ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.5281/zenodo.15739462

Differentiation of Gallbladder Stones According to Their External Structure and Cross-Sectional Morphological Features such as Color, Size and Shape

Safra Kesesi Taşlarının Dış Yapılarına ve Renk, Boyut ve Şekil Gibi Kesitsel Morfolojik Özelliklerine Göre Ayırt Edilmesi

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Abstract

Gallstones are gravel-like substances that form over time inside the gallbladder and are one of the most painful biliary tract diseases in adults, and are known to have a high incidence. Bile is composed of water, cholesterol, fats, bile salts, proteins, and bilirubin. While bile can usually dissolve cholesterol, crystals that can eventually turn into stones can form in the presence of too much cholesterol. It is difficult to define a clear classification system according to the types of an ideal gallstone, but we can generally group them according to their main chemical composition. Key factors in the etiopathogenesis of gallstone disease can be determined by chemical, structural, and elemental composition analyses. Different complementary analytical techniques, both microscopic and spectroscopic, can be applied, and the ultrastructure of gallstones and the identification of trace elements can also be done. The aim of this study was to investigate the structural features of gallstones in sixty patients accepted for cholecystectomy from Erzincan and surrounding regions. In this study, gallstone samples were collected from 60 patients who were hospitalized in Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital General Surgery Clinic and underwent cholecystectomy due to symptomatic gallbladder disease without evidence of gallbladder malignancy between February 2025 and April 2025. The external and cross-sectional morphological features of gallstones, such as color, size, shape, and number of stones, were examined with the naked eye, and their photographs were taken with high sensitivity and resolution. Selected gallstones showing distinct stratification as core, middle layer, and shell were separated according to these factors. In addition, when the structure of the stones was examined, concentric layers containing alternating dark and light-colored bands were observed, emphasizing the importance of compositional changes in bile during the formation of gallstones.

Keywords: Gallbladder stone, External structure, Cross-sectional morphological feature.

Öz

Safra kesesinin içinde zamanla oluşan çakıl benzeri maddeler olan safra taşları, yetişkinlerde en ağrılı safra yolu hastalıklarından biridir ve yüksek oranda görüldüğü bilinmektedir. Safra, su, kolesterol, yağlar, safra tuzları, proteinler ve bilirubinden oluşur. Safra genellikle kolesterolü çözebilirken, çok fazla kolesterol varlığında sonunda taşa dönüşebilen kristaller oluşabilir. Safra kesesi taşı hastalığının etiyopatogenezindeki temel faktörler kimyasal, yapısal ve elementel kompozisyon analizleri ile belirlenebilir. Hem mikroskobik hem de spektroskopik olmak üzere farklı tamamlayıcı analitik teknikler uygulanabilir ve safra kesesi taşlarının ultra yapısı ve eser elementlerin tanımlanması da yapılabilir. Bu çalışmada Erzincan ve çevre bölgelerden kolesistektomi için kabul edilen altmış hastanın safra taşının yapısal özelliklerini araştırma amaçlanmıştır. Bu çalışmada Şubat 2025 ile Nisan 2025 tarihleri arasında Erzincan Binali Yıldırım Üniversitesi Mengücek Gazi Eğitim ve Araştırma Hastanesi Genel Cerrahi Kliniğinde yatan ve safra kesesi malignitesi kanıtı olmaksızın semptomatik safra kesesi hastalığı nedeniyle kolesistektomi yapılan 60 hastadan safra taşı örnekleri toplandı. Safra kesesi taşlarının renk, boyut, şekil ve taş sayısı gibi dış ve kesitsel morfolojik özellikleri çıplak gözle incelendi ve fotoğrafları yüksek hassasiyet ve çözünürlükle çekildi. Çekirdek, orta tabaka ve kabuk olarak belirgin katmanlaşma gösteren seçilmiş safra taşları bu faktörlere göre ayrıldı. Ayrıca taşların yapısı incelendiğinde koyu ve açık renkli bantları dönüşümlü olarak içeren eş merkezli katmanlar gözlenmiş olup, safra taşlarının oluşumu sırasında safradaki bileşimsel değişimlerin önemi vurgulandı.

Anahtar Kelimeler: Safra kesesi taşı, Dış yapı, Kesitsel morfolojik özellik.

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Geliş Tarihi/Received 30.04.2025
Kabul Tarihi/Accepted 19.06.2025
Yayın Tarihi/Publication Date 27.06.2025

Cite this article
Çimen, O., Keskin Çimen, F., Durdağı, S., & Gedik, H. (2025) Differentiation of gallbladder stones according to their external structure and cross-sectional morphological features such as color, size and shape. *Journal of Anatolian Physics and Astronomy*, 4(1), 24-31.

Introduction

Gallstones, also known as cholelithiasis, are masses that form in the gallbladder or biliary tract caused by abnormally high cholesterol or bilirubin levels in bile (Jones et al., 2025). Gallstones are a common disease in general (approximately 10-20% of the global adult population), with >20% of people with gallstones (usually in adulthood) developing symptoms (including biliary colic or infections) during their lifetime (Lamert et al., 2016). While black pigment stones can be caused by chronic hemolysis, brown pigment stones usually develop in blocked and infected bile ducts (Trotman B. W., 1991). Although the localization of gallstones in the bile ducts is more important than the composition for treatment, determining their structure is also important for subsequent treatment planning. Gallstone disease is defined by the occurrence of symptoms or complications caused by gallstones in the gallbladder and/or bile ducts (Internal Clinical Guidelines, 2014). From a clinical perspective or treatment algorithms, those with asymptomatic stones are usually not classified as having gallstone disease (Lee et al., 2022). Gallstone disease is among the gastrointestinal conditions associated with high costs (Shaffer et al., 2006).

In some cases, increased bilirubin levels can lead to the formation of stones. The process of gallstone formation can be summarized as follows: hard chemical particles that form and develop inside the gallbladder after a complex series of events, including bile supersaturation, nucleation, stone accumulation, and stone growth. Each gallstone has its own causes and formation mechanisms. Factors that affect the composition of gallstones can be classified as follows: age, diet, geographic region, obesity, weight loss, and ethnicity. It is difficult to define a clear classification system according to the types of an ideal gallstone, but we can group them generally according to their main chemical composition.

While gallstones are classified according to their composition and location, more than 90% of gallstones are mainly composed of cholesterol gallstones (Gu et al., 2020). Other types of stones (less than 10%) are represented by black and brown pigment stones (Sebghatollahi et al., 2023). In other words, gallstones (cholecystolithiasis) consist of cholesterol and black pigment gallstones (consisting of polymerized calcium bilirubinate). Brown pigment stones containing bilirubin and calcium fatty acid soaps are formed in infected bile ducts (Carey, 1992). Another classification type of bile duct stones is made as extrahepatic stones (choledocholithiasis) and intrahepatic stones (hepatolithiasis) (Tazuma, 2006).

Some of the risk factors for gallstones are female gender, age, pregnancy, physical inactivity, obesity, and overnutrition (Di Ciaula et al., 2018; Jukić et al., 2023). Although factors associated with metabolic syndrome also increase the risk of developing gallstones, primary prevention can be achieved through lifestyle changes (Portincasa et al., 2006). Diagnosis is mainly based on clinical symptoms, abdominal ultrasonography, and liver biochemistry tests. Symptoms usually precede the onset of the three common and potentially life-threatening complications of gallstones (acute cholecystitis, acute cholangitis, and biliary pancreatitis) (Lamert et al., 2016). Although knowledge of the genetics and pathophysiology of gallstones has increased thanks to recent studies, current treatment algorithms remain predominantly invasive and surgical (Costa et al., 2024). Therefore, our future efforts should focus on new preventive strategies, especially in at-risk patients.

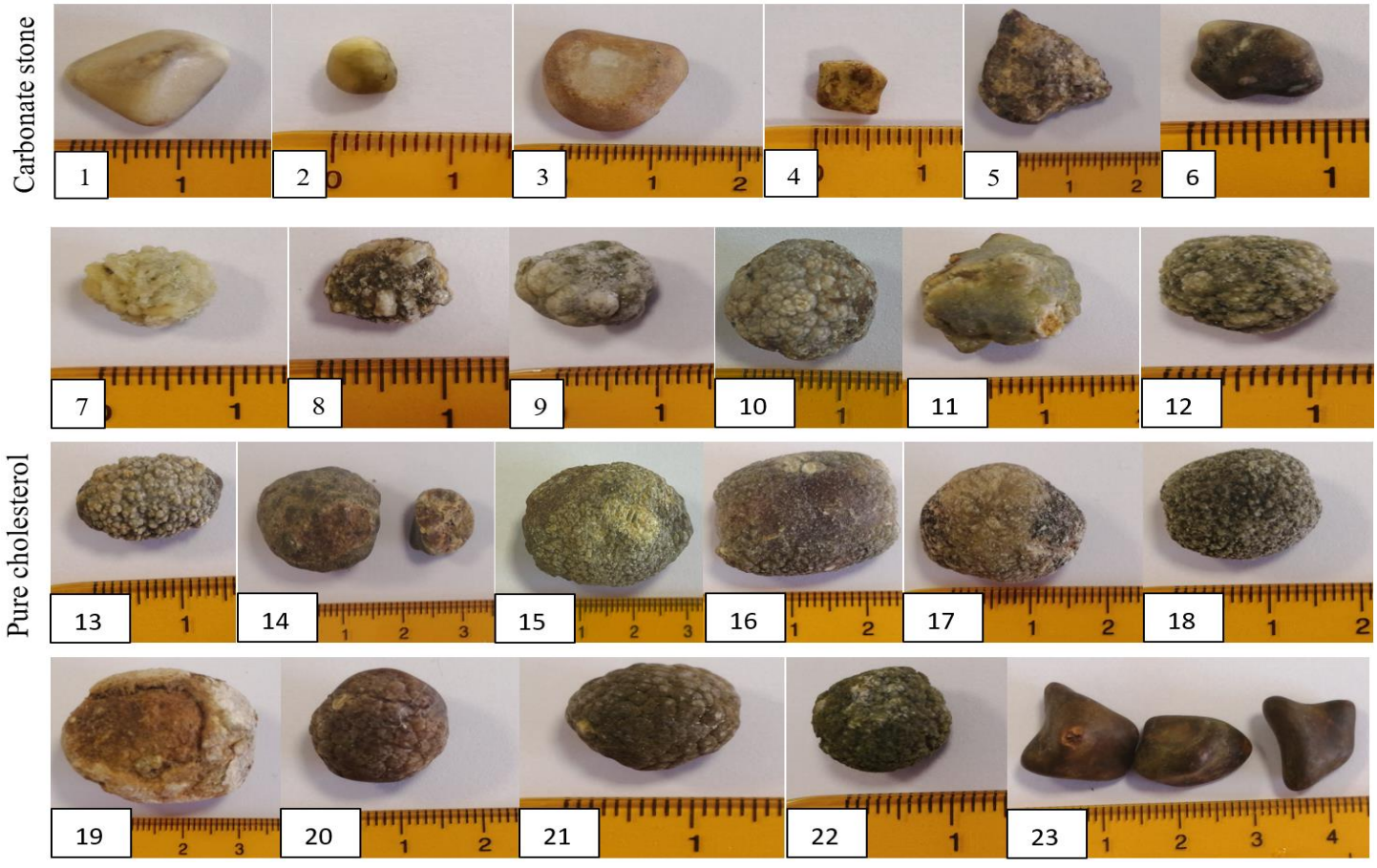
Methods

Gallstone classification systems vary in terms of nomenclature and are divided into different categories based on changes in geographical location, stone composition, structure, and color. Traditionally, gallstones are divided into three main types: (a) Cholesterol stones, (b) Pigment stones and, (c) Mixed stones, but with the advancement of scientific technology, new gallstone classes such as calcium carbonate stones, phosphate stones, calcium stearate stones, protein stones, and cystine stones have been added to these three classification systems (Jones et al., 2025). The reasons for the characteristic variation of gallstones within the population of a single country have not yet been elucidated. Studies on gallstones will be able to determine the causal factors and suggest preventive measures against stone formation. This study was conducted to examine the structures of gallstones collected as a result of operations performed on patients living around Erzincan.

Gallstone samples were collected from 60 patients hospitalized in the Department of General Surgery, Mengücek Gazi Training and Research Hospital, Erzincan Binali Yıldırım University, between February 2025 and April 2025, who underwent cholecystectomy due to symptomatic gallbladder disease without evidence of gallbladder malignancy. All patients were included in the study after obtaining written and informed consent. Ethical approval for the study was obtained from the Non-Interventional Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (Date: 09.01.2025, No: 2025-

01/03). All these patients had regular liver function tests and normal complete blood counts before surgery. Sample preparation steps play a critical role in gallstone analysis; therefore, potential errors were minimized through the precision demonstrated in sample preparation. The extracted gallstone samples were placed in sterile containers immediately after surgery. The stones were cleaned with deionized water and air-dried on sterile gauze. The dried samples were then transferred to dry bottles. The samples were then cleaned using isopropyl alcohol (70%) to remove blood clots on the surface and washed several times with deionized water. All collected gallstones were then dried in an oven at 50 ºC for one hour to remove moisture, and photographs were taken of the dried stone samples. After photography, they were placed back into storage bottles, and multiple gallstones removed from a patient were considered as a single sample. Large stones (>10 mm) were cut using a sterile saw to access the core. Core and surface samples were photographed separately.

The external and cross-sectional morphological features of gallstones, such as color, size, shape, and number of stones were examined with the naked eye, and their photographs were taken with high sensitivity and resolution. Selected gallstones showing distinct layering as core, middle layer, and shell were separated according to these factors. All photographs taken are given in Figure 1.



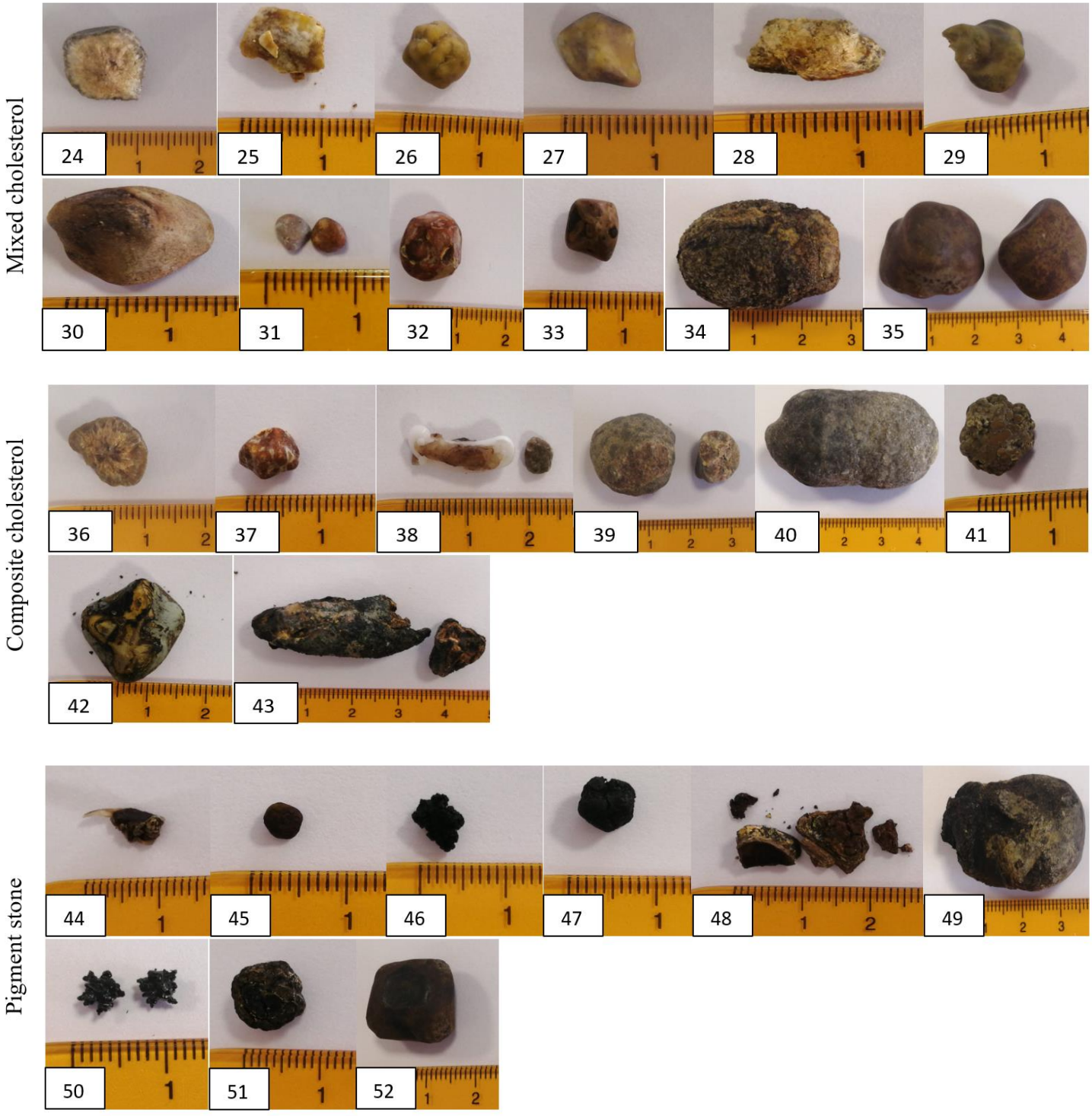


Figure 1. Classification of the different types of gallstones examined as pure cholesterol stones, mixed cholesterol stones, compound cholesterol stones and, pigment stones and images representing each type.

Results

The appearance, shape, size, color, number of stones, and distinct morphological and structural features of gallstones vary. The examined samples were initially divided into five groups according to morphological and internal structural features as pure carbonate stones (n=6; 11.5%), pure cholesterol stones (n=17; 32.7%), mixed cholesterol stones (n=12; 23.1%), composite cholesterol stones (n=8; 15.4%) and pigment stones (n=9; 17.3%) (Table 1). Table 1 shows the distribution of investigated gallstones by gender, age, and stone group. The percentage of female patients is 61.5%, and the percentage of male patients is 38.5%. It has been determined that the risk of gallstone disease is higher in female patients. While 40.6% of

female patients were observed to have carbonate cholesterol-type stones, most male patients were observed to have composite cholesterol group stones. When determining cholesterol stones, a range was taken in which the surfaces were smooth, and their colors mainly varied from pale white or yellow to a yellowish-brown. When determining pigment stones, dark colors dominated by different black and brown tones were primarily taken as the basis. When determining mixed stones, situations with various colors, such as yellow to brown, black and greenish were mainly taken as the basis (Liu et al., 2002; Ha et al., 2018; Jayasoma et al., 2022).

Table 1. İnformation about the studied gallstones and the distribution of gallstone types**.**

	Female	**Male**
Number of the samples	32	20
Sample (%)	61.5	38.5
Mean age (year)	52.28	52.45
Min age (year)	24	37
Max age (year)	80	68
>50 years old (%)	50	60
Mean age of the pure cholesterol group	47.4	55.3
Mean age of the mixed cholesterol group	56.3	46.7
Mean age of the composite cholesterol group	61.7	48.8
Mean age of the pigment stone group	60.5	57.9
Mean age of the carbonate stone group	48.6	39
Samples from the pure cholesterol group (%)	40.6	20
Samples from the mixed cholesterol group (%)	28.1	15
Samples from the composite cholesterol group (%)	9.4	25
Samples from the pigment stone group (%)	6.3	35
Samples from the carbonate stone group (%)	15.6	5

The sizes and number of stones examined in this study were found to be quite variable. While the diameter of pure cholesterol stones varied between 0.77 and 3.2 cm, it was determined that most of them showed a polyhedral shape (Figure 1). The situation was different in cholesterol stones that were extracted as a single piece, and a generally spherical or oval structure was encountered. On the other hand, it was observed that most of the pigment stones in the examined samples were amorphous and brittle, with diameters smaller than 3 mm, and they were composed of many stones (Figure 1). While the small-sized pigment stones predominantly exhibited a rough surface appearance, the others showed an irregular shape. No clear conclusion could be reached in the shapes of the mixed stones; different types were recorded as irregular, round, or oval. In fact, many stones varying in size from a few mm to 2 cm were observed. Most of the cholesterol and mixed stones showed a dark-colored core resembling the core of the stone.

When examining the photographs of the gallstones, it is evident that the stones coded as 1, 2 and 3 are white carbonate stones with sharp surfaces. 16 stones coded between 7 and 23 are included in the pure cholesterol class due to their white/whitish color and rough surfaces, and their structure mainly consists of cholesterol. Gallstones coded between 24 and 35 are classified as mixed cholesterol stones due to their yellowish/yellowish-light brown color. Cholesterol is the most abundant phase following bilirubin salts and is concentrated more in the periphery than in the center of the stone, as shown in Figure 2. Stones coded between 36 and 43 are included in the compound cholesterol group because their outer surfaces are rough and dark brown (Peter et al., 2020; Kim et al., 2003). Although these stones are similar in structure to mixed cholesterol stones, cholesterol, and bilirubin remain the primary components in them. The reason for the black color observed in the central parts of gallstones is the presence of Cu in the form of copper bilirubinate (Singh et al., 2020; Liu et al., 2025) (Figure 2, like stone number 13). Gallstones, coded between 34 and 52, are easily distinguishable by their black, irregular shapes and have a harder structure than other stones.

When we look at their cross-sectional views, a radial arrangement of mineral/minerals can be seen in the middle region (Figure 2). Generally, a three-layered structure (shell, middle part, and core) and a concentric ring pattern with different thicknesses were observed, contrasting between these regions.

Figure 2 shows the cross-sectional views of the gallstones examined, and random circular arrangements were observed in some stones, such as 7, 8, 9, and 10. In addition, the yellow-brown crystallization and structure of these stones were found in layered concentric deposits, and an aggregate with a dense shell and a distinct central part was detected. In stones between 1 and 6, especially in stone number 5, a rare morphological structure, such as aragonite helictites (mineral aggregates with branched cylindrical or conical dendritic extensions) or corallites (aggregates of individual dendrite branches and clusters joined to each other, located between them) was encountered.

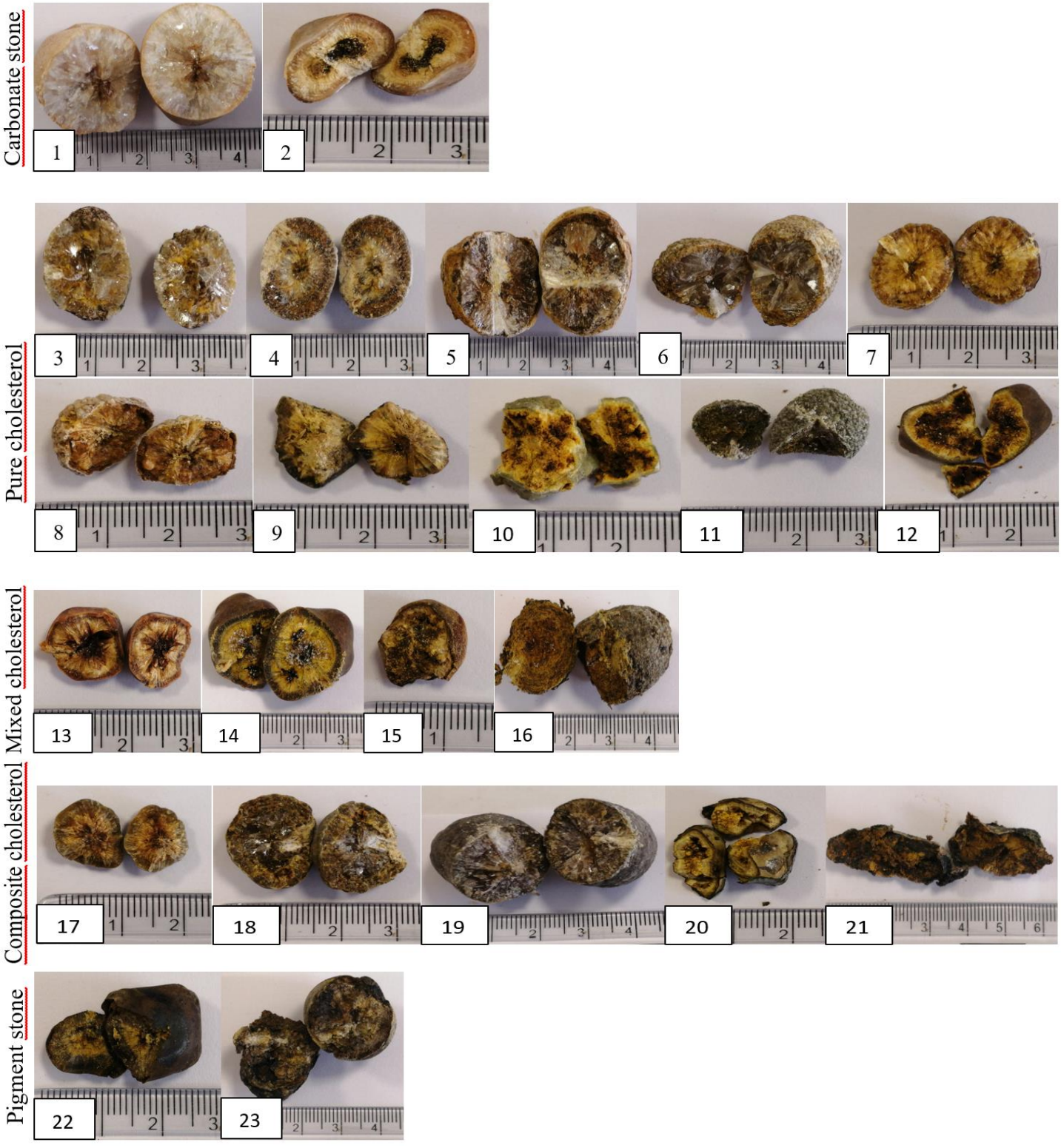


Figure 2. Section pictures after the gallstones are cut.

Conclusions

Based on this study, as previously stated by Jayasoma et al., (2022), we can say that the dark and light-colored layered structure of the stones may be related to some behaviors of the individuals, such as eating and drinking habits, quality of drinking water consumed, medication intake and dietary supplements and that the compositional structure of each gallstone is different. The direction of stone growth originates from the nucleus and follows a radial pattern outward. This study has shown that the formation mechanism of each stone has a unique microstructural feature.

To date, it has been established that gallstone disease has a direct relationship with the patient's age and gender, nutritional and obesity factors, heredity, gallbladder, and biliary tract dyskinesia, and violation of water and salt balance in the organism (Nakeeb et al., 2002; Parra-Landazury et al., 2021; Stinton et al., 2012; Pak et al., 2016). With the increasing incidence of this disease, the potential for intravital diagnosis of the phase and chemical composition of gallstones is growing. We emphasize the need for research to enhance the effectiveness of organ-preserving techniques in gallstone treatment.

Gallstone analysis is a test performed to find out what gallstones are made of, and this information helps the doctor develop a plan to reduce the risk of developing more stones in the future. In addition to determining the type of gallstone, high levels of different kinds of molecules in the urine or blood can also indicate which type of stone you have. Stone analysis can be used to determine whether you have another stone, i.e., recurrence. In addition, gallstone analysis can inform dietary changes, medication intake can be determined, and appropriate supplements (vitamins, etc.) can be recommended. Furthermore, fluid consumption (water, milk, cola, etc.) can be adjusted as needed. In other words, recurrence can be prevented by informing you of the necessary changes to your health conditions and daily life.

This study, conducted to investigate the structures of gallstones in the population of Erzincan and its surrounding districts, is part of a multidisciplinary research conducted between the Department of Surgical Medical Sciences, the Department of Medical Pathology, and the Faculty of Arts and Sciences at Erzincan Binali Yıldırım University. With the correct information provided by physicists and chemists analyzing gallstones, physicians will be able to help determine the cause(s) of stone formation and growth. After evaluating possible metabolic diseases or risk factors, physicians can make rough estimates about the stone structure without resorting to analysis by looking at metabolic disorders, and blood, and urine biochemistry. It is essential to have information about the components of gallstones to provide stone-specific treatment, identify the etiology of stone formation, prevent recurrence, and offer individualized treatment.

Etik Komite Onayı: *Bu çalışma için etik komite onayı Erzincan Binali Yıldırım Üniversitesi'nden (Tarih: 09.01.2025, Sayı: 2025-01/03) alınmıştır.*
Hasta Onamı: *Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.*
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: *Konsept – SD, HG, OÇ, FKÇ; Tasarım – SD, HG, OÇ, FKÇ; Denetim - SD, HG, OÇ, FKÇ; Kaynaklar – SD, HG, OÇ, FKÇ; Malzemeler - OÇ, FKÇ; Veri Toplama ve/veya İşleme – SD, OÇ, FKÇ; Analiz ve/veya Yorum - FKÇ, SD; Literatür Taraması - SD, OÇ,; Yazma - OÇ, SD; Eleştirel İnceleme - OÇ, SD.*
Çıkar Çatışması: *Yazarlar, çıkar çatışması olmadığını beyan etmiştir.*
Finansal Destek: Bu çalışma için herhangi bir kurumdan finansal destek alınmamıştır.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Erzincan Binali Yıldırım University (Date: 09.01.2025, No: 2025-01/03).*
Informed Consent: *Written informed consent was obtained from patients who participated in this study.*
Peer-review*: Externally peer-reviewed.*
Author Contributions: *Concept - SD, HG, OÇ, FKÇ; Design- SD, HG, OÇ, FKÇ; Supervision- SD, HG, OÇ, FKÇ; Resources- SD, HG, OÇ, FKÇ; Materials - OÇ, FKÇ; Data Collection and/or Processing- SD, OÇ, FKÇ; Analysis and/or Interpretation- FKÇ, SD; Literature Search- SD, OÇ,; Writing Manuscript-OÇ, SD; Critical Review-OÇ, SD..*
Conflict of Interest: *The authors have no conflicts of interest to declare.*
Financial Disclosure: *No financial support was received from any institution for this study.*

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