Ca(H_2PO_4)_2$ $4CO(NH_2)_2$) and 1 sample contained cholesterol dibromide ($C_{27}H_{44}Br_{20}$) (Table 1).

The chemical analysis (FTIR) results revealed that, struvite, apatite, whitlockite, newberyite, carbon apatite, hydroxyl-apatite, calcium phosphate carbonate, aragonite, calcium bilirubinate, aluminum silicate, barium acetate, aragonite, calcite, and palmitic acid in the samples. Sample 24: Aragonite-type calcium carbonate absorption peaks of 1456, 1048, 854, and 698 cm^{-1} were determined. Sample 27: Protein absorption peaks of 1612, 1551, 1403, 1245, and 103 (cm^{-1}) were determined. Sample 51: Bilirubinate absorption peaks of 1612, 1554, 1435, 1243, and 1049 cm^{-1} were determined (Figure 5).

Table 1. The content, chemical formulas, and crystal systems determined in the results of XRD analysis of the samples

Çizelge 1. *Örneklerin XRD analizi sonuçlarında belirlenen içerik, kimyasal formüller ve kristal sistemleri.*

Sample no	COMPOUNDS		Crystal System
	Name	Molecular Formula	
1-2-4-5-6-7-8-13-14-15-16-17-18-19-20-21-22-23-25-26-28-29-30-32-34-35-36-37-39-40-46-48-54-55-56-58-59	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Newberyite	$MgHPO_4(H_2O)_3$	Orthorhombic
3-31-33-38-41-42-44-45-47-49-50-52-5 3	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Cholesterol	$C_{27}H_{46}O$	
	Newberyite	$MgHPO_4(H_2O)_3$	Orthorhombic
9-12	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Newberyite	$MgHPO_4(H_2O)_3$	Orthorhombic
	Struvite	$MgHPO_4(H_2O)_6$	Orthorhombic
24	Aragonite	$Ca(CO_3)$	Orthorhombic
43	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Cholesterol	$C_{27}H_{46}O$	
	Newberyite	$MgHPO_4(H_2O)_3$	Orthorhombic
	Struvite	$MgHPO_4(H_2O)_6$	Orthorhombic
57-61-67-68	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
60-62-63-64-66-	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Newberyite	$MgHPO_4(H_2O)_3$	Orthorhombic
	Calcium Hydrogen Phosphate Urea	$C_4H_{16}N_8O_4 \cdot Ca(H_2PO_4)_2$ / $Ca(H_2PO_4)_2 \cdot 4CO(NH_2)_2$	Monoclinic
65-69	Cholesterol (5-cholesten-3beta-ol)	$C_{27}H_{46}O$	
	Calcium Hydrogen Phosphate Urea	$C_4H_{16}N_8O_4 \cdot Ca(H_2PO_4)_2$ / $Ca(H_2PO_4)_2 \cdot 4CO(NH_2)_2$	Monoclinic

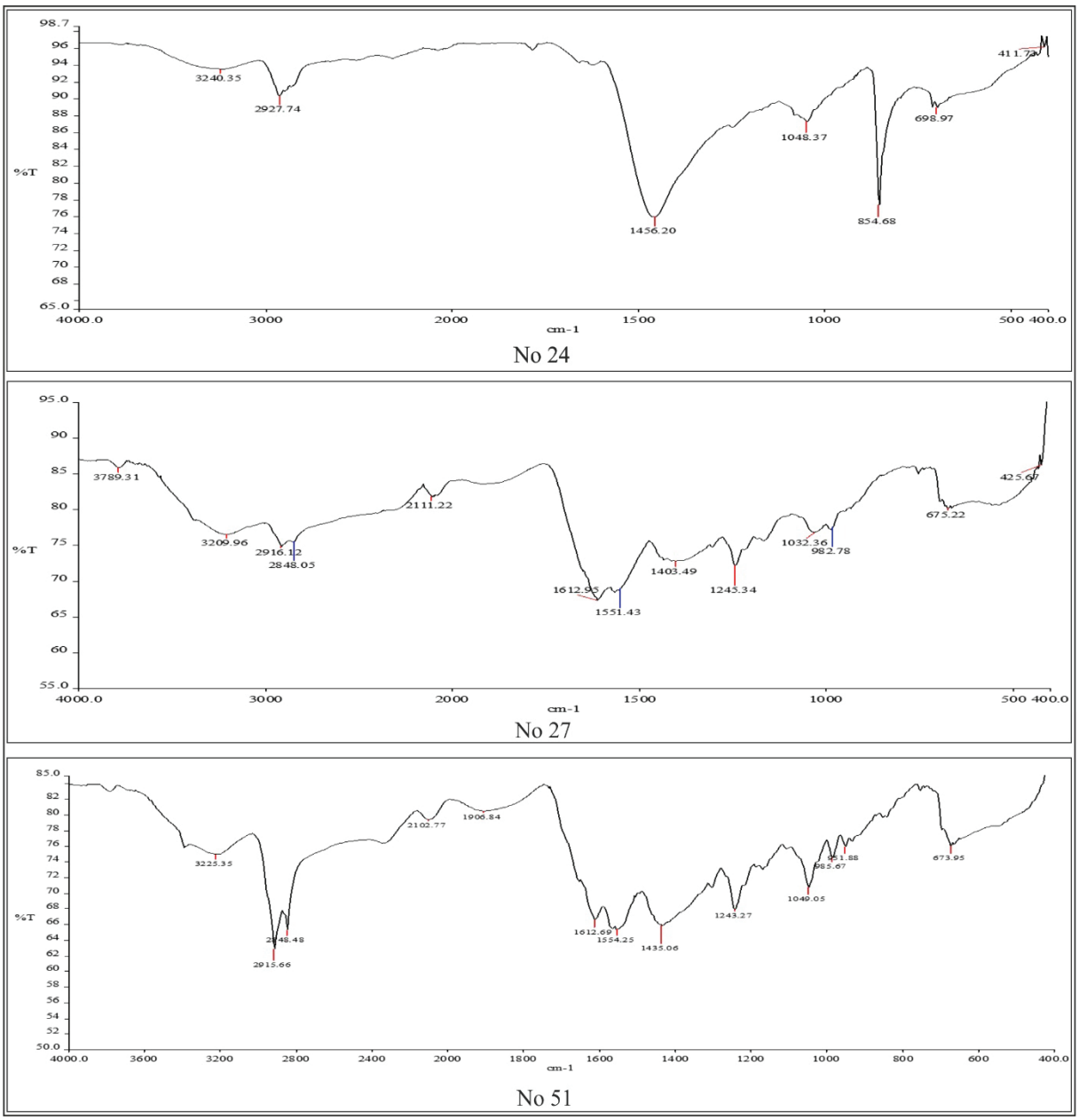

Figure 5. FTIR analysis peaks of the samples 24, 27, and 51.
Şekil 5. *24, 27 ve 51 numaralı örneklerin FTIR analizi pikleri.*

Dietary habits play an important role in this gallbladder disease, which is generally rare in the world. The environment in which individuals live, the food they eat and the concentrations of heavy metals in the water they drink are also important. Stress, age, improperly functioning thyroid hormones and structural changes in cancer cells can all contribute to the disease. Environmental triggers are particularly important in the development of gallbladder cancer (Hundal and Shaffer, 2014) Newberyite formation in the gallbladder is especially common when the pH

of the individual's bile is low and the amount of magnesium in the bile is high. Newberyte formation can also occur when there is a failure in the breakdown of magnesium during the patient's metabolism. Struvite can develop in association with the presence of ammonium phosphate in the individual's bile (Ortega et al., 1997; Glasdam et al., 2016; Wang et al., 2017). Ammonium phosphate is found in plant fertilizers and is readily soluble in water. Newheryite and struvite are involved in the complex mechanisms of production and destruction in the human body. Therefore, identifying the source of these newberyite and struvite minerals is crucial understanding the mechanisms of gallstone formation. There are many reasons for the development of stones in the gallbladder. The stones are formed as a result of the chemical imbalance in the bile produced by the liver to digest fats. When bile is released from the liver, it is about 800-1200 ml and 97% of it is water. This fluid is stored in the gallbladder until it reaches a concentration of 100-200 ml. The residue includes bilirubin, biliverdin, bile salts, bile acids, lipids (phospholipids, cholesterol and triglycerides), electrolytes, and certain enzymes. Over time, changes in the density of the components in the bile lead to the formation of gallstones (Kleiner et al., 2002; Liu et al., 2002; Wang et al., 2009; Altinkaya et al., 2011; Bazzano et al., 2011; Qiao et al., 2013; Yu et al., 2013; Usai et al., 2017; Dorvash et al., 2018; Hanafi et al., 2018; Gazali et al., 2019). In gallstones, any of the bile components alone can form crystals. Bile components can also form crystalline structures together. Gallstones can form together with their own components (Tazuma, 2017; Zhou et al., 2018; Önür and Beyler, 2001).

Among the methods used in gallstone disease, ultrasonography is used to accurately determine the point prevalence of gallstone disease. Using this method, it is the most preferred method especially to determine the point prevalence. This method can be given as the best epidemiologic screening method using this method; it is possible to understand the size and number of stones. Methods such as ultrasound, computed tomography and magnetic resonance imaging (MRI) or Endoscopic Ultrasonography (EUS), ERCP (Endoscopic Retrograde Cholangiopancreatography) etc. provide good information about the physical characteristics of the gallbladder, the shape and presence of gallstones and the diagnosis of the disease. However, these methods cannot accurately determine the mineral and chemical content of gallstones. In tests performed with ultrasound technology under laboratory conditions on individuals, it is based on the principle of sending the sound waves of the method through transmitter and converting the returning wavelength into an image. It is important that the patient fasts for at least 6 hours before the examination. The method is effective in detecting stones after fasting. Magnetic Resonance Cholangiopancreatography (MRCP) is a non-invasive imaging method that can provide detailed information about the anatomy and diseases of the gallbladder and biliary tract. After 6 hours of fasting, a 20 to 30 minute examination process is performed to reveal conditions such as stones, stenosis, tumor compression in the bile and pancreatic ducts and is used for diagnostic purposes only (Soto, 1996; Portincasa et al., 2006; Mandarano and Jim, 2008; Göbel et al., 2011; Ye et al., 2016; Kurtul et al., 2017). Laparoscopic cholecystectomy is the gold standard surgical treatment of gallstone disease and a commonly performed procedure in general surgery Identification of the mineral content of gallstones will contribute to the development of treatment methods (Nan et al. 2023). Owing to the identification of environmental factors and metabolic processes contributing to the formation of minerals detected in the content of gallstones and revealing the construction-destruction mechanisms of these minerals in the body, treatment will be available. Pigment stones were observed in only a few individuals of the study group.

Pigment stones were observed in a few individuals of the study group. Pigment stones in the gallbladder can be seen in different colors. These stones, especially black and brown pigment stones, have been observed in two forms. In this study, one out of every four pigment stones was identified as black pigment stones and FTIR analysis revealed the presence of calcium bilirubinate and protein. Black pigment stones consist either composed entirely of calcium bilirubinate or of polymer-like complexes containing calcium, copper and numerous amounts of mucin glycoprotein. These stones occur most commonly in cases of cirrhosis and chronic hemolysis (Sleisenger and Fordtran, 1998). Scientific studies have determined that the main elements of saffron are sodium, potassium and phosphorus (Golovanova et al. 2006). However, it is clear that calcium is the dominant element, especially in gallstones. It is the other components that make a difference to the chemical composition. In particular, the content and distribution of microelements in gallstones are important in this regard. These differences are determined by the environmental factors or regional characteristics of the individual. Brown pigment stones are composed of calcium salts, unconjugated bilirubin and varying amounts of cholesterol and protein; it is often associated with infection. However, regional nutrition types also gain importance. The bacteria in the biliary system secrete the B-glucuronidase enzyme, which hydrolyzes gluconic acid from conjugated bilirubin, which then leads to the formation of calcium salts by conjugated bilirubin, deconjugated bile acids and saturated fatty acids (Skar et al., 1989: Bistgani and Imani, 2013).

In the mineralogical analysis of gallstones samples from the studied region, newberyite and struvite minerals were found to be the most common minerals. Considering the XRD diffractogram data interpretations of the samples, newberyite was formulated as observed in the analysis images of the samples, which were available for cross-sectioning for SEM examination. SEM images of cholesterol gallstones were seen as cholesterol clumps consisting of overlapping plaques (as described in Trotman et al., 1974). Previous studies have reported that cholesterol content in frequently consumed foods influence the formation of gallstones (Baysal et al., 1991). In the mineralogical analysis of gallstone samples from the studied region, newberyite and struvite minerals were found to be the most common. Considering the XRD diffractogram data interpretations of the samples, newberyite was formulated as $MgHPO_4(H_2O)_3$, and struvite was formulated as $MgHPO_4(H_2O)_6$. Magnesium plays a role in the formation of more than 300 biochemical reactions in our body (Grubbs and Maguire, 1987; Altura, 1991; Ismail and Ismail, 2016). It has been proven that the element has positive effects on the heart, nervous system, and immune system (Finkelman et al., 2001; Selinus et al., 2005). Phosphorus is the most common element in the human body after calcium. Excessive amounts of these elements in the body lead to impairments in the digestive system and renal functions as well as many diseases related to the immune system (Bijvoet, 1977). The intake of these elements in the body is related to dietary habits, it occurs due to the consumption of potable water and the environment we live in (Varol et al., 2008; Moctezuma-Velázquez et al., 2017).

It is known that marble quarries in Antalya province pollute potable and irrigation water resources (Antalya Provincial Environmental Status Report, 2017). Due to the heavy metals pollution of water resources in residential and industrial zones, resulting from industrial production, mining and other enterprises not using appropriate operating methods, and improper disposale of operational waste, these pollutants can enter the body with essential elements for the organism, which are constantly required, such as water and air, and they can cause disease (Athanasiadou et al., 2013). Scientific studies of

gallstones from patients in various parts of the world have shown similar rich pigment stones in metal contaminated areas. Gallstone samples from patients in metal-polluted environmental areas were generally Ca-bilirubinate (Parviainen et al., 2018). Previous studies also suggest that pigment stones get higher metal concentrations that cholesterol stones, and that metals have an impact on the formation of pigment stones (Suzuki et al., 1975; Suhara et al., 1998; Ashok et al., 2003; Rautray et al., 2007; Palchik and Moroz, 2005; Omer, 2011; Sharma et al., 2015; Weerakoon et al., 2015; Butanovs et al. 2021). In particular, Ca, Cu, Fe, Mg, Mn, Pb, and Zn concentrations tend to increase in pigment stones (Suzuki, 1966). Randomly floating sediments in bile as an adjuvant, together with proteins that induce stone formation, act as a nucleate (Chuang et al., 2012). Numerous studies indicate that the presence of bacteria is related calcium bilirubinate (Vitetta et al., 1989; Flores et al., 2003; Güdücüoğlu et al., 2004; Petakovic et al., 2002; Kose et al., 2018). The presence of palmitic acid as a result of the reaction of bacteria with bile enzymes has been revealed in previous studies (Robins et al., 1982; Vitetta et al., 1989; Mir et al., 2013). Calcium carbonate gallstone samples are very rare in the literatures. They have been reported as few as 1/20,000 in previous studies (Griffith, 1978; Moe, 2008).

CONCLUSION

64% of the 69 patients in our study were female patients and 36% were male patients. 95% of the samples were cholesterol stones and 5% of them were pigment stones. They have been classified as cholesterol stones, mixed cholesterol stones and brown and black pigment stones. Moreover, they have been classified as cubic, morular, oval, anhedral, fragmented, prismatic, and dendritic in terms of their form. The maximum number of gallbladder stones (437) and the largest gallstone sample (4.4 cm) were obtained from the female patients. This study supports the fact that in the world health sector, overweight, female, fair-haired (blonde), over forty in age and fertile individuals are those potentially to be ill. Therefore, preventive measures should be taken in individuals who will be affected by personal characteristics that cannot be changed and especially by adverse environmental factors. Strategies for personal health measures and healthy environmental conditions should be established before gallstones form or become lodged in the biliary tract. The samples 24, 27, 31, and 51 were determined as pigment stones. The color of these four samples ranges between dark green and black. The sample 27 is in the dendritic type and it developed by branching outward from the central nucleus. The outer surfaces of Sample 31 and 51 are coated with a dark crust, which can be broken down quickly and are smooth. Sample 24 has an oval form with a morular texture on its surface. The presence of bacteria was observed in SEM images of Sample 51. FTIR analysis of samples showed the presence of calcium bilirubinate, cerumen, protein, bilirubin, palmitic acid and ammonium urate. In the literature, there are studies that reveal the presence of bacteria having as an impact on the presence of palmitic acid and palmitate. The FTIR analysis of Sample 24 showed the presence of barium acetate and aluminum silicate. The common feature of both these chemicals is that they are used in some paints. When harmful chemicals entering the body cannot be removed, they can accumulate in organs and cause disease. Also, calcium bilirubinate together with aragonite were detected, which is related to the presence of bilirubinate and bacteria. Calcium carbonate, which is rare in the literature, was rarely observed in the samples. Among the 69 samples in this study, only one $CaCO_3$ polymorph aragonite gallstone was found. Aragonite, vaterite, and calcite are various polymorphs of carbonate and their varieties can be transformed into each other by changing the conditions supporting

the formation mechanisms. It is possible to make many interpretations of the mechanisms supporting the formation of stones that contain $CaCO_3$ in humans. According to XRD results of the gallbladder samples, 25 of which were taken from male patients and 44 from female patients, newberyite, struvite, aragonite, cholesterol dibromide, and calcium hydrogen phosphate were found in 59, 3, 1, 1, and 9 samples respectively. Cholesterol, cholesterol derivatives (5-cholesten-3beta-ol), which are an important steroid and found in all mussels, is the main component of cholesterol dibromide gallstones. Examining the XRD analysis data of the samples 9, 12, and 43, the newberyite mineral was observed together with struvite mineral.

The formation mechanisms of gallstones can only be understood by forming control groups that will be followed up for a long time and examining the characteristics of these control groups (nutrition, stress, environment, environmental conditions, hormones, age, ete.). In future studies, the relationship of these data with each other should be evaluated concerning the determined gallstone sample (cholesterol, pigment). For example, an individual with diabetes is more likely to have gallstones. Significant data can only be obtained in the studies after collecting all the history of the individuals.

GENİŞLETİLMİŞ ÖZET

Bireyler bulundukları çevresel ortamdaki kirleticilerin etkisine maruz kalabilirdiler. Bu kirleticilere maruz kalan bireyler sağlık sorunları riski taşırlar. Ayrıca, muhtelif araştırmalar obezite, yaş, doğurganlık, ırk, beslenme vb. kişisel faktörlerin de sağlık sorunları üzerindeki ilişkilerinden bahsetmişlerdir. Kişisel ve çevresel etkilerle ortaya çıkabilecek olası sağlık sorunlarından birisi, safra kesesi taşlarının oluşumudur. Morfoloji ve içeriklerine göre "kolesterol taşları, pigment taşları, kalsiyum karbonat taşları, fosfat taşları, kalsiyum stearat taşları, protein taşları, sistin taşları ve karışık taşlar" şeklinde isimlendirilirler. Çalışmanın amacı, Antalya bölgesinde yaşayan bireylere ait safra taşı örneklerinin fiziksel, kimyasal ve mineralojik özelliklerinin belirlenmesidir. Bu özelliklerin belirlenmesi, hastaların tedavisine fayda sağlayacak olup, bölge halkının kişisel ve çevresel faktörleri dikkate almasını sağlayacaktır. Araştırma için kullanılan safra taşı örnekleri, "safra kesesi taşı" tanısı sebebiyle ameliyat edilen 25 erkek ve 44 kadın hastadan, hasta hakları prosedürüne uygun şekilde temin edilmiştir. Her bir örnek literatüre uygun laboratuvar koşullarında hazırlanarak analiz edilmiştir. Fiziksel özellikler jeoloji laboratuvarında belirlenmiş, mineralojik özelliklerin belirlenmesi için XRD (X-Işını Difraktometre Cihazı) ile SEM (Taramalı elektron mikroskobu) analizleri ve kimyasal özelliklerinin saptanabilmesi için FT-IR (Fourier Dönüşümlü Kızılötesi Spektroskopisi) analizi gerçekleştirilmiştir.

Sarı, beyaz, siyah, kahverengi, yeşil renklerde gözlenen safra taşları, orta sert ve yumuşak dayanım göstermiştir. Oval, kübik, öz şekilsiz, dendritik yüzeyli, prizmatik ve parçalı şekiller sunan, 0,1 ile 4,4 cm arası çaplarında bulunan örnekler kolesterol taşı, mixed kolesterol taşı ve pigment taşı (numune 24, 27, 31, 51) olarak sınıflanmıştır (Şekil 1 ve 2). En fazla sayıda safra kesesi taşı (437 adet) ve en büyük safra kesesi taşı örneği (4,4 cm) kadın hastalardan temin edilmiştir. SEM ve FTIR analizlerinde örnekler kolesterol taşı, karbonat apatit, kalsiyum karbonat, kalsiyum fosfat karbonat, kalsiyum bilirubinat ve protein belirlenmiştir (Şekil 3 ve 4). Bazı kollesterol safra taşlarının (numune 16) SEM görüntüleri, üst üste plakalardan oluşan kolesterol yığınları olarak izlenmişlerdir. Pigment taşında da (numune 51) bakteri tespit edilmiştir (Şekil 4a). XRD analizlerinde 59 adet Nevberyit (MgHPO4(H2O)3), 3 adet Struvit ($MgNH\ 4PO_4(H_2O)_6$), 1 adet Aragonit ($Ca(CO_3)$),

9 adet Kalsiyum hidrojen fosfat üre ($C_4H_{16}N$ $8O_4{\cdot}Ca(H_2PO_4)_2$ / $Ca(H_2PO_4)_2$ $4CO(NH_2)_2$) ve 1 adet Kolestrol dibromid ($C_{27}H_{44}Br_{20}$) gözlenmiştir (Çizelge 1). Önemli bir steroit olan ve tüm kaslarda bulunan kolesterol ve türevi Kolesterol (5-cholesten-3beta-ol), kolesterol dibromid safra taşlarının ana bileşenidir. Nevberyit minerali ile birlikte Struvit minerali bulunduğu örnekler gözlenmiştir (numune 9, 12, 43). FTIR analizlerinde struvit, apatit, vitlokit nevberyit, karbon apatit, hidroksil apatit, kalsiyum fosfat karbonat, aragonit, kalsiyum bilirubinat (numune 25, 26, 27, 28), alüminyum silikat, baryum asetat, aragonit, kalsit, palmitik asit (numune 29) belirlenmiştir. Çevresel etkinin varlığını gösteren 24 nolu örneğin FTIR analizlerinde, bazı boyaların içinde kullanılan baryum asetat ve alüminyum silikat varlığı izlenmiştir. Bu zararlı kimyasalların vücuttan atılamadığında organlarda birikerek olası bu türlü hastalıklara sebebiyet verebileceği düşünülmüştür. Ayrıca aragonit ile beraber kalsiyum bilirubinat tespit edilmiştir ki daha önceki çalışmalar, bilirubinat varlığı ile bakteri varlığının ilişkili olduğunu ortaya koymuştur. Literatürde, bu tip örneklere nadir rastlanmaktadır. Alınan 69 adet örneğin % 64'ü kadın, %36'sı erkek hastalara aittir. Örneklerin %95'i kolestrol, %5'i pigment taşıdır.

Bu çalışma ile de dünya sağlık sektöründe, Fat (şişman), Female (kadın), Fairy (sarışın), Forty (40 yaş üstü), Fertile (doğurgan) bireylerin potansiyel hasta oldukları olgusunu desteklemiştir. Bu çalışmada literatürde nadir görülen bilirubinat ile bakteri varlığının ilişkisini belirlemek önemli bir bulgudur. Ayrıca, safra taşı içinde baryum asetat ve alüminyum silikat varlığı çevresel ortamdaki kirleticiler ile olan ilişkiyi ortaya çıkarması, bu çalışmanın önemini artırmıştır.

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REFERENCES

Altinkaya, N., Koc, Z., Alkan, O., Demir, S. & Belli, S. (2011). Multidetector computed tomography diagnosis of ileal and anthropologic gallstone ileus. *Turkish Journal of Trauma and Emergency Surgery, 17*(5), 461-463. https://doi.org/10.5505/tjtes.2011.50103

Altura, B. M. (1991). Basic Biochemistry and Physiology of Magnesium; A Brief Review, *Magnes Trace Element, 10*, 167-71 .

Antalya Provincial Environmental Status Report. webdosya.csb.gov.tr/db/ced/icerikler/antalya_2017_cevre_durum_raporu 20180705142911.pdf (2017).

Ashok, M., Rautray, T., Nayak, P., Vijayan, V., Jayanthi, V. & Narayana, K. S. (2003). Energy-dispersive X-ray fluorescence analysis of gallstones. *Journal of Radioanalytical and Nuclear Chemistry, 257*(2), 333-335. https://doi.org/10.1023/A:1024735930031

Athanasiadou, D., Godelitsas, A., Sokaras, D., Karydas, A. G., Dotsika, E., Potamitis, C., ... & Becker, U., 2013. New insights into the chemical and isotopic composition of human-body biominerals. I: Cholesterol gallstones from England and Greece. *Journal of Trace Elements in Medicine and Biology*, *27*(2), 79-84. https://doi.org/10.1016/j.jtemb.2012.08.004

Baysal, A., Kececioglu, S. & Arslan, P. (1991). Compositionof Foods, Turkish Dietetic Association Publication No 1.*Yenicag, Basın-Yayın San*, Ankara, Turkey.

Bazzano, L. A., Thompson, A. M., Tees, M. T., Nguyen, C. H. & Winham, D. M. (2011). Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *21*(2), 94-103. https://doi.org/10.1016/j.numecd.2009.08.012

Bijvoet, Olav L. M. (1977). Kidney function in calcium and phosphate metabolism. In: Metabolic bone disease. *Academic Press*, p. 49-140. https://doi.org/10.1016/B978-0-12-068701-5.50008-7

Bistgani, M. M. & Imani, R. (2013). Bacteria isolated from patients with cholelithiasis and their antibacterial susceptibility pattern. *Iranian Red Crescent Medical Journal*, *15*(8), 759 https://doi.org/10.5812/ircmj.3883

Butanovs, E., Zolotarjovs, A., Kuzmin, A. & Polyakov, B. (2021). Nuclear Instruments and *Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. https://doi.org/10.1016/j.nima.2021.165736

Cen, L., Pan, J., Zhou, B., Yu, C., Li, Y., Chen, W. & Shen, Z. (2018). Helicobacter Pylori infection of the gallbladder and the risk of chronic cholecystitis and cholelithiasis: A systematic review and meta analysis. *Helicobacter*, *23*(1), Article no: e12457. https://doi.org/10.1111/hel.12457

Chuang, S. C., Hsi, E. & Lee, K. T. (2012). Mucin genes in gallstone disease. *Clinica Chimica Acta*, *413*(19-20), 1466-1471. https://doi.org/10.1016/j.cca.2012.06.015

Cohen, S. Z., Creeger, S. M., Carsel, R. F. & Enfield, C. G. (1984). Potential pesticide contamination of groundwater from agricultural uses. *Treatment and Disposal of Pesticide Wastes,* 259, 297. https://doi.org/10.1021/bk-1984-0259.ch018

Damalas, C. A., Telidis, G. K. & Thanos, S. D. (2008). Assessing farmers' practices on disposal of pesticide waste after use. *Science of the total environment, 390*(2-3), 341-345. https://doi.org/10.1016/j.scitotenv.2007.10.028

Dorvash, M. R., Khoshnood, M. J., Saber, H., Dehghanian, A., Mosaddeghi, P. & Firouzabadi, N. (2018). Metformin treatment prevents gallstone formation but mimics porcelain gallbladder in C57Bl/6 mice. *European Journal of Pharmacology*, *833*, 165-172. https://doi.org/10.1016/j.ejphar.2018.06.002

Finkelman, R. B., Skinner H. C. W, Plumlee, G. S. & Bunnell, J. E. (2001). Medical Geology. *Geotimes,* 20-23

Flores, C., Maguilnik, I., Hadlich, E. & Goldani, L. Z. (2003). Microbiology of choledochal bile in patients with choledocholithiasis admitted to a tertiary hospital. *J Gastroenterol Hepatol*, *18*(3):333-6. https://doi.org/10.1046/j.1440-1746.2003.02971.x

Gazali, Z., Thakur, S. N. & Rai, A. K. (2019). Compositional study of gallbladder stone using photoacoustic spectroscopy. *Optics & Laser Technology*, *111*, 696-700. https://doi.org/10.1016/j.optlastec.2018.09.003

Glasdam, S. M., Glasdam, S. & Peters, G. H. (2016). The importance of magnesium in the human body: a systematic literature review. *In Advances in Clinical Chemistry*,*73*, 169-193. https://doi.org/10.1016/bs.acc.2015.10.002

Golovanova, 0. A., Pakhik, N. A., Berezina, N. Y., & Yudina, L. N. (2006). Comparative characteristics of the mineral and microelement composition of gallstones extracted from patients in the Novosibirsk and Omsk regions. *Chemistry for Sustainable Development, 14,* 125- 131.

Göbel, T., Kubitz, R., Blondin, D., & Häussinger, D. (2011). Intrahepatic type II gall bladder perforation by a gall stone in a CAPD patient. European journal of medical research, *16*(5), 213-216. https://doi.org/10.1186/2047-783X-16-5-213

Griffith, D. P. (1978). Struvite stones. *Kidney International,* Vol. 13, 372 - 382. https://doi.org/10.1038/ki.1978.55

Grigor'eva, I. N. (2020). Gallstone disease, obesity and the Firmicutes/Bacteroidetes ratio as a possible biomarker of gut dysbiosis. *Journal of Personalized Medicine*, *11*(1), 13. https://doi.org/10.3390/jpm11010013

Grubbs, R. D. & Maguire, M. E. (1987). Magnesium as a regulatory cation: *Criteria and Evajuation. Magnesium*, *6,* 113-27.

Güdücüoğlu, H., Bozkurt, H., Bayram, Y., Yaman, G., Berktas, M. (2004). Microorganisms isolated from bile samples of the patients who

had cholecystectomy and their antibiotics susceptibilities. *Genel Tıp Dergisi (Journal of General Medicine), 14*(1), 13-7.

Hanafi, N. I., Mohamed, A. S., Kadir, S. A., Hamimah, S. & Othman, M. H. D. (2018). Overview of bile acids signaling and perspective on the signal of ursodeoxycholic acid, the most hydrophilic bile acid, in the heart. *Biomolecules*, *8*(4), 159. https://doi.org/10.3390/biom8040159

Hu, H., Shao, W., Liu, Q., Liu, N., Wang, Q., Xu, J., ... & Gu, A. (2022). Gut microbiota promotes cholesterol gallstone formation by modulating bile acid composition and biliary cholesterol secretion. *Nature Communications, 13*(1), 252. https://doi.org/10.1038/s41467-021-27758-8

Hundal, R., & Shaffer, E. A. (2014). Gallbladder cancer: epidemiology and outcome. Clinical Epidemiology, 99-109. https://doi.org/10.2147/CLEP.S37357

Huner, U., Gulec, H. A. & Damar Huner, I. (2017). Effect of gas type and application distance on atmospheric pressure plasma jet-treated flax composites. *Journal of Reinforced Plastics and Composites, 36*(17), 1197-1210. https://doi.org/10.1177/0731684417703490

Iordanidis, A., Garcia-Guinea, J., Giousef, C., Angelopoulos, A., Doulgerakis, M. & Papadopoulou, L. (2013). Characterization of gallbladder stones from cholelithiasis patients of Northern Greece, using complementary techniques. *Spectroscopy Letters*, *46*(4), 301-306. https://doi.org/10.1080/00387010.2012.728552

Ismail, A.A. & Ismail, N.A. (2016). Magnesium: A mineral essential for health yet generally underestimated or even ignored. *J. Nutr. Food Sci, 6*(2). https://doi.org/10.4172/2155-9600.1000523

Kabakci D., Yalcin M. G. & Elpek G. O. (2016). The Determination of the Mineralogical and Chemical Composition of Gallstones (Antalya) and Assessment in Terms of Medical Geology. *69th Geological Congress of Turkey*, 11-15 April 2016, 46-47. ISBN: 978-605-01-0869-9

Kleiner, O., Ramesh, J., Huleihel, M., Cohen, B., Kantarovich, K., Levi, C. & Mordechai, S. (2002). A comparative study of gallstones from children and adults using FTIR spectroscopy and fluorescence microscopy. *BMC Gastroenterology, 2*(1), 3. https://doi.org/10.1186/1471-230x-2-3

Kose, S. H., Grice, K., Orsi, W. D., Ballal, M. & Coolen, M. J. L. (2018). Metagenomics of pigmented and cholesterol gallstones: the putative role of bacteria. *Scientific Reports,* 8, Article 11218. https://doi.org/10.1038/s41598-018-29571-8

Kurtul, Y. H., Sahin, G., Ekin, E. E., Erok, B. & Adas G. T. (2017). Diagnostic Contribution of Magnetic Resonance Cholangiopancreatography in Biliary Obstruction: Additional Findings and Misdiagnosis. *JAREM*, *7*, 135-9. https://doi.org/10.5152/jarem.2017.1371

Lammert, F., Gurusamy, K., Ko, C. W., Miquel, J. F., Mendez-Sanchez, N., Portincasa, P. & Wang, D. Q. H. (2016). Gallstones. *Nature reviews Disease primers*, *2*(1), 1-17. https://10.1038/nrdp.2016.24

Liu, G., Xing, D., Wang, H. & Wu, J. (2002). Vibrational spectroscopic study of human pigment gallstones and their insoluble materials. *Journal of Molecular Structure*, *616*(1-3), 187-191. https://doi.org/10.1016/S0022-2860(02)00330-7

Mandarano, G. & Sim, J. (2008). The diagnostic MRCP examination: overcoming technical challenges to ensure clinical success. *Biomedical Imaging and Intervention Journal*, *4*, Article e28. https://doi.org/10.2349/biij.4.4.e28

McBride, M.B. (1995). Toxic metal accumulation from agricultural use of sludge: are USEPA regulations protective?. *Journal of Environmental Quality*, *24*(1), 5-18. https://doi.org/10.2134/jeq1995.00472425002400010002x

Mir, M. A., Malik, U. Y., Wani, H. & Bali, B. S. (2013). Prevalence, pattern, sensitivity and resistance to antibiotics of different bacteria isolated from port site infection in low risk patients after elective laparoscopic cholecystectomy for symptomatic cholelithiasis at tertiary care hospital of Kashmir. *International Wound Journal*, *10*(1), 110-113. https://doi.org/10.1111/j.1742-481X.2012.00963.x

Moctezuma-Velázquez, C., Gómez-Sámano, M. Á., Cajas-Sánchez, M. B., Reyes-Molina, D. L., Galindo-Guzmán, M., Meza-Arana, C. E., Cuevas-Ramos, D., Gómez-Pérez, F. J. & Gulias-Herrero, A., (2017). High dietary magnesium intake is significantly and independently associated with higher insulin sensitivity in a Mexican-mestizo

population: a brief cross-sectional report. *Revista de Investigación Clínica*, *69*(1), 40-46. https://doi.org/10.24875/ric.17002086

Moe, S.M. (2008). Disorders involving calcium, phosphorus, and magnesium. Primary Care: *Clinics in Office Practice*, *35*(2), 215-237. https://doi.org/10.1016/j.pop.2008.01.007

Molinero, N., Ruiz, L., Milani, C., Gutiérrez-Díaz, I., Sánchez, B., Mangifesta, M., Segura, J., Cambero, I., Campelo, A.B., García-Bernardo, C.M., Cabrera, A., Rodríguez, J.I., González, S.I., Rodríguez, J.M., Ventura, M., Delgado, S. & Margolles, A. (2019). The human gallbladder microbiome is related to the physiological state and the biliary metabolic profile. *Microbiome*, *7*(1), 100. https://doi.org/10.1186/s40168-019-0712-8

Nan, X., Prabha, R. D., Rashid, M. & Patel, B. (2023). Journey of a stone-thirty years on. *Journal of Surgical Case Reports, 2*, rjad069. https://doi.org/10.1093/jscr/rjad069

Omer, L. S. (2011). Quantitative analysis in (33) traces metals in human gallstones by ICP-AES. *International Journal of Chemistry*, *3*(2), 105. https://doi.org/10.5539/ijc.v3n2p105

Ortega, R. M., Fernández-Azuela, M., Encinas-Sotillos, A., Andres, P. & Lopez-Sobaler, A. M. (1997). Differences in diet and food habits between patients with gallstones and controls. *Journal of the American College of Nutrition*, *16*(1), 88-95. https://doi.org/10.1080/07315724.1997.10718655

Önür, N. D. & Beyler, A. R. (2001). Safra Asitleri Metabolizmasi. *Ankara Universitesi Tıp Fakültesi Mecmuası*, *54*(1). (in Turkish).

Palchik, N. A. & Moroz, T. N., (2005). Polymorph modifications of calcium carbonate in gallstones. *Journal of Crystal Growth*, *283*(3-4), 450-456. https://doi.org/I10.1016/j.jcrysgro.2005.05.035

Parviainen, A., Marchesi, C., Suarez-Grau, J. M., Garrido, C. J., Perez-Lopez, R., Nieto, J. M. & Cobo-Cardenas, G. (2018). Unraveling the impact of chronic exposure to metal pollution through human gallstones. *Science of the Total Environment*, *624*, 1031-1040. https://doi.org/10.1016/j.scitotenv.2017.12.224

Parviainen, A., Suarez-Grau, J. M., Perez-Lopez, R., Nieto, J. M., Garrido, C. J. & Cobo-Cardenas, G. (2016). Combined microstructural and mineralogical phase characterization of gallstones in a patient-based study in SW Spain-Implications for environmental contamination in their formation. *Science of the Total Environment*, *573*, 433-443. https://doi.org/10.1016/j.scitotenv.2016.08.110

Petakovic, G., Korica, M, Gavrilovic, S., 2002. Bacteriologic examination of gallbladder contents, *Med Pregl*, *55*(5-6):225-8. https://doi.org/10.2298/mpns0206225p

Pichugina, A. A., Tsyro, L.V. & Unger, F. G., (2018). IR Spectroscopy and X-Ray Phase Analysis of the Chemical Composition of Gallstones. *Journal of Applied Spectroscopy*, *84*(6), 1024-1029. https://doi.org/10.1007/s10812-018-0581-0

Portincasa P., Moschetta A., Petruzzelli M., Palasciano G., Di Ciaula A. & Pezzolla A., (2006). Gallstone disease: symptoms and diagnosis of gallbladder stones. *Best Practice & Research Clinical Gastroenterology, 20*, 1017–29. https://doi.org/10.1016/j.bpg.2006.05.005

Qiao, T., Ma, R. H., Luo, X. B., Yang, L. Q., Luo, Z. L. & Zheng, P. M. (2013). The systematic classification of gallbladder stones. *PLOS One, 8*(10), Article e74887. https://doi.org/10.1371/journal.pone.0074887

Ramya, J. R., Arul, K. T., Epple, M., Giebel, U., Guendel-Graber, J., Jayanthi, V., Sharma, M., Rela, M. N. & Kalkura, S. N. (2017). Chemical and structural analysis of gallstones from the Indian subcontinent. *Materials Science and Engineerin*g, *C, 78*, 878-885. https://doi.org/10.1016/j.msec.2017.04.004

Rautray, T. R., Vijayan, V. & Panigrahi, S. (2007). Analysis of Indian pigment gallstones. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Ato*ms, *255*(2), 409-415 https://doi.org/10.1016/j.nimb.2006.12.147

Robins, S. J., Fasulo, J. M. & Patton, G. M., (1982). Lipids of pigment gallstones. *Biochimica et Biophysica Acta, 712*, 21–25. https://doi.org/10.1016/0005-2760(82)90079-0

Selinus, 0., Alloway, B., Centeno, J. A., Finkelman, R. B, Fuge, R., Lindh, U. & Smedley, P. (2005). *Esential Medical Geology.* 115-594. https://doi.org/10.1007/978-94-007-4375-5

Sharma, R., Soy, S., Kumar, C., Sachan, S. G. & Sharma, S.R. (2015). Analysis of gallstone composition and structure in Jharkhand region. *Indian Journal of Gastroenterology*, 34(1), 29-37. https://doi.org/10.1007/s12664-014-0523-6

Skar, V., Skar, A. G. & Bratlie, J. (1989). Beta-glucuronidase Activity in the Bile of Gallstone Patients Both with and without Duodenal Diverticula. *Scandinavian Journal of Gastroenterology*, *24*, 205. https://doi.org/10.3109/00365528909093038

Sleisenger & Fordtran. (1998). *Gastrointestinal and Liver Disease*, *6th ed.,* p. 631-644.

Soodan, R. K., Pakade, Y. B., Nagpal, A. & Katnoria, J. K. (2014). Analytical techniques for estimation of heavy metals in soil ecosystem. A tabulated review. *Talanta*, *125*, 405-410. https://doi.org/10.1016/j.talanta.2014.02.033

Soto, J. A., Barish, M. A., Yucel, E. K., Siegenberg, D., Ferrucci, J. T. & Chuttani, R. (1996). Magnetic resonance cholangiography: comparison with endoscopic retrograde cholangio pancreatography. *Gastroenterology 110*(2), 589-97. https://doi.org/10.1053/gast.1996.v110.pm8566608

Srinivasan, K. (2016). BiologicaJ activities of red pepper (Capsicum annuum) and its pungent principle capsaicin: a review. *Critical Reviews in Food Science and Nutrition*, *56*(9), 1488-1500 https://doi.org/10.1080/10408398.2013.772090

Stinton, L. M. & Shaffer, E. A. (2012). Epidemiology of gallbladder disease: cholelithiasis and cancer. *Gut and liver*, *6*(2), 172. https://doi.org/10.5009/gnl.2012.6.2.172

Suhara, M., Matsuda, K. & Sera, K., (1998). Elemental concentrations of four types of human gallstones by PIXE analysis. *International Journal of PIXE*, *8*(01), 73-88. https://doi.org/10.1142/S0129083598000091_

Suzuki, N., (1966). On bilirubin-metal complex compounds in relation to black pigments of gallstones. *The Tohoku Journal of Experimental Medicine*, *90*, 195-205. https://doi.org/10.1620/tjem.90.195

Suzuki, N., Nakamura, Y., Kobayashi, N. & Sato, T. (1975). On metal elements in pure pigment gallstones. *The Tohoku Journal of Experimental Medicine*, *116*(3), 233-240. https://doi.org/10.1620/tjem.116.233

Tazuma, S. (2017). Bile Acids and Gallstones: Epidemiology, Pathogenesis, Diagnosis, and Management. *In Bile Acids in Gastroenterology* (pp. 93-107). Springer, Tokyo. https://doi.org/10.1007/978-4-431-56062-3_6

Trotman, B.W., Ostrow, J.D., Soloway, R.D., Cheong, E.B. & Longyear, R.B. (1974). Pigment vs cholesterol cholelithiasis: comparison of stone and bile composition. *The American Journal of Digestive Diseases*, *19*, 585–90. https://doi.org/10.1007/BF01073011

Unisa, S., Jagannath, P., Dhir, V., Khandelwal, C., Sarangi, L. & Roy, T.K. (2011). Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. *HPB*, *13*(2), 117-125. https://doi.org/10.1111/j.1477-2574.2010.00255.x

Usai, D., Maritan, L., Dal Sasso, G., Artioli, G., Salvatori, S., Jakob, T. & Salviato, T. (2017). Late Pleistocene/Early Holocene Evidence of Prostatic Stones at Al Khiday Cemetery, Central Sudan. *PLOS One*, *12*(1), e0169524. https://doi.org/10.1371/journal.pone.0169524

van Dam, R. M., Hu, F. B. & Willett, W. C. (2020). Coffee, caffeine, and health. *New England Journal of Medicin*e, *383*(4), 369-378.

Varol, S., Davraz, A. & Varol, E. (2008). Yeraltı suyu kimyası ve safra etkisinin Tıbbi Jeoloji açısından degerlendirilmesi. *TAF Preventive Medicine Bulletin*, *714*, 351-356. (in Turkish)

Vitetta, L., Sali, A., Moritz, V., Shaw, A., Carson, P., Little, P. & Elzarka, A. (1989). Bacteria and gallstone nucleation. *Australian and New Zealand Journal of Surgery*, *59*(7), 571-577. https://doi.org/10.1111/j.1445-2197.1989.tb01633.x

Wang, D.Q., Cohen, D.E. & Carey, M.C. (2009). Biliary lipids and cholesterol gallstone disease. *Journal of Lipid Research, 50 (Supplement),* S406-S411. https://doi.org/10.1194/jlr.R800075-JLR200

Wang, J., Ma, X. Y., Feng, Y. F., Ma, Z. S., Ma, T. C., Zhang, Y., Li, X., Wang, L. & Lei, W. (2017). Magnesium ions promote the biological behaviour of rat calvarial osteoblasts by activating the PI3K/Aktsignalling pathway. *Biological Trace*

Element Research, *179*(2), 284-293. https://doi.org/10.1007/s12011-017-0948-8

Weerakoon, H., Navaratne, A., Ranasinghe, S., Sivakanesan, R., Galketiya, K.B. & Rosairo, S. (2015). Chemical characterization of gallstones: an approach to explore the aetiopathogenesis of gallstone disease in Sri Lanka. *PLOS One, 10*(4), Article e0121537. https://doi.org/10.1371/journal.pone.0121537

Yalcin, M. G. (2013). Examining the gallstones in Nigde Province. *2nd Medical Geology Workshop,* 283-284. https://doi.org/10.13140/RG.2.1.3911.9121

Yalcin, M. G., Yuksel, S., Akcadag, T., Copuroglu, I. & Aslan, E. (2011). The Observation of Gall Bladder Stone with Petrography and FTIR Method, *64th Geological Congress of Turkey*, 53-55.

Ye, F., Shen, H., Li, Z., Meng, F., Li, L., Yang, J., Chen, Y., Bo, X, Zhang, X. & Ni, M. (2016). Influence of the biliary system on biliary bacteria revealed by bacterial communities of the human biliary and upper digestive tracts. *PloS one, 11*(3), Article e0150519. https://doi.org/10.1371/journal.pone.0150519

Yu, J. K., Pan, H., Huang, S. M., Huang, N. L., Yao, C. C., Hsiao, K. M. & Wu, C. W. (2013). Calcium content of different compositions of gallstones and pathogenesis of calcium carbonate gallstones. *Asian Journal of Surgery*, *36*(1), 26-35. https://doi.org/10.1016/j.asjsur.2012.06.001

Yuan, S., Gill, D., Giovannucci, E. L., & Larsson, S. C. (2022). Obesity, type 2 diabetes, lifestyle factors, and risk of gallstone disease: a Mendelian randomization investigation. *Clinical Gastroenterology and Hepatology*, *20*(3), Article e529-e537. https://doi.org/10.1016/j.cgh.2020.12.034

Zhou, J. S., Chen, H. J., Ji, H., Shi, X. C., Li, X. X., Chen, L. Q., Du, Z. Y. & Yu, H. B. (2018). Effect of dietary bile acids on growth, body composition, lipid metabolism and microbiota in grass carp (Ctenopharyngodonidella). *Aquaculture nutrition*, *24*(2), 802-813. https://doi.org/10.1111/anu.12609

Zuo, M., Rashid, A., Wang, Y., Jain, A., Li, D., Behari, A., Kapoor, V.K., Koay, E.J., Chang, P., Vauthey, J.N., Li, Y., Espinoza, J.A., Roa, J.C., Javle, M. (2016). RNA sequencing-based analysis of gallbladder cancer reveals the importance of the liver X receptor and lipid metabolism in gallbladder cancer. Oncotarget, 7(23), 35302. https://10.18632/oncotarget.9181