

## Gallstones associated with bacterial incidence

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### Abstract :

**Objective:** To study the correlation between the gallstones accumulation and bacterial incidence .

**Patients and methods :** This study was carried out for the period July 2009 to February 2010 in Ramadi General hospital in Ramadi City. Forty patients were diagnosed clinically as having gallstones were submitted to surgical operation .Stones were collected for culture, all the information study patients were recorded.

**Results :** 40 stones were collected , male to female ratio was 1:2.8 , age ranged between (30-60) years old. The stones were diagnosed based on the morphology, size and color , cholesterol stones were found in 25(62.5%) and pigment stones in 15(37.5%).

70% appeared no growth of pathogenic microorganism while 30% of these stones show bacterial growth of the following bacteria, *Escherichia coli* 12(48 %), *Klebsiella pneumoniae* 4 (16% ) , *Pseudomonas aeruginosa* 3 (12 %), *Salmonella enterica enterica (S.Typhi)* 4 (16%) and *Staphylococcus aureus* 2 (8%) .

**Conclusion :** It was concluded that bacteria actually constitute a substantial portion of the stone and are more than just a remote source of  $\beta$ -glucuronidase.

**Key words :** Gallstones , Bacteria

### Introduction :

The gallbladder is located below the edge of the liver, gallstones may accumulate within the gallbladder ,a condition known as chronic cholelithiasis .Gallstones are the commonest biliary pathology, which occurs in both sexes, quite often at earlier age even in childhood and more common in old age .In Europe , 30% of females over 60 years of age have gallstones however, two thirds are asymptomatic <sup>(1)</sup>.In U.S.A., 15 million people have gallstones and about 350.000 gallbladder were removed during a one year only<sup>(2)</sup> . Bacterial DNA is present in all brown pigment stones but only occasionally in black pigment stones or stones with a high cholesterol content (70-95%)<sup>(3,4)</sup>. Previous studies have suggested that bacteria may have a special role in the pathogenesis and clinical manifestation of pigment gallstone disease. The other <sup>(5,6)</sup> have shown that infectious complications are much more common in patients with pigment than with cholesterol gallstone disease. However , the role that bacteria play in the formation of pigment gallstones remains incompletely defined .The aim of this study was set out to culture bacteria from core of gallstones in patients undergoing cholecystectomy .

### Patients and Methods :

A sample of (40) patients , was selected and admitted to the Ramadi General Hospital for the period July 2009 to February 2010. In all Forty consecutive patients, all of whom had stones in their gallbladder.

The patient age was ranged between 30 years to 60 years old with mean (45.52Y)

### Gallstone sample :

After cholecystectomy the gallbladder was opened and the largest stone was transferred to a culture bottle and immediately transported for culture. In the Microbiology Department the whole stone was immersed in 70% ethanol for 10 min for surface sterilization, it was then bisected with sterile precautions and the core was scooped out for culture on Blood agar, Chocolate agar ,Salmonella-Shigella agar and MacConkey agar, cultures were incubated aerobically environment for 24 hours at 37C°. All study isolates were bacteriologically identified and confirmed by biochemical test<sup>(7,8)</sup>.

### Results :

The percentage of each type of gallstones was calculated and was related to age and sex. Out of 40 gallstones, 25(62.5%) was cholesterol gallstone and 15(37.5%) pigment gallstone. The patients that participated in this study were 32 females (80%) and 8 males (20%). Their age ranged between 30-60 years with mean of (45.52 Y).The male to female ratio was 1:2.8. The male ratio to female which have pigment gallstone at (25%), and (42.8%) respectively and at the range age 43.93 year, and the male ratio to female which have cholesterol gallstone at (41.6%), and (71.4%) respectively at range age 50.36 year. Table (1)

**Table 1 : Percentage of occurrence of gallstones in both sexes .**

Type of gallstones	Male number (%)	Female number (%)	Mean of age(years)	Total number	The percentage
Cholesterol gallstone	5 (41.6%)	20 (71.4%)	50.36	25	62.5%
Pigment gallstone	3 (25%)	12 (42.8%)	43.93	15	37.5%
Total number	8	32	45.52	40	100%

Further, the study was revealed that out of 23 (92%) isolates of gram negative and 2(8%) isolates of gram positive organisms from the two types of gallstones, *Escherichia coli* the commonest organisms isolated

12(48 %) followed by *Klebsiella pneumoniae* 4 (16%), *Salmonella enterica enterica (S.Typhi)* 4 (16%), *Pseudomonas aeruginosa* 3 (12%) and *Staphylococcus aureus* 2 (8%) . Table 2.

**Table 2 : Percentage type of gallstones according to species isolated from the stones.**

Species of bacteria	Pigment gallstones	Cholesterol gallstones	Total number
<i>Escherichia coli</i>	8 (32%)	4 (16%)	12 (48%)
<i>Klebsiella pneumoniae</i>	2 (8%)	2 (8%)	4 (16%)
<i>Pseudomonas aeruginosa</i>	3 (12%)	0	3 (12%)
<i>S.Typhi</i>	4 (16%)	0	4 (16%)
<i>Staphylococcus aureus</i>	2 (8%)	0	2 (8%)

Also, result showed that out of 12(30%) of gallstones were positive for culture, 10 (83.3%) from pigment

gallstones and 2 (16.6%) from cholesterol gallstones . (Table 3)

**Table 3: Percentage of bacterial positive culture according to type and numer of gallstones .**

Type of gallstones	Number of gallstones	The positive bacterial culture	The percentage (%)
Cholesterol gallstones	25	2	16.6%
Pigment gallstones	15	10	83.3%
Total	40	12	30%

### Discussion :

The study showed that the mean age for the occurrence of gallstones was 45.52 years which is not significantly different from that found by other studies 39.9 years. The present study showed that the male to female ratio who they had pigment gallstones which concerned with bacterial found was (25%),(42.8%) respectively this showed the higher proportion than other kinds of gallstones and the age rate (43.93Y) , whereas the male to female ratio who they had cholesterol gallstones was (41.6%),(71.4%) respectively and the age rate (50.36Y) which is not significantly different from that found by some authors who observed that the pigment gallstone was found mostly in age more than 50 years<sup>(8,9)</sup>.The male to female ratio was 1:2.8 which agrees with other studies<sup>(10)</sup>.

Under the field of role the bacteria to form gallstones, our results showed that the ratio of cholesterol gallstone was (62.5%), in other hand the pigment gallstone was formed (37.5%) from the total number, this sovereignty in the ratio of cholesterol gallstone consistent with a number of authers<sup>(9,11)</sup> were observed that the ratio of cholesterol gallstone was (74.68%) on the other hand the pigment gallstone was formed (13.29%) from 960 patients .

The present study was showed that the bacterial isolates from the core of 12 gallstones only at rate (30%), 10 (83.3%) from pigment gallstones and 2(16.6%) from cholesterol gallstones, this result was

consistent with the observation recorded by<sup>(9)</sup> when he examined (225) cases with the cholecystectomy he found (31.2%) were positive culture, and<sup>(4)</sup> was observed the positive culture ratio of pigment gallstone was (100%) whereas cholesterol gallstone was (47%) , thus emphasises the relationship between pigment gallstone formation with the bacterial infection of gallstone cases .

The study was shown 23 (92%) gram-negative pathogens were isolated as followed *E. coli* ,to be the commonest organisms isolated 12 (48%) followed by *Klebsiella spp.* 4(16%), *S.Typhi* 4(16%),and *Pseudomonas aeruginosa* 3 (12%). Whereas 2(8%) gram-positive

pathogen was isolated followed by *Staphylococcus aureus*. Enteric organisms had often been suspected of caused cholelithiasis, and intestinal flora had frequently been recovered followed intervention on the biliary tree<sup>(12,13)</sup>.The acquisition of certain outer membrane characteristics can distinguish these bacteria from normal gut flora and may explain their selective colonization of the biliary tract<sup>(4)</sup> .

Under the same filed, the bacterial infection could lead to calcium bilirubinate precipitated by the elaboration of the enzyme  $\beta$ -glucuronidase, which deconjugates bilirubin diglucuronide<sup>(14,15)</sup>. Monoconjugated and unconjugated bilirubin were much less soluble in bile and precipitated as calcium bilirubinate crystals. Our results were in agreement with those obtained from the study carried out by<sup>(4,16)</sup>

who observed the same types of bacteria were isolated from bile fluid and gallstones .

Cetta(9) has proposed that bacterial phospholipase may also provide the catalyst action for the precipitation of calcium palmitate, which has been observed in some gallstones that were associated with gallbladder bile infection<sup>(17)</sup>. Hence, it is likely that specific bacteria have a central role in gallstones formation because of their association with the pigment and fatty acid components of these stones.

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The study concluded that bacteria actually constitute a substantial portion of the stone and are more than just a remote source of  $\beta$ -glucuronidase, and bacteria were responsible for the formation of the majority of black as well as brown stones. In addition to this primary role of bacteria in pigment precipitation and agglomeration, these findings also explained why sepsis were more common in gallstone disease, since the stones serve as a sanctuary that protects the bacteria from endogenous and exogenous antibacterial factors .

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## حصى المرارة المرتبطة بالاخماج الجرثومية

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### الملخص

**الهدف:** دراسة العلاقة بين الخماج الجرثومية وتكوين حصى المرارة.

**المرضى وطرق العمل:** أجريت الدراسة بين تموز ٢٠٠٩ إلى شباط ٢٠١٠ في مستشفى الرمادي العام في مدينة الرمادي على ٤٠ مريضا شخصت لديهم حصاة المرارة واخضعوا للعملية الجراحية ، اجري الزرع البكتيري لجميع الحصوات ، سجلت جميع العوامل ذات العلاقة بالمرضى .

**أ لنتائج:** جمعت ٤٠ حصى ،نسبة الرجال الى النساء (٢،٨:١) وكانت أعمار المرضى تتراوح بين ٣٠-٦٠ سنة شخصت نوعين من الحصى بالاعتماد على المظهر الخارجي والحجم واللون هما حصى الكوليستيروول (٢٥،٥%) وحصى الصبغة (١٥،٥%) .أوضحت النتائج ان ٧٠% من الحصى كانت سالبة للزرع الجرثومي و ٣٠% كانت موجبة للزرع الجرثومي.وشملت الأنواع التالية: *Escherichia coli* 12(48%) ، *Klebsiella pneumoniae* 4(16%) ، *S. Typhi* 4 (16%)، *Pseudomonas aeruginosa* 3(12%) ، *Staphylococcus aureus* 2 (8%)

**الاستنتاج:** استنتج من هذه الدراسة إن البكتريا تشكل جزء أساسي في تكوين حصى المرارة وهي أكثر من أن تكون مجرد مصدر محفز لإنزيم  $\beta$ -glucuronidase