

Bacteriological and Immunological study of Cholecystectomy patients .

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ABSTRACT:

The most prevalent complication of gallstone is chronic cholecystitis. The aim of the present study was to determine the responsible microorganisms association in gallbladder inflammation in patients undertook cholecystectomy and determine the relationship of serum interleukin-12 concentration and some hematological parameter with gall bladder infaction.

Cholecystectomy was achieved in 107 patients. Collected samples transferred to laboratory inappropriate media, then cultured on selective media to isolate the possible causative bacteria.the levels of interleukin-12 , PCV, WBC,HB were determined.

bacterial growth was shown in 94.4% of all cultured samples , of which 87.6% gram negative bacteria (*E.coli, pseudomonas,Enterobacter,Klebsiella,salmonella, shigella, Acintobacter, Citrobacter,Proteus*).and 12.4% grame positive bacteria(*Enterococcus feacales,Staphylococcus. areuse,Staphylococcus.epidermidis,Bacillus,Streptococcus.pneumonia, and Streptococcus viridians*).

the resulte explain increase in levels of interleukin-12 and variation in hematological parameters compaired with control. The study also revealed three types of stones depending upon their colouer(36 cholesterol, 26 mixed, 34 pigment) . High percentage of bacteria was isolated from pigment stone,where as cholesterol stone show no bacterial growth.

دراسة مناعيه وبكتيرييه للمرضى للمجرى لهم عملية استئصال المراره

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الخلاصه:

يعد التهاب المراره من اكثر المضاعفات الشائعه لحصى المراره. الهدف من الدراسة هو تحديد الاحياء المجهرية المرافقه للمرضى للمجرى لهم عملية استئصال المراره. وتحديد بعض معايير الدم ذات العلاقه بالتهاب المراره وتحديد مستوى تركيز الانترلوكين-12

جمعت العينات من (107) مريض اجري لهم عملية استئصال المراره في مستشفى الحله التعليمي العام، ونقلت العينات الى المختبر بواسطة وسط زرعي محضر مسبقا، ثم زرعت العينات على اوساط زرعيه مختلفه لغرض عزل وتشخيص اهم المسببات البكتيرييه لالتهاب وحصى المراره.

اظهرت النتائج نمو بكتيري في 94.4% من العينات. 87.6% منها هي بكتيريا سالبه لصبغة غرام (*E.coli*, *Pseudomonase*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella*, *Acintobacter*, *Citrobacter*, *Proutes*) و 12.4% هي بكتيريا موجبه لصبغة غرام (*Enterococcus*, *Staph.aruse*, *Staph.epidermidis*, *Bacillus* *Strep.pneumonia*, *Strep.viridans*)

بينت النتائج زياده في مستوى تركيز الانترلوكين -12 وتنوع في معايير الدم عند مقارنتها بالسيطره. قسمت الحصى الى ثلاث مجاميع اعتمادا على اللون والقوام (36 حصى كوليستروليه، 26 حصى مختلطه، 34 حصى صبغيه). احتوت الحصى الصبغيه على نسبه عاليه من البكتيريا بينما كانت نسبه البكتيريا اقل او معدومه في الحصى الكوليستروليه.

INTRODUCTION:

Acute cholecystitis is an infection of the biliary tract, which results from bile stasis due to chronic obstruction. The obstruction is usually attributed to gallstones in 80% of cases. The causes of acalculous cholecystitis include biliary structures, human immunodeficiency virus cholangiopathy, biliary parasites and primary sclerosing cholangitis. Other causes include complicated cases of burns, trauma, major surgery, diabetes and unusual bacterial infections of the gallbladder (*Salmonella* spp. or *Vibrio cholerae*) and other systemic infections (tuberculosis and syphilis) (Drugs and Therapeutics Bulletin, 2005). Biliary obstruction causes an increase in ductal pressure, resulting in bacterial proliferation and dissemination. Bacterial infection is the most common type of acute cholangitis, with a Gram-

negative preponderance. Gram-positive and anaerobic are uncommon causative agents. Viral and fungal agents are rare (Greenberger *et al.*, 1998).

In an enteric fever endemic country like India, *Salmonella enteric* serovar *Typhi* and *S. Paratyphi A* are among the major biliary pathogens. Enteric fever persists for many years after convalescence and increases the risk of hepatobiliary malignancy. Even with prompt diagnosis and treatment acute cholangitis can lead to septicemia and complications like emphysema, gangrene, perforation and chronic cholecystitis (Csendes *et al.*, 1996). Abnormality or inflammatory diseases of gallbladder usually necessitate cholecystectomy (Vitetta *et al.*, 2000). Obstruction of cystic duct that is mainly associated with gallstones is the main reason of acute cholecystitis. Gallstones are usually chemically classified as cholesterol or mixed or pigment stones (Chandran *et al.*, 2007). The most prevalent complication of gallstone is chronic cholecystitis occurs in approximately 4% of cases. It affects subjects aged 30-40 years and presents with nausea and vomiting, pain, fever and chills (Eslami *et al.*, 2007). Prior investigators have proposed the following causative pathogens in cholecystitis: *enterococci* 23%, *pseudomonas* 1%, *Salmonella typhi* 2-5% and other gram negative bacteria such as *E. coli* 41.1% (Al-Khafeji, 2006). *Salmonella typhi* causes typhoid fever and *Salmonella paratyphi* (Malini *et al.*, 2008) is associated with paratyphoid fevers. Humans as the original source of infection, distribute the organism in the society (Vitetta *et al.*, 2000).

Gallstones may manifest themselves with dramatic clinical features of obstructive jaundice and acute cholecystitis, or they can stay silent or be minimally symptomatic and be discovered only as incidental findings. They may be located in any part of the biliary tract, but are primarily found in gallbladder and less often in the common bile duct or intrahepatic ductal system. Once gallstones are discovered, they may grow, shrink, or remain the same size for years. Additional stones may form, and existing stones may dissolve or be passed. Gas can appear within gallstones on radiography and disappear later. Despite these well documented observations of dynamic structural changes in the development of gallstones, gallstones are traditionally arbitrarily divided, at the time of cholecystectomy, according to their chemical composition and color, into three main groups: cholesterol, mixed and brown pigment gallstones form as a product of bacterial infection (Swidsinski and Sum, 2001).

AIMS OF THE STUDY

The present study aimed to detect the responsible microorganisms in patients who underwent cholecystectomy and determine levels of interleukin -12, WBC, HB, PCV, and their relationship with this disease.

MATERIALS AND METHODS

The study included 107 patients, with age ranged (18-70) years undergoing cholecystectomy at the general teaching Hilla Hospital from November 2011 to June

2012 .Gallstones from 107 patients of cholelithiasis were collected after cholecystectomy .95 gallstones were collected from16 males and 92 females. The stones were divided into3 groups depending upon their colour: pale yellow and whitish stones as cholesterol calculi, black and blackish brown as pigment calculi and brownish yellow or greenish with laminated features as mixed calculi (Eslami *et.al.*,2007) . The other relevant information about the patients such as age, sex , number of calculi , and patient family history and presence of any other disease were obtained from hospital records. The various physical parameters of stones such as number, shape, size, texture and cross-section were noted. Brian heart infusion broth were used as transferred media for gallbladder specimen. Swabs for outer and inner surface of gallstone and swabs for bile and whole gallbladder were used for aerobic bacterial culturing . the swabs were directly cultured onto eight solid media (Nutrient Agar , Chocolate Agar , Blood Agar Base, MacConky Agar , *Salmonella-Shigella*Agar (S-S Agar) , Simmon’S Citrate Agar , Eosin Methylin Blue Media , Manitol Salt agar).the plates were incubated aerobically and examined after 24-48 hours.All bacterial isolated were identified by routine laboratory methods included morphological, microscopical examination and biochemical test.(Macfaddin,2000 ; Finegold and Baron,1986).

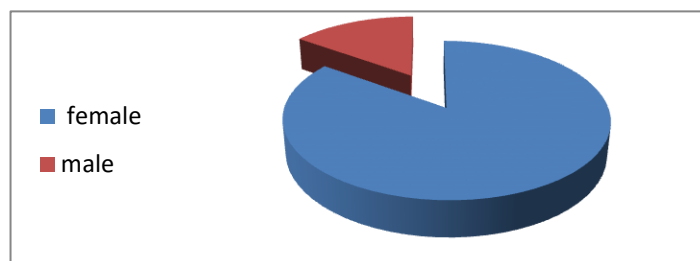
Levels of interleukin-12 were determined by Enzyme – Linked Immunosorbent Assay (ELISA) using Ebioscience(U.S.A) kit (Baggiolini *et. al.*,1994).Determination of the WBC , PCV , HB, were carried out for patients and healthy control (Lewis *et.al.*,2001).

STATISTICALLY ANALYSIS

The data analyzed by using T-test and least significance defferances between control and patient ($p>0.05$) to compare between treatment(Niazi , 2004) .

RESULTS AND DISCUSSION

This study carried on 107 cholecystectomy patients from November 2011 and june 2012 cholecystectomy was carried on 107 pateints :92(85%) were women, 16(15%) men. Similar finding have been observed in prior studies (Vaishnavi *et.al.*,2005).figure(1)



Figure(1): distribution of cholecystectomy with gender.

(Rains,1964) advocated that concentration of bile salts in bile is reduced by estrogen and thereby making it lithogenesis.(Horn,1965)postulated that under the influence of female sex hormone, the muscle may relax, biliary passage dilates and duodenal content of pancreatic secretion regurgitates into gallbladder and promote conditions which favor the formation of gallstones. Females had a greater risk of gallstone disease,especially if they had used oral contraception and/or had four or more children (Moore,1984).

The biliary calculi collected from 95 gallstone patients were divided into 3 groups based on their colour: cholesterol calculi,pigment calculi , and mixed calculi . The commonest type of gallstone was cholesterol stone 36 (37%), followed by pigment stone 33 (34%), and mixed stone 26(27%). Out of the total number of stones collected, the incidence of gallstone was higher in age group 41-50 years (Table 1). These results are in agreement with the results of related study carried out in Babyl by Al-Khafeji, (2006) who showed that the most common type of gallstone was of cholesterol type (40%) followed by pigment stone (35%), and mixed stone (25%). While the results of similar studies done in other countries like the study done in southern India by Jayanthi *et.al.*,(1998) who showed that the most common type of gallstone was of pigment stone (63.8%) followed by mixed type (34.8%) and then by cholesterol stone (1.9%) . the comparison of our data with those in similar studies in different countries may not be valid because of geographic , dietary and ethnic differences(Bedirli *et al* ,2001).

Table(1): incidence of different types of gallstone in relation to age

Age group (years)	Type of gallstone			Total gallstone
	mixed	pigment	cholesterol	
10-20	2	1	1	4
21-30	1	9	3	13
31-40	6	4	11	21
41-50	10	10	8	28
51-60	4	7	8	19
61-70	3	2	5	10
Total	26(27%)	33(34%)	36(37%)	95

Cholestrol calculi size ranged 0.2-2.4 cm and 0.4-3.2 cm with an average of 0.97 cm and 1.50 cm, mixed calculi size range was 0.1-2.5 cm and 0.1-3.0 cm with an average of 0.6 cm and 0.93 cm , while the size of pigment calculi was 0.1-1.9 cm and 0.2-2.5 cm with an average of 0.69 cm and 1.01 cm table 2 . The size of different types of calculi in this study was agree with previous studies (Pundir *et.al.*, 2001

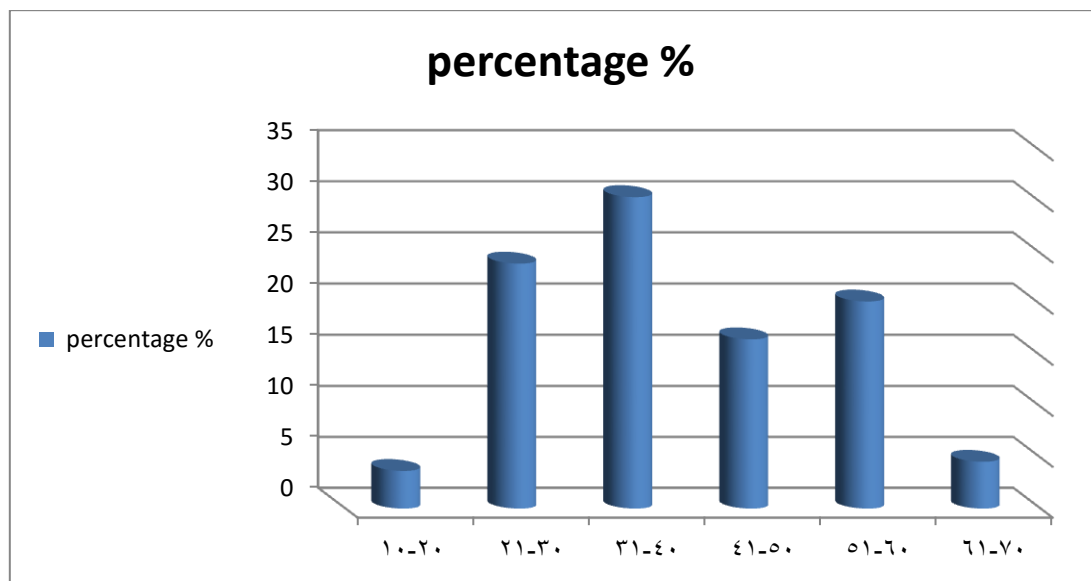
;Chandran *et.al.*, 2007). The difference in size of stone may be due to type of stone and time length of its formation.

Table 2. Size (cm) in different type of gallstone

Type of calculi	Size range cm	average
Cholesterol	0.2-2.4 and 0.4-3.2	0.97 and 1.50
Mixed	0.1-2.5 and 0.1-3.0	0.6 and 0.93
Pigmen	0.1-1.9 and 0.2-2.5	0.69 and 1.01

Cholesterol calculi were soft with soft surface, while pigment calculi were soft with rough surface. Mixed calculi had both rough and smooth surface. Cholesterol calculi showed radiations like spokes of wheel with a nuclear cross-section. Pigment calculi were amorphous, while mixed had laminated features. These results are comparable to reports from Singapore (Ti JK and Yuen R, 1985).

This study shows that cholecystitis appears in different age groups from (10-70) years. However, the incidence of inflammation increases in the (31-40) years. Figure (2)



Figure(2): distribution of gallstone among age period

Age period 31-40 showed high percentage of calculi and the mean age of cholecystectomy was 39 years. Mean age of cholecystectomy was reported 42 and 35 years in two other studies that is in agreement with ours (Al-khajeji, 2006 ; Jayanthi *et.al.*, 1998).

Bacteriological study for different sites of gallbladder showed appearance of bacterial isolates in all sites with high percentage in inner surface of gallstone 96.3%. The presence of bacteria within the gallstones positively correlates with the

presence of calcium bilirubinate table 3 . Bacteria could be demonstrated within brown pigment stones and in the brown pigment portions of cholesterol gallstones via bacterial culture, electron microscopy and molecular-genetic methods (Csendes *et.al.*,1994).

Table 3. Incidence of bacterial isolates in different locations of gallbladder

Site of culture	Bacterial growth	Number of isolate	Percentage%
Whole gallbladde	+ve growth	102	95.3%
	-ve growth	5	4.7%
Gallstone outer surface	+ve growth	102	95.3%
	-ve growth	5	4.7%
Gallstone inner surface	+ve growth	103	96.3%
	-ve growth	4	3.7%
Bile	+ve growth	26.75	25%
	-ve growth	80.25	75%

This study reveals that culturing of bile specimen show low rate of bacterial growth . the presence of bacteria in bile varies according to the severity of the biliary tract disease and obstruction, reaching nearly 100% in cases with acute cholangitis(Csendes *et.al.*,1996). In cases of cholangitis, bacteria are found more often in the gallbladder wall and within gallstones than in the bile (Hanche *et.al.*,1986).Bacteria are gallbladder and gallstones (Csendes *et.al.*,1994) and in all patients with cholangio carcinoma and hepatolithiasis (Chijiwa *et.al.*,1993).

Both gram positive and gram negative bacteria are found in gallbladder culture. The commonest Gram negative bacteria were found *E.coli*18.6% ,*Enterobacter*15.8% ,while Gram positive bacteria appear with low rate (table 4).

5.6% of cultured samples did not any bacterial growth, however, they could be infected with viruses or fungi. Similarly, Bonnet et al reported viruses as a relatively common causative agent (Bonnet *et.al.*,2001).

E.coli was detected in 20 samples (18.6% of gram negative-infected subjects). Tseng *et al.*,(2000) found *E.coli* in 57% of their cases . In our study, the most prevalent gram positive isolate was *Strep.fecalis* and *Staph.arues*(6.5% *Strep.fecalis* and 3.7% *Staph arues* of 12.4% gram positive bacteria).(Csends *et.al.*,1996 ; Bedirli *et.al.*,2001) reported *Enterococci* as the most prevalent gram positive bacteria , another study demonstrated coagulase negative *staphylococci* as the most prevalent gram positive cocci (3.6%)(Miranda *et. al.*,2001). Mean while, brucella and aeromonas were also reported (Lau *et. al.*,2000).

Table (4) total number of bacteria isolates from gallbladder

	Bacterial species	No.	Percentage%
Gram negative bacteria 87.6%(94)	<i>E.coli</i>	20	18.6
	<i>Enterobacter spp.</i>	17	15.8
	<i>Klebsiella</i>	16	14.9
	<i>Salmonella</i>	9	8.4
	<i>Pseudomonas spp</i>	7	6.5
	<i>Acintobacter</i>	7	6.5
	<i>shigella</i>	5	4.6
	<i>Citrobacter</i>	4	3.7
Gram positive bacteria 12.4% (13)	<i>Proteus</i>	4	3.7
	<i>Strep.fecalis</i>	7	6.5
	<i>Staph.arues</i>	4	3.7
	<i>Enterococcus spp.</i>	3	2.8
	<i>Strep.viridans</i>	2	1.8
	<i>Staph. epidermidis</i>	1	0.9
	<i>Bacillus spp.</i>	1	0.9

The mechanism of increased gallstone formation by bacterial betaglucoronidase has been proposed by prior researchers. The production of betaglucoronidase by bacteria causes sedimentation in gallbladder and results in gallstones formation.

The bacterial species found in the bile of patients with biliary disease (both gallstones and cholangitis) indicate that the intestinal flora is a main source of bacterobilia. *Escherichia coli (E.coli)*, *Streptococci* or *Enterococci*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Proteus* are the species most frequently isolated from aerobic cultures. *Clostridium* and *Bacteroides* species are often found in anaerobic isolates(Brook,1989).

The result explain increace in levels of interleukin-12 of patients according to control by using Enzyme – Linked Immunosorbent Assay (ELISA)(Baggiolini *et al.*,1994) .highest concentration of interleukin-12 was show in age (41-50) about 8.868 ± 13.38 pg/mlcompairted with control 5.061 ± 1.99 pg/ml,while the low concentration of interleukin -12 appear in age (61-70) about 3.856 ± 0.568 pg/mlcompairted with control 4.006 ± 0.321 pg/ml.table(5).

Most cytokines presents in cells in a precursor form , antigens such as pathogenic bacteria and their components are able to induced activation of cytokines to active forms (Roitt *et al* ,2001). IL 12 plays an important role in immune response through activation of T lymphocytes and mediates enhancement of the cytotoxic activity of NK cells in different type of inflammation (Temblay *et.al* ,2007) .

Table(5) concentration of IL-12 in patients undergo cholecystectomy

Age	groups	Concentration of intrleukin-12 pg/m M± SD
10-20	patient	6.315 ± 2.014
	control	3.632 ± 0.088
21-30	patient	*6.236 ± 5.430
	control	3.560 ± 0.455
31-40	patient	*7.148 ± 10.821
	control	5.095 ± 1.896
41-50	patient	*8.868 ± 13.38
	control	5.061 ± 1.99
51-60	patient	6.887 ± 10.14
	control	5.86 ± 3.10
61-70	patient	*3.856 ± 0.568
	control	4.06 ± 0.321

*there is significance defferances of p>0.05

Levels of WBC increase compared with control and age group (31-40) showed highly increase in WBC value about 17000 ± 26970 compared with control 7833 ± 1089 . where as HB and PCV levels decrease in their value specially with age between (61-70). Table(6).

Human blood components formed a critical signal for any abnormalities resulted by invading of foreign agents or inflammation, these invaders led to changes in levels of blood parameters such as WBC, PCV, phagocytes percentage as a result of defense mechanism (Wu *et al*, 2003).

Table(6) some hematological parameter in patient with cholecystitis

age	groups	WBC M \pm SD	PCV M \pm SD	HB M \pm SD
10-20	patient	* 16500 ± 19004	* 35.33 ± 4.61	* 10.77 ± 0.30
	control	7425 ± 359.39	37.00 ± 5.03	11.05 ± 0.67
21-30	patient	7190 ± 1680.9	36.00 ± 2.23	11.00 ± 0.50
	control	5700 ± 537.48	38.20 ± 3.08	12.3 ± 1.46
31-40	patient	* 17000 ± 26970	* 38.00 ± 1.73	* 11.66 ± 0.32
	control	7833 ± 1089	41.2 ± 3.90	12.2 ± 1.30
41-50	patient	* 7033 ± 1042.8	36.6 ± 2.30	11.1 ± 0.26
	control	5944 ± 1184.3	38.8 ± 3.25	12.1 ± 1.46
51-60	patient	* 8450 ± 831.6	* 38.8 ± 2.38	* 11.93 ± 0.20
	control	7750 ± 2258.44	41.00 ± 3.80	12.1 ± 0.36
61-70	patient	8000 ± 946.4	* 37.33 ± 2.08	* 11.44 ± 0.25
	control	5875 ± 946.04	40.50 ± 6.55	12.8 ± 0.46

*there is significance differences of $p > 0.05$

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