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INTERNATIONAL JOURNAL OF CURRENT MEDICAL SCIENCES

RESEARCH ARTICLE

ISSN: 2249- 1465

International Journal of Current Medical Sciences - Vol. 5, Issue, 1, pp. 14-18, January, 2015

HISTORICAL REVIEW ON CHOLELITHIASIS

Kirti Rani

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida (UP) India

A R T I C L E I N F O

Article History:

Received 7th, December, 2014
Received in revised form 19th, December, 2014
Accepted 10th, January, 2015
Published online 28th, January, 2015

Key words:

Biliary calculi, cholelithiasis, bile ducts, gall bladder, cholecystectomy.

A B S T R A C T

The gall stone formation (cholelithiasis) is well known global health problem since very early times. Various biochemical and patho-physiological factors have been reported to be associated with gall stone disease such as stasis of bile, obstruction in bile duct, infection in bile, obesity, oestrogen level, inflammation of gall bladder valve, defective function of liver and gall bladder, reduction in bile salt concentration, change in cholesterol and bilirubin concentration and various precipitating factors for stone formation. Risk factors of cholelithiasis include overweight, age (near or above 40 years), female-menopausal age and racism with risk history of cholelithasis as found more prevalent in Caucasians than in people of other races. A lack of melatonin could significantly contribute to gallbladder stones, as melatonin inhibits cholesterol secretion from the gallbladder to enhances the conversion of cholesterol to bile and is an antioxidant, capable of reducing oxidative stress to the gallbladder. Clinical researchers believe that gallstones may be caused by a combination of risk factors, including inherited body chemistry, body weight, gallbladder motility with cholesterol crystallization and dietary habits. Role of various metal ions e.g. copper, iron, calcium, phosphate, sodium and potassium were also found to be responsible for nucleation of gall stone and their settling in gall stone bladder in cholelithiasis patients.

INTRODUCTION

Gallstones may be asymptomatic, even for years and are called "silent stones" and do not require treatment. Symptoms, commonly occurs to appear once the stones reach a certain size (>8 mm) (Pundir *et al*, 2002). A characteristic symptom of gallstones is a "gallstone attack", in which a person may experience intense pain in the upper-right side of the abdomen, accompanied by nausea and vomiting which lasts up to 30 minutes to several hours. A patient may also experience referred pain between the shoulder blades or below the right shoulder and these symptoms may resemble as symptoms occur in "kidney stone attack" usually at night. Other symptoms include abdominal bloating, intolerance of fatty foods, belching, gas, and indigestion (Pundir *et al*, 2002 and Dhull, 1966). The absence of such risk factors (overweight, age near or above 40, female-menopausal state and lack of melatonin) does not, however, preclude the formation of gallstones. No clear relationship has been proven between diet and gallstone formation, however, low-fiber, high-cholesterol diets and diets high in starchy foods have been suggested as contributing to gallstone formation (Pundir *et al*, 2001). Other nutritional factors that may increase risk of gallstones include rapid weight loss, constipation, too much fasting, low protein rich diet and low intakes of the nutrients folate, magnesium, calcium, and vitamin C. On the other hand, wine and whole-grain bread may decrease the risk of gallstones. Pigment gallstones are most commonly seen in the developing countries with their risk factors for pigment stones include hemolytic anemias (such as sickle-cell disease and hereditary spherocytosis), cirrhosis, cholestasis and biliary tract infections (Maki, 1966). People with erythropoietic protoporphyria (EPP) are at increased risk to develop gallstones at early age (Kovi, 1995). Additionally, prolonged use of proton pump inhibitors, called group of drugs which are used in treatment of peptic ulcers and pylori lead to decrease gallbladder function, potentially leading to gallstone formation. Cholesterol gallstones develop when bile contains high concentration of cholesterol as compared to bile salts. Besides it, two other factors play important role in causing gallstones, first, is how often and how well the gallbladder contracts for incomplete and infrequent emptying of the gallbladder may cause the bile to become over-concentrated and contribute to gallstone formation. The second factor is the presence of proteins in the liver and bile that either promote or inhibit cholesterol crystallization into gallstones (Dhull, 1966 and Cetta, 1978). In addition, increased levels of the estrogen in

Corresponding author:* **Kirti Rani
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida (UP) India

pregnancy or due to induced hormone therapy, use of estrogen-containing hormonal contraception therapy may increase cholesterol levels in bile and decrease gallbladder movement, resulting in gallstone formation. Cholesterol gallstones can sometimes be dissolved by oral ursodeoxycholic acid, but it may be necessary for the patient to take this medication for up to two years. Obstruction of the common bile duct with gallstones can sometimes be relieved by endoscopic retrograde sphincterotomy (ERS) following endoscopic retrograde cholangiopancreatography (ERCP). Gallstones can be broken up using a procedure called extracorporeal shock wave lithotripsy (often simply called "lithotripsy"), which is a method of concentrating ultrasonic shock waves onto the stones to break them into tiny dissolvable pieces. Cholecystectomy (gallbladder removal) has a 99% chance of eliminating the recurrence of cholelithiasis (Pundir *et al*, 2001 and Pundir *et al*, 2002). The lack of a gallbladder may have no negative consequences in many people, However, in 10 and 15% of patients, a condition called postcholecystectomy syndrome is found to be occurred which may cause gastrointestinal distress and persistent pain in the upper-right abdomen, with 10% chance of developing chronic diarrhea too. Choledocholithiasis is the presence of gallstones in the common bile duct and condition causes jaundice and liver cell damage, and requires treatment by cholecystectomy and/or endoscopic retrograde cholangiopancreatography. The diagnosis of choledocholithiasis is suggested when the liver function blood test shows an elevation in bilirubin and serum transaminases (Dhull, 1966, Pundir *et al*, 2002 and Sharma, 2012).

Historical background

It is well known that as much as 10% of human population suffers from gall stone disease and yet its cause remains virtually obscure till now. It was found that its more prevalence was coined in Northern part of India. Analysis of gall stones had always presented a problem because of presence of bile pigment and inorganic compounds. Various methods have been proposed including stepwise solvent extraction, x-ray diffraction analysis, infra-red absorption spectra, and thin layer chromatography. These methods are complimentary to each other but not a complete solution of cholelithiasis (Nakayama, 1968). In the present study, we have used the simple, sensitive and specific enzymic/colorimetric method for quantitative analysis of gall stones as well as serum of gall stone patients after cholecystectomy. These methods can be applied as small as 30mg of a gall stone sample and provide information of wide variety of compounds such as total cholesterol, total bilirubin, inorganic phosphate, calcium and iron. Atomic spectrophotometry has been employed for analysis of iron in both the gall stone and serum. Gall stone have been classified into three classes based on their physico-chemical characteristics: cholesterol stone, pigment stone and mixed stones. Cholesterol stone are formed in gall bladder, while pigment stones are formed in bile canaliculi of liver and mixed stones are formed in entire biliary tract and ultimately grow and settle down in gall bladder (Udupa *et al* , 1968). Chemical analysis of these gall stones revealed that cholesterol stone has calcium salt of cholesterol as major constituents, while pigment stone has calcium bilirubinate as chief constituent and mixed stone have salts of both cholesterol and bilirubin as principle constituents. The other chemical constituents are to be found in gall stones are calcium phosphate, calcium carbonate, calcuim salt of fatty acids, mucin, glycoprotein, phospholipids (lecithin, lysolecithin and cephalin) and some trace elements like sodium, potassium, copper, magnesium, mangenese and iron (Nakayama, 1967). The major constituents of bile are bile salts, pigment acids, cholesterol, protein, calcium and phospholipids. Biliary cholesterol is found to be present in unconjugated form and its concentration is unrelated to serum cholesterol level (Womack *et al*, 1963).

Morphological and chemical analysis of biliary calculi

Chemical analysis of gall stone might be helpful in finding etiopathogenisis of gall stone disease (cholelithasis) and different chemical composition of gall stones have been reported from different countries and from various regions of India, which are as follows:

In foreign countries

The crystalline composition of biliary calculi from American and Australian patients was determined by x-ray powder method and crystalline composition of gall stones from Germany, India, Kuwait, South Africa, Sweden and USA has subsequently been determined. The results were presented together with a statistical survey carried out on all available relevant data for the eight countries concerned (Sutor and wooley, 1969). The crystalline composition of gall stones were analyzed from Australia, England, Germany, India, Kuwait, South Africa, Sweden & USA by X-ray powder method. Out of 11 compounds identified, the three cholesterol monohydrate, anhydrous cholesterol and cholesterol II-constituted 71% of the total crystalline material in the stone, calcium carbonate contributed 15% and calcium palmitate contributed 6%. Smaller amount of apatite, sodium chloride, whitlockite and alpha-palmitic acid were also found. Gall stones from Germany, Sweden and Australia were almost comparable and more cases of carbonated gall stones were reported from England, while from South Africa, less cholesterol and more calcium phospahte and calcium palmitate stone formers are reported. Stones from Kuwait patients had a large amount of calcium palmitate and slightly more calcium palmitate and slightly more calcium carbonate (Sutor and Wooley , 1971).

Singapore: The chemical composition of biliary calculi in patients was determined which were operated upon over a three month period by a micro-analytical technique and equilibrium swelling tests. Twelve patients had biliary calculi was confirmed to the gall bladder and the main component of six calculi was cholesterol. In the other 6 patients, the dark brown to black pigment calculi contained bilirubin and calcium and a small quantity of cholesterol, but the major constituent was a insoluble pigment with swelling characteristics of a bilirubin polymer. These cholesterol and pigment calculi had similarities in composition to gall stones of Western Patients. In 13 patients with cholingitis, the brown, soft, pigment calculi found in the dilated bile ducts had biirubin

as the main component was considerably less but the lower swelling ratio suggested more cross linkage compared with black gall bladder stones. Difference in chemical composition between pigment gall bladder and bile duct calculi supported the concept that western type cholelithiasis and oriental cholingitis occurred as separate pathological and biochemical physiology of cholelithiasis entities in Singapore patients (Ti and Yuen, 1985)

Japan: The quantitative micro-analytical technique for gall stones was done which was based on the preliminary separation of gall stones constituents into subgroups with solvent partition and silica gel column chromatography followed by gas chromatography. The method was required only 35mg of serum or bile sample and provided the information on wide variety of gall stone constituents i.e. cholesterol and its esters, bilirubin, free fatty acids, glycerides, phospholipids, bile acids, calcium, sodium, potassium and phosphorus. Cholesterol ester was present in traces and cholesterol was found between 11% to 98% free fatty acids along with palmitic acid. Phospholipids and bile acid were also found to be present (Nakayama, 1967 & 1968).

Budapest: In 48 the infants who were suffered from cholelithiasis, the gall stones were developed mostly on the basis of predisposing disease and the spontaneous stone dissolution was to be found for prevalence of this disease in early age due to having family/racism history of cholelithiasis (Kovi *et al* ,1995).

Genova: The most important factors associated with cholesterol gall stones were found in Low-Density-Lipids with pathophysiological conditions called, hypercholesterolemia and hypertriglyceridemia, while the most frequent factors associated symptom was dyspepsia. Only the black pigmented and mixed gall stones were associated with jaundice and pancreatits (Cariatia *et al*, 1994).

Siena: The gall stones were classified which had interesting implications for the diagnosis and therapeutic purpose and were divided according to the type namely, cholesterol single and cholesterol multiple (Cetta, 1978).

In India

In India, occurrence of gall stones with excess of calcium phosphate was reported by Sutor and Wooley (1971). The report on physico-chemical study of gall stones from different parts of India are as follows.

Haryana: In Haryana, cholesterol was reported higher in cholesterol gall stones, bilirubin and calcium were higher in pigment gall stones and phosphate and iron content were reported higher in mixed gall stones (Pundir *et al*, 2001). The correlation between serum of gall stones of the patients of cholelithiasis was studied after laproscopic cholecystectomy with healthy persons and they were reported the significant increase in the total bilrubin and total iron in serum of cholesterol gall stones. Hence, they were also reported the significant increase in total cholesterol, total bilirubin and total inorganic phosphate in serum of pigment gall stones as well as significant increase in total bilirubin and toal inorganic phosphate level in serum of mixed gall stones (Pundir *et al, 2002*). The cholesterol was found to be major constituents in cholesterol calculi and formed in gall bladder. Bilirubin and calcium was reported major constituent of pigment calculi and these calculi were used to formed in bile canaliculi of liver. The mixed calculi were formed in entire biliary tract and then finally settled in gall baldder (Pundir *et al*, 2002). It was also reported the increased bile acid level in bile and serum of gall stone patients by using two new developed methods which were based on immobilization of 3-α hydroxysteroid dehydrogenase and diaphorase onto arylaime glass beads and alkylaime glass beads respectively (Rani K. *et al* 2004 & 2006).

Patiala: A study of 250 cases of patients having biliary tract disease was recorded and carried out with regard to their case history, physical and laboratory investigations, surgery and follow up. It was found that especially females were frequently affected, majority of cases were 3rd to 5th decade of their life (Singh *et al*, 1980). The rise in risk factors in context of age was showed decline in incidence with majority of cases (48.45%) were vegetarians and other cases (8.8%) belonged to middle and poor class. Pain in right upper quadrant of the anterior abdominal wall was the commonest symptoms, in about half the cases it got aggravated by fatty meals and fatty deposition was felt in right hypochondrium in 26.9%. Radio-opaque calculi were present in 8% of cases and biliary calculi were demonstrated by oral cholecystography, radiography could detect calculi in 47 (25.4%) cases and it overall diagnostic success rate was low (56.8%). Ultrasonography was proved more significant tool for its diagnosis and bile culture was observed positive in 8.8% of cases only for *E.Coli, proteus, Klebsiella, staphylococci or paracolon* with right subcostal incision. It was also observed that chornic cholecystitis with cholelithiasis was diagnosed in 26%, incidence of carcinoma in 2.8% and in 5 out of 7 cases malignancy, the mortality was quite low and thus acceptable (Singh *et al*, 1980).

Aligarh: The morphological changes of gall bladder were also studied in 415 cholecystectomy specimens. There was a preponderance of females (male to female ratio 1:6.5) and mean age of the cases was 43.6 years. Most of the cases (63.4%) were in the 4th and 5th decades of life and average duration of illness was observed 2.8 years. Associated cholelithiasis was found to be present in 85.3% cases and gall stones were of mixed variety in 78.2% cases, cholesterol type in 15.3% cases and both types in 65% cases (Tyagi *et al*, 1992).

Kanpur: A quantitative chemical analysis of gall stones was carried in 24 female patients and two categories of stones were identified. The first category included ten cholesterol stones with 93.38% cholesterol and only 0.17% bilirubin. The second category had mixed stones with cholesterol below 65%. They were sub divided in two groups (a) six stones with 0.5% bilirubin and (b) eight stone with 0.5% bilirubin. The calcium of cholesterol and mixed stones was comparable while phosphorus level in cholesterol stones was found to be low by over 30% in mixed type of stones (Bansal *et al*, 1992).

Aurangabad: It was reported that common bile duct stone was a rare entity and associated with a very rare asymptotic conditions called, congenital absence of gall bladder (Takalkar *et al*, 1996).

Guwahati: Patient cases with 160 cholecystectomy at Bharatpur clinic, Guwahati, were analyzed over a period of 13 years from 1982 to 1994. An attempt was made to find out the pattern and distribution of disease in the North Eastern Part of country regarding age, sex presentation, type of calculi and encountered anomalies. Most of findings were found to be comparable with the finding of similar series from other parts of country. A low male-female ratio (1:2.5) a positive history of oral contraceptive steroid intake in 90.9% female, a low mortality (0.6%) and higher incidence of extra hepatic biliary anomalies encountered (4.3%) were some of the salient features noticed for this study (Goswami, 1999).

Varanasi: The biochemical studies of the stones removed from gall stone patients were reported and classify them into three well known categories e.g. cholesterol stones (single or multiple), calcium bilirubinate stones, and mixed or infected stones. In the cholesterol stones, 80% cholesterol was found, the remaining were calcium, protein and small quantities of bilirubin. On the other hand, calcium bilirubinate stones have only calcium bilirubinate and protein. The mixed stones have all the components in variable quantities (Udupa *et al*, 1968).

Calcutta: The composition of different macroscopic types of gall stones was re-examined by X-ray diffraction analysi and study was indicated that macroscopic appearances were not related to their chemical constituent. The major constituents of stones were cholesterol & calcium oxalate, while the rest were a mixture of variety of calcium carbonate, billirubin and apatite. (Raha *et al*, 1996).

Mumbai: All gall stone patients were found to be had acute and chronic cholecystitis, biliary ducts obstruction, cholingitis and pancreatitis. Many of these complications as well as the presence of gall bladder stones were found to be in the common bile duct and hepatic duct without any clinical complication required ductal exploration (Bhansali, 1979).

Chemical analysis of serum of cholelithiasis patients

No relationship had been reported for the principle chemical constituents of gall stones such as total cholesterol, bilirubin, inorganic phosphate, calcium and iron with those of the serum of gall stones patients after cholecystectomy. However, bilirubin fractions have been found important indicator of cholestatic pediatric patients in reported cases (Ito F, 1995). The increase in serum bilirubin deconjugate fraction was also coined a significant early marker for cholestatic in chosen samples of rats (Mesa *et al*, 1997). Serum cholesterol concentration had been found not to have any correlation to bile cholesterol which depends only to limited extent on bile acid pool size and bile secretion in rats (Small, 1968). Chemical analysis of bile of gall stone patients was done to interpret microanalysis of bile acid and its role in etiology of cholelithaisis. It was found the presence of cholesterol crystal in bile of 83% cases, calcium bilirubinate crystal in bile of 77% cases and cholesterol as well as calcium bilirubinate crystals in 24% cases (Dhull, 1966). Study on various constituents of gall bladder bile was also done by quantitative microanalysis for cholesterol, total lipids, lecithin, fatty acids, phospholipids, bile acid and bilirubin (Nakayama, 1967). Gall bladder bile from patients of cholelithiasiswas reported for cholesterol crystal that develop within 48 hrs, whereas bile from persons without stone did not develop crystals and this study had also confirmed that abnormality within nucleus was due to gall bladder rather than defect in liver. Gall bladder might be accelerated nucleation of gall stones by addition of nucleating factors or by removal of absorption of antinucleating agents normally present in bile (Sedaghat and Grundy, 1980). Calcium present in bile is found to be a critical event in the formation of cholesterol gall stones and lead to the concept that if decrease free calcium in bile is to be found that might would have reduce the calcium lithogenecity. The excess of calcium concentration could have caused the solubility product of calcium in bile be exceeded and calcium might be precipitated (Sutor and Wolley, 1971). Calcium precipitation could have formed the nucleus on which cholesterol could precipitate to form stone (Lamont, 1983). Biliary cholesterol in the free esterified form and its concentration is bile was reported unrelated to serum of gall stone patients (Small, 1980). Various chemical constituents had play major role in precipitation of cholesterol nucleus in cholelithiasis such as biliary phospholipids include lecithin (90%), lysolecithin (3%), ethanolamine (1%), cholic acid, dihydroxycholic acid, deoxycholic acid and lithocholic acid with amino acids (glycine and toluene) to form salt, namely, glycocholate and taurocholate (small and Admirand, 1968). Bile samples and gall stone were also analyzed quantitatively for their composition and direct correlation was found to existed between the chemical composition of bile and gall stones of chosen gall stone patients (Lamont *et al*, 1968). However, a direct correlation was found between level of protein, calcium and cholesterol in bile samples and the various types of gall stones but no correlation was clearly observed in the level of bilirubin, bile acid in bile samples of various groups of gall stone (Dhull, 1966 and Pundir, 2002).

Role of metal ions in gall stones

The role of metal ion in gall stone formation had received scant attention in previous clinical reports and a small amount of copper and iron in the thin black-shell of sulphur around the central dark inclusion body the gall stone was also found (Been, 1977). scribed that protein pigment complex of gall stone contained copper and high molecular weight compounds which induced protein-albumin exit in bile (Maki, 1966). Gollan (1975) showed that copper might occur in gall stone as a consequence of precipitate of its protein ligand. Bile acids are conjugated with taurine or the amino acid glycine, or with a sulfate or a glucuronide, and are then stored in the gallbladder, which concentrates the salts by removing the water lead to gall bladder blockage due to gall stone formation.

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