

RESEARCH ARTICLE

Determination of Chemical Composition of Gallbladder Stones and their Association with Induction of Cholangiocarcinoma

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Abstract

Gallstone disease is a major surgical problem in many populations; it is probably related to diet, especially excessive consumption of meat. The objective of this study was to determine the chemical composition of gallstones and their association with neoplastic changes including cholangiocarcinomas in cholecystectomised patients. The chemical composition of gallstones from 40 patients (8 males and 32 females) was analyzed. This is a prospective study performed in Baquba teaching hospital in the period from 1/10/2012 to 1/1/2013 in which we collected the gallstones for the patients who underwent cholecystectomy, whether open or laparoscopic. The stones were classified according to their chemical composition as a mixed stones (MS), and examined using a stone analysis set (chemical qualitative method) for calcium, magnesium, phosphate, uric acid and oxalate which was used reagent for qualitative determination of main individual components of stones. The results of this study showed the highest incidence of gallstones in the age group 40-49 was 13 cases followed by 11, 8 and 4 cases for age groups 30-39, 50-59, 20-29 and 60 and above, respectively. The chemical analysis showed the majority of gallstones were mixed, 38 containing calcium followed by 37 cases with uric acid, 28 with magnesium, and 25 and 22 stones with oxalate and phosphate, respectively. Microscopically, we confirmed neoplastic changes (17.5%) as cholangiocarcinomas (CCCs) (7.55%) and dysplastic cells of carcinoma in situ in 4 (10%), 31 (77.5%) cases were chronic cholecystitis and 2 (5%) cases were acute cholecystitis with empyema out of bile duct disorders patients. In conclusion, majority of cases had mixed gallstones that involved five and four of inorganic chemicals of calcium, magnesium and phosphate, the highest incidence of gallstones in age group 40-49 years old was 13 cases, and neoplastic changes were confirmed (17.5%) including CCCs, (7.5%) and dysplastic cells of carcinoma in situ (10%), while 31 (77.5%) cases were chronic cholecystitis.

Keywords: Chemical analysis - gallstones - calcium - magnesium - phosphate - uric acid - oxalate

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Introduction

Chemical composition of gall stones is essential for aetiopathogenesis of gallstone disease. Cholelithiasis or gallbladder stones are one of the major surgical problems in many hospital admission and surgical interventions (Jaraari et al., 2010). This problem is probably related to obesity, cardiovascular disorders (CVD), metabolic syndrome, and dietary habits, especially excessive consumption of meat, which is known to contain large amounts of cholesterol. Obese individuals with a BMI-30 kg/m² have 95% cholesterol-dominant gallstones and are at a high-risk for cholesterol stones (CS) (Schafmayer et al., 2006).

A quantitative chemical analysis of total cholesterol, total bilirubin, fatty acids, triglycerides, phospholipids, bile acids, soluble proteins, calcium, sodium potassium, magnesium, copper, oxalate and chlorides as minor components were found in all types of calculi (Chandran et al., 2007). Studies on gallstone composition carried out in different parts of the world indicate a close link

with dietary habits and ethnicity (Pundir et al., 2001; Verma et al., 2002; Chandran et al., 2007). Other studies have shown that dietary intake of total calories in the form of carbohydrates and fats were associated with high triglyceride levels in gallstone patients (Lee et al., 2012). Gallstone formation is relatively increased with consumption of dietary fats rich in saturated fatty acids (Tsai et al., 2004; Jaraari et al., 2010).

Cholangiocarcinomas (CCCs) are highly malignant tumour that can arise anywhere in the biliary tract which considered a second most common type of primary liver cancer after hepatocellular carcinoma. The aetiology is unknown, but predisposing conditions include primary sclerosing cholangitis, diabetes and obesity (Charatcharoenwitthaya et al., 2008). In addition, intrahepatic stones (hepatolithiasis), bile duct adenoma or papilloma, choledochal cysts, hepatitis C and possibly tobacco smoking that in association with primary sclerosing cholangitis could be involved in some cases (Welzel et al., 2007). Some of these conditions are thought to increase the risk of CCCs by inducing chronic

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inflammation or increasing bile duct pressure (Nordenstedt et al., 2012). Gallstones might induce biliary inflammation and cholecystectomy is typically followed by dilation of the bile ducts (Hussain, 2013). However, there are no reports on the chemical composition of gallstones in Iraqis though a sizeable population suffers from gallstones and neoplastic changes. This study describes an extensive quantitative analysis of gallstones, including calcium, magnesium, phosphate, uric acid and oxalate and their association to induce neoplastic changes. Hence, the objective of this study was to determine the chemical composition of gallstones and their association to induce neoplastic changes and cholangiocarcinoma in the cholecystectomy patients.

Materials and Methods

Gallstone extraction

This is a prospective study held in Baquba teaching hospital in the period from 1/10/2012 to 1/1/2013. Expert surgeons from the department of surgery extracted the gallstones during surgery. Initially, the gallbladder from patients was surgically removed whether by using a Laparoscopic Cholecystectomy or open procedure. The gallstones were extracted from the gallbladder and preserved under sterile conditions. Gallstones samples were collected from 40 (8 males and 32 females) patients for this study. The study was approved by the local Scientific and Ethic Committee of the institute and consent was obtained from all patients to use the isolated stones for research purpose.

Chemical Analysis of Gallstones and Tissues Specimens Collection

Stones were examined using stone analysis set (chemical qualitative method) of calcium, magnesium, phosphate, uric acid and oxalate. Gallstones analysis kit was used for qualitative determination of main individual components, which is manufactured by BIOLABO SA 02160, Maizy, France. The biopsy tissue specimens were fixed in formalin, embedded in paraffin, and 4 µm sections were prepared. The sections for histological examination were stained with haematoxylin and eosin (H and E).

Table 1. Shows the Number of Gallstones Incidence Rate Related the Gallstones Chemical Composition

NO of cases	Stones' chemical composition	Chemical frequency
10 (25.0%)	Ca, uric acid, Mg, PO ₄ and oxalate	Ca 38
15 (37.5%)	Ca, uric acid, Mg and oxalate	Uric acid 37
11 (27.5%)	Ca, uric acid and PO ₄	Mg 28
2 (-7.5%)	Mg and Ca	Oxalate 25
1 (-7.5%)	Mg and uric acid	PO ₄ 22
1 (2.5%)		PO ₄

*Ca: Calcium; Mg: Magnesium; PO₄: Phosphate

Table 2. Shows the Disease Incidence rate Related the Gallstones Chemical Composition with Different age Group

Disease	Incidence	Chemical Qualitative	Age Group
Cholangiocarcinoma	3 (7.5%)	calcium, magnesium, phosphate, uric acid and oxalate	50-62
Chronic cholecystitis and carcinoma <i>in situ</i>	4 (10.0%)	calcium, magnesium, phosphate, uric acid and oxalate	40-59
Chronic cholecystitis	31(77.5%)	2-4 chemicals	20-65
Acute cholecystitis	2 (5.0%)	2-4 chemicals	20-29

Results

An interesting finding in this study is that the females are at higher risk of cholelithiasis development than males, in a ratio of female: male (8:1). The results of this study showed the cholelithiasis highest incidence in the age group 40-49 was 13 cases followed by 11, 8 and 4 cases for age groups 30-39, 50-59, 20-29 and 60-more years old out of 40 cases respectively.

The chemical analysis showed the majority of gallstone were mixed, 38 gallstones contain calcium followed by 37 cases had uric acid, 28 stones contain magnesium, 25 and 22 stones contain oxalate and phosphate respectively. 10 (25%) gallstones compose of five chemicals included calcium, uric acid, magnesium, phosphate and oxalate, 15 (37.5%) gallstones composed of four chemicals were calcium, uric acid, magnesium and oxalate, 11 (27.5%) gallstones composed of three chemicals of calcium, uric acid and phosphate, 3 (7.5%) gallstones composed of two chemicals, which one contain magnesium and uric acid, and other contain magnesium and calcium), and only one (2.5%) gallstone composed of one chemical which is phosphate (Table 1).

Histopathology diagnosis confirmed that neoplastic changes was 17.5% as low-differentiated adenocarcinoma (cholangiocarcinoma) (7.5%) and dysplastic cells of carcinoma *in situ* (10%) as non invasive carcinomas and other 31 (77.5%) cases were chronic cholecystitis, and 2 (5%) cases were acute cholecystitis with (empyema) (Table 2). Microscopically, the cholangiocarcinoma (CCCs) composed of cells that resemble biliary epithelium. The cells were cuboidal or columnar, with a

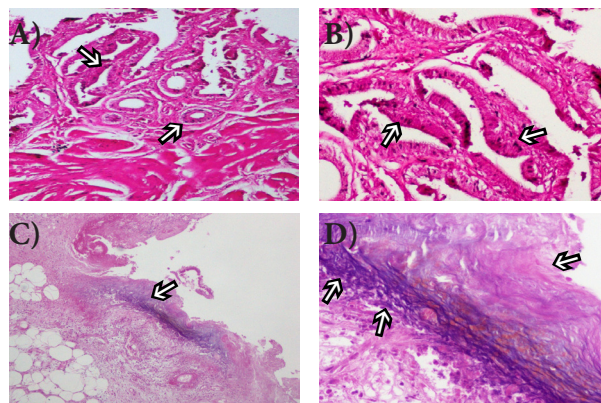


Figure 1. A and B) Biopsy from gallbladder after cholecystectomy showing Low-differentiated adenocarcinoma (cholangiocarcinomas), cells resemble biliary epithelium were cuboidal or columnar, with a moderate amount of clear or slightly granular cytoplasm. Most of their nuclei were vesicular (arrows), X10 and 40, H and E. **C and D)** Biopsy from bile duct showing erosive, ulcerated and follicular chronic cholecystitis (thin arrows) and dysplastic cells of the simple of carcinoma *in situ* (thick arrows), X 10 and 40, H and E

moderate amount of clear or slightly granular cytoplasm. Most of their nuclei were vesicular, different in size, hyperchromatic and mitoses were frequently seen (Figures 1A and 1B). The dysplastic lesions, identified in erosive, ulcerated and follicular chronic cholecystitis were in the four cases of carcinoma in situ (Figures 1C and 1D).

Discussion

Gallstone disease is still one of the most common digestive diseases with an overall prevalence ranges from 10% to 15% in adults and most costly digestive diseases requiring hospital admission, since its prevalence. Medical expenses for gallstone treatment exceeded \$4 billion in facility charges in 2004 in the United States (Everhart and Ruhl, 2009). Cholesterol disease, one of the commonest digestive diseases in western countries, is induced by an imbalance in cholesterol metabolism, which involves intestinal absorption, hepatic biosynthesis, biliary output of cholesterol, and its conversion to bile acids (Agostino et al., 2003).

Since the pathogenesis of gallstones is not clearly understood, its analysis-using chemical and spectroscopic techniques have provided some clues. The results of stones analysis suggested that the chemical composition of varies considerably. This is clear black stone had a higher bilirubin and lower cholesterol content than other type of stone it can be deduced that the bilirubin content of black stones was much higher than that of brown stones in adults (Portincasa et al., 2008). The frequent of metabolic abnormalities such as atherosclerosis, obesity, metabolic syndrome, and gallstone disease are related to impaired cholesterol homeostasis. The current view that such abnormalities gain clinical relevance only during adulthood and elderly age is dramatically changing. Both genetic and epigenetic studies suggested a very early onset of chronic disease already in utero. Starting from these particular metabolic conditions, a better understanding of mechanisms resulting in chromatin remodeling in response to environmental stimuli acting on the epigenome may offer new options for therapy of cholesterol cholelithiasis and better possibilities for primary prevention in subjects at risk (Agostino et al., 2003). The results of this study were agreed with pervious report that mentioned the major of chemical constituent stones is calcium compounds. Chemical composition of gallstones is essential for aetiopathogenesis of gallstone disease (Chandran et al., 2007). Mixed stones composed from including calcium, uric acid, magnesium, phosphate and oxalate, this result constant with pervious finding that also mentioned the pure uric acid stones are relatively rare (Morton et al., 2002). Reduced urinary pH could be an important risk factor for uric acid stone formation (Gianfrancesco et al., 2003). In this study, the majority of stones recovered from patients were composed of calcium, oxalate and uric acid. This might be because of a protein (29 kDa protein) which plays a major role in epitaxial deposition of calcium oxalate over uric acid core (Naseem et al., 2007). The results of study agreed with pervious analysis that showed the calcium content was significantly higher in mixed stones. An interesting

finding in this study is that the females are at higher risk of cholelithiasis development than males, in a ratio of female: male (7.3:1). This finding is attributed to high percentage of patients with high risk factors such as multiparity, use of oral contraceptives, obesity and family (Taher, 2013).

Gallstones might induce biliary inflammation and cholecystectomy is typically followed by dilation of the bile ducts (Hussain, 2013), which might also cause inflammation and thereby possibly increase the risk of CCCs. Previous studies addressing gallstones or cholecystectomy in relation to extrahepatic CCCs have revealed contradictory results, and there are potential associations with intrahepatic CCCs (Nordenstedt et al., 2012).

The results of this study showed that neoplastic changes was 17.5%, this result was agreed with previous studies that reported the differentiation often between a real dysplasia (neoplastic) from atypical epithelial hyperplasia in the case of cholecystitis can be difficult because of inflammation and ulceration (Stancu et al., 2007; Tran et al., 2012). Our results are situated, only (7.5%) low grade cholangiocarcinomas and (10%) chronic cholecystitis-dysplasia (carcinoma in situ) from the total of cases presenting chronic cholecystitis, this findings were disagreed with previous studies that found less than 1% was chronic cholecystitis-dysplasia (Stancu et al., 2007 Attraplsi et al., 2013). Often the differentiation between a real dysplasia (neoplastic) from atypical epithelial hyperplasia in the case of cholecystitis can be difficult because of inflammation and ulceration. This might be due to the extremely high incidence of ulcerated and erosive cholecystitis with important destructive lesions of the gallbladder mucosa (Stancu et al., 2007; Reshetnyak, 2012). In conclusion, majority of cases had mixed gallstones that involved five and four of inorganic chemicals of calcium, magnesium, uric acid and phosphate. The highest incidence was 13 gallstones cases in the age group 40-49 years, neoplastic changes (17.5%) including CCCs, (7.5%), dysplastic cells of carcinoma in situ (10%) and other 31(77.5%) cases were chronic cholecystitis.

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