Biochemical and demographical study of lipid profile in sera of patients with gallstone

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Abstract

Thirty patients with gallstone [aged 25-55 years]and thirty age-and sex-matched healthy subjects as control group were involved. Those patients were intended to undergo surgical removal of gallbladder in the surgical ward- Al Sadder Teaching Hospital and AL-Hakeem Hospital in Najaf city during the period from July/2009 to December /2009. The proportion of female was [86%] compared to the male t [14%]. The ratio of female: male was 5:1. Blood samples were obtained from all patients prior to surgery. Serum was obtained by usual methods and analyzed for lipid profile test [total cholesterol [TC], triglyceride [TG], high density lipoprotein-cholesterol [HDL-C], low density lipoprotein cholesterol [LDL-C] and very low density lipoprotein cholesterol [VLDL-C]}. The results showed that there were no significant differences between gallstone patients and control regarding HDL-C [$p>0.05$] but there was highly significant [$p<0.05$] difference in the concentrations of total cholesterol [TC], triglyceride [TG] and VLDL-C comparing with the control group. The study also showed that the sex has a significant effect [$p<0.05$] on the levels of TC, LDL-C, and VLDL-C in sera of patients with gallstone whereas, non significant sex variation had found with respect to HDL-C level. On the other hand, it was also found that there was a significant $p<0.05$] age variations with respect to serum levels of TC, TG and VLDL-C with effect with respect to both HDL-C and LDL-C levels in sera of patient with gallstone. Additionally, all items of lipid panel found to be significantly [$p<0.05$] varied with respect to the BMI. Similarly, smoking was found to be important cause and revealed a significant [$p<0.05$] difference regarding all parameters- but not LDL-C of lipid components.
The objective of this study was to estimate the serum lipid profile, in sera of patients with gallstone and investigate their relationship with demographic factors.

Key words: gallbladder, gallstone, lipid profile test

دراسة كيموحيوية وديموغرافية للدهون في مصول مرضى حصى المرارة

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الخلاصة

تضمنت الدراسة ثلاثون مصابا بحصى المرارة تراوحت أعمارهم بين (٢٥ – ٥٥) سنة وثلاثون شخصاً اصحاء بنفس الأعمار ونفس الجنس للمقارنة . تم الحصول على أمصال المرضى عند مجيئهم الى ردهة

العمليات في مستشفى الصدر التعليمي ومستشفى الحكيم في مدينة النجف الاشرف لاستئصال المرارة ، للفترة من تموز / ٢٠٠٩ الى كانون الاول /٢٠٠٩ . كانت نسبة الاناث (٨٦%) مقارنة بالذكور (١٤ %) ، اي حوالي ١:٥ .

جمعت العينات قبل اجراء العملية لغرض قياس مستوى الكولسترول والدهون الثلاثية والدهون عالية الكثافة والدهون واطئة الكثافة وواطئة الكثافة جدا. اظهرت نتائج الدراسة عدم وجود فروق معنوية [[p>0.05 بين مرضى حصى المرارة مقارنة بمجموعة السيطرة فيم يتعلق بتركيز الدهون عالية الكثافة لكن كان هناك زيادة معنوية (p < ٠.٠٥) في تركيز الكولسترول و الدهون الثلاثية والدهون واطئة الكثافة جدا مقارنة بمجموعة السيطرة.كذلك اظهرت الدراسة ان للجنس تأثير معنوي(p < ٠.٠٥) في مستويات الكولسترول و الدهون الثلاثية، و الدهون واطئة الكثافة والدهون واطئة الكثافة جدا في أمصال مرضى حصى المرارة مقارنة بالسيطرة بينما لم يكن هناك أي فرق معنوي في تركيز الدهون عالية الكثافة. من جهة اخرى وجد ان هناك تغايرعمري معنوي (p < ٠.٠٥) فيما يتعلق بمستويات الكولسترول، الدهون الثلاثية والدهون واطئة الكثافةجدا مع عدم وجود فروق معنوية(p>0.05) لمستويات الدهون الثلاثية عالية الكثافة والدهون الثلاثية واطئة الكثافة لمرضى الحصى مقارنة بالسيطرة. إضافة الى ذلك اظهرت جميع فحوصات الدهون فروق معنوية(p < ٠.٠٥) بالاعتماد على دليل كتلة الجسم BMI. كذلك بالنسبة للمدخنين اظهرت الدراسة فروق معنوية (p < ٠.٠٥) في مستويات الدهون لمرضى حصى المرارة باستثناء الدهون واطئة الكثافة.

كلمات مفتاحية: المرارة ، حصى المرارة ، اختبار الدهون

Introduction

Gallstones are very common diseases, they are hard pieces of stone like material, round, oval faceted commonly occurring in the gallbladder or the bill duct.[1] Women are much more likely than men to develop gallstones and about 1 to 5 older adults have gallstones.[2] In woman as an effect of female sex hormones on hepatic function.[3]Also oral contraceptive therapy may lead to increase formation of gallstone.[4] Bile duct stones diseases is relatively rare in children.[5] The majority of patients with gallstones are asymptomatic during their life.[6] These people are said to have so-called silent gallstones with no associated pain.[7] The incidence of gallstones increases with age.[5,7]Gallstones are classified into three types according to their chemical composition cholesterol , pigment, and mixed stones.[8] Most stones originate in the gallbladder and travel distally into the common duct, however, if the common bile duct is partially obstructed, stones can form there as well.[9] Most Cholecystitis are made up of cholesterol, calcium carbonate calcium bilirubinate , or a mixture of these.[6] Gallstones are believed to form, when the concentration of cholesterol exceeded that which can be held in mixed micelles solution with bile acids and phospholipids. Cholesterol gallstones result from the secretion by the liver of bile supersaturated with cholesterol. This results in cholesterol crystallization and stone growth within the gallbladder, which can be exacerbated by gallbladder stasis. This may occur in association with obesity, high-caloric and cholesterol-rich diets, or drugs [for example., clofibrate] and may result from increased activity of hydroxyl methyl glytarel Co-A (HMG-CoA) reductase, the rate-limiting enzyme of hepatic cholesterol synthesis, and increased hepatic uptake of cholesterol from blood.[9] In patients with gallstones, dietary cholesterol increases biliary cholesterol secretion[10]. The composition of the bile salt pool may also influence the ability to maintain cholesterol in solution.[11] Patients over age 60 and those who have had numerous bowel operations [particularly in the region where the small and large bowel meet] are at especially high risk.[10] The main function of gallbladder is to concentrate and store hepatic bile during the fasting state and to deliver bile in to the duodenum in response to the meal.[12] In the gallbladder, the bile is concentrated by absorption of water. The degree of this concentration is shown by the increase in the

concentration of solids liver bile is 97% water, whereas the average water content of gallbladder bile is 89%.[13] Cholesterol supersaturation of bile essential for cholesterol stone formation in many individuals in whom such supersaturation occurs will never develop stones.[14] A number of lipoprotien have been reported as positive crystallizing factors. Gallbladder motility represents a further factor that may influence the cholesterol crystallization from supersaturated bile. There is evidence from animal models that gallbladder stasis leads to cholesterol crystallization mediated by hypersecretion of mucin.[7] In all population of the world , women are almost twice as likely as men to experience cholelithiasis.[15] Gender is one of the most powerful influences on gallbladder, they are more common in females during their fertile years as in males.[6] Biliary cholesterol saturation increases with age due to a decline in the activity of cholesterol 7-αhydroxylase , the rate limiting enzyme for bile acid synthesis.[16] In the elderly , bile acid synthesis is reduced , biliary cholesterol output is increased and cholesterol saturation of bile increase both in women and men.[17] People with diabetes generally have high levels of fatty acids triglycerides. These fatty acids may increase the risk of gallstones.[18].

Material and methods

Patients and control

During the period from July/2009 to December /2009, thirty patients with symptomatic gallstone [4male and 26females] with ages ranged between (25-55) year were taken from surgical ward-AL-Sadder teaching and AL-Hakeem hospitals in Najaf city.

The control group consisted of people who were chosen from medical staff and relative who were free from signs and symptoms of gallstone disease, liver disease, lipid disorders, diabetes mellitus and hypertension. They were 25 females and 5 males.

Five ml of blood by vein puncture were drawn from each fasting patient and control .The sample was left at room temperature for 10 minutes to clot then was centrifuged for 15 minutes at 3000 xg for 5 min. The sera was separated and kept in deep freeze until analysis.

Stone Samples

Gallstones from 30 patients [2 men and 28 women] of cholelithiasis were collected after cholecystectomy. The stones were divided into 3 groups depending their color: 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. The other relevant information about the patients such as age, sex, smoking and number of calculi were obtained records from hospital. The various physical parameters of stones such as number, shape, size, texture and cross-section were noted.

Qualitative analysis of Gallstone

The stones were washed by distilled water and dried, then they were powdered in a pestle and mortar to detected the organic and inorganic compounds. [19]

Cholesterol test

To detect cholesterol, 10mg of stone powder were dissolved in 1 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min, and then stone solution was used for detection of cholesterol by adding H_2SO_4. Tow layer of color will form (chloroform layer, red-blue color and acid layer, green color).

Calcium test

To detect calcium,10 mg of stone powder were dissolved in 1 ml HCl in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water.[20] The tube was kept in boiling water bath then 0.5 ml of ammonium oxalate were added, if misty (positive) or not(negative).

Oxalate test

To detect the oxalate 10 mg stone powder were dissolved in 1 ml of HCl in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water.[21] The tube was kept in boiling water bath for 1hr then 0.5 ml of potassium permanganate were added and left for ten mint for appearing color[positive]

Phosphate test

To detect phosphate,10 mg stone powder were dissolved in 1 ml of HCl in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water.[21] The tube was kept in

boiling water bath for 1hr then 0.5 ml of Molybedate was added followed by 1ml of ascorbic acid and left for five mint to blue deep color (Positive)or not (Negative).

Carbonate test

To detect $CO_3^=$ 10mg stone powder were dissolved in nitric acid if buzz and bubbling were seen (positive) or not (Negative).[22]

Determination of Serum Total Cholesterol,triglycerides and HDL-cholesterol concentrations

Total cholesterol, triglycerides and HDL-cholesterol were determined by enzymatic method according to spinreact kits.

Statistical Analysis

Results are expressed as Mean±SD. student T-test was used to analyze results by using SPSS 17. p-Value less than 0.05 was considered significant.

Results and discussion

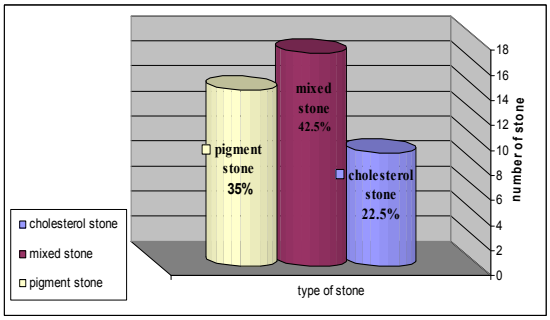
Chemical Constituents of gallstone

The detection of chemical constituents in gallstone has shown that it contains cholesterol, calcium, Phosphate, carbonate, oxalate.

The results demonstrated that the percentage of the type of gallstones were 42.5% as mixed stone ,35% pigment stone and 22.5% as cholesterol stone **(Fig-1).** These results don't agree with the results of related study carried out in south of Iraq by [14] who showed that the most common type of gallstone was of cholesterol type (42.86%) followed by pigment stones (38.6%) and then mixed stone (63.8%).[14] The comparison of our data with this study may not be valid because of geographic, dietary and ethnic differences.

Determination of serum lipid profile

This study showed that the cholesterol affected more in patient with gallbladder disease and the total cholesterol , triglyceride and very low density lipoprotein concentration had significant($p<0.05$) increase in patient with cholesterol calculi compared with control. High density lipoprotein and low density lipoprotein concentration did not show significant difference compared with control **(Table-1)**. These results were in confirmation with the earlier report (Atman, 2006). he is study relation between lipid profile and gallstone formation 2006.[23].Super saturation of cholesterol is believed to be due to abnormal production of bile from liver. The concept of cholesterol supersaturation as a basis for gallstone formation has been emphasized for cholesterol stones, which are composed of mainly cholesterol.[24] Increased cholesterol reduces gallbladder emptying.[25]Although gallstones are formed from supersaturation of cholesterol in the bile, high total cholesterol levels themselves are not necessarily associated with gallstones. [24,25]Some evidence suggests that high levels of triglycerides may impair the emptying actions of the gallbladder. The mechanism by which cholesterol stones form is not fully understood, but are likely as the result of a complex alteration in hepatobiliary function.[10] Bile containing cholesterol stones has an excess of cholesterol relative to bile salts and phospholipids. Thus allowing cholesterol crystals to form. Such bile is termed 'supersaturated' or 'lithogenic'.The concentration of bile salts in bile is reduced by oestrogens, and also by factors which interrupt the intrahepatic circulation of bile salts (e.g. ileal disease, resection or bypass and cholestyramine therapy). These conditions are all associated with an increased incidence of stones, but there are still some people with cholesterol supersaturation who remain free of stones, suggesting that there are other factors are importance.[14]



(Figure-1) percentage of types of gallstone

Demographical study

Sex factor

The incidence of this disease was found to be higher in females 86% than in males 14% the ratio of female to male was 5:1. The results revealed that there was a significant ($P < 0.05$) increase in the concentrations of male cholesterol, TG,VLDL and LDL comparing with female , whereas there wasn't any significant difference in the concentration of HDL between

male and female **(Table-2)**. It is tempting to explain the increased frequency of gallstone in woman as an effect of female sex hormones on hepatic function , bile secretion and gallbladder function.[26] In contrast to earlier reports , result of studies of biliary lipids during the menstrual cycle show no effect on cholesterol saturation.[27] Bile tends to be more saturated in woman than in men and this cannot be explained by differences in body weight or age.[28] The increased number of pregnancies is associated with an increased risk of gallstones believe that the progesterone component rather than the estrogen is responsible for the changes in biliary lipids. Bile is more saturated during the second and third trimester of pregnancy. [29]The use of oral contraceptive also induces an increased risk of gallbladder disease. Oral contraceptive therapy may be associated with an increase in cholesterol saturation.[11] Oestrogen increases the biliary of cholesterol secretion and the lithogenicity of bile , there may be stimulation of hepatic lipoprotein receptors and increased hepatic cholesterol uptake.[26]Synthesis of chendeoxycholic acid is inhibited and the pool of chenodeoxycholic acid is reduced thus , the overall effect of oesterogen is to increase biliary cholesterol and promote the secretion of lithogenic bile.[7] A part from an effect on bile chemistry , hormones also influence gallbladder function which , in turn , might affect biliary lipids secretion or predispose the organ to cholecystitis. In pregnancy the fasting and residual gallbladder volumes are larger and the emptying rate is slower than in non pregnant women.[5,30] The effect of female sex hormones on the biliary system is complex and diverse but , by adversely influencing cholesterol and bile salt sectarian in to bile as well as gallbladder function.[31] They predispose to cholesterol gallstone disease. The strong relationship of femininity and parity to cholesterol gallstone does not exist for pigment lithiasis. Both men and women are affected equally.[32] The results of our study are very much in agreement with the above view , whereby the incidence of pigment stones in both sexes is almost equal . Bile cholesterol increases with age and is raised in women, particularly those taking the contraceptive pill.[26]

Age factor

The patient with gallstones under study were classified into two groups depending on their age. The results showed that there was significant ($p< 0.05$) increase in concentration of triglyceride in aging group (25-40) year and significant ($p<0.05$) decrease in concentration of cholesterol and VLDL but there were no Significant ($p>0.05$) differencesin the concentration of HDL and LDL between the two groups **(Table-3)**. This study revealed that in both sexes, hospitalization of patients for gallstones were above 20 years age, and the significantly increasing with age , more so in females and there after gradually declined. The affected patients over 60 were rare. The low incidence of gallstone disease over the age of 60 might be due to partly the fact that old people are poor candidate for surgery and anesthesia in addition to lesser easy access of medical assistance to this age group. The proportion of patients below 60 years was high. That is, operation was performed at an earlier stage of the disease thus favoring the admission of young patient with fewer anticipated complication. In general, gallstones of any type are rare before the age of 10 years.[14]Many factors contribute to gallstones in children's . About 25% of children with gallstones have hemolytic disease, bowel resection and heart disease.[9] The prevalence of gallstones remains rare until the onset of adolescence and then begins to increase in frequency[34] ,particularly in females.[4] The results revealed that there is consistent evidence that gallbladder disease is more common in females at all ages ,although the female :male proportion increases slightly with age.[9]

Obesity factor (BMI)

The patient with gallstones under study were also classified into two groups depended on their obesity factor (BMI :kg/m^2) above thirty obese and under thirty non obese. The result showed that there was ahighly significant ($P<0.01$)decrease in the concentration of cholesterol, triglyceride , HDL and a significant decrease in the concentration of VLDL in obese group and significant ($p<0.05$) increase in concentration of LDL comparing with non-obese subjects **(Table-4)**. Obesity is a major risk factor for gallstones([14,35) The most important factors that influence excretion and

concentration of lithogenic and inhibitory substances are diet and related metabolic disorders. Increasing incidence of urolithiasis in world countries in the last decades is due to changes in lifestyle. Factors raises particular attention to dietary habits and nutritional status of stone formers. Larger body size(BMI) was suggested to be associated with a higher risk of stone formation.[14]A risk factor for the development of recurrent stones may be overweight or obesity and associated dietary pattern. However, the mechanisms for this effect are still unclear.[10] Also a large clinical study showed that being even moderately overweight increases the risk for developing gallstones. The most likely reason is the amount of bile salts in bile is reduced, resulting in more cholesterol.[36]Because obesity is a risk factor, people should aim to maintain an ideal body weight. Otherwise there is no specific diet for gallstone disease. Very obese individuals who are attempting drastic weight reduction are at risk for developing gallstones. They should lose weight under medical supervision.[35]

Smoking factor

The patients with gallstones under study were classified into two groups [smokers and non smokers],the results showed that there was significant ($p< 0.05$) increase in concentration of cholesterol and significant ($p<0.05$) decrease in concentration of triglyceride ,Also significant decrease in HDL and VLDL in smoker patients ($p<0.01$)comparing with non-smoker patients but there were non Significant [$p>0.05$]differences in concentration of LDL between two groups **(Table-5)**Smoking affects every part of the body, with cholesterol being no exception. It raises "bad" cholesterol to a high degree while lowering the "good" cholesterol. It also puts you at risk for heart attack, stroke and cardiac arrest because high cholesterol in the veins and arteries. The good news is that once you quit smoking, your bad cholesterol level will diminish almost instantly.[37] When you smoke, you lower your high-density lipoprotein (HDL) cholesterol, which is a bad thing
because HDL is associated with low blood pressure
and low risk of artherosclerosis (clotting of the blood vessels) .[38] When there is artherosclerosis, oxygen-rich blood can't move easily through the blood vessels and take the oxygen to the heart or brain. This eventually leads to considerable physical damage or death. [39]

Other diseases

This study found some patients suffering from other diseases plus gallstone such as sugar increase, blood pressure, heart disease, kidney disease and anemia. So the patients with gallstones under study were classified into two groups depending on their other disease. The result showed that there was significant ($p< 0.05$) increase in concentration of cholesterol and significant ($p< 0.05$)decrease in concentration of triglyceride,HDL and VLDL in patients with diseases group comparing with those without diseases group but no significant($p>0.05$) differences in concentration of LDL between two groups (**Table-6)**. People with diabetes are at higher risk for gallstones and have a higher-than-average risk for a calculous gallbladder disease (without stones)[38] Gallbladder disease may progress more rapidly in patients with diabetes, who tend to suffer worse infections.[39]

(Table-1) The concentraction of lipid profile in sera of control and patients

Group	No.	Cholesterol [Mean ±SD]	TG [Mean ±SD]	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
Patient	30	275.43±35.89*	186.63 ± 67.21*	43.83 ± 7.75	37.03 ± 13.45*	192.21± 41.38
Control	30	252.87 ± 34.67	156.77 ± 29.56	46.93 ± 6.27	31.23 ± 5.98	184.26± 35.19

*** Significant difference** at $P< 0.05$
****Highly significant difference** at $P< 0.01$

(Table- 2) The concentration of lipid profile in sera of male and female

Group	No	Cholesterol [Mean ±SD]	TG [Mean ±SD] .	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
Male	4	259.00±41.721*	201.00± 82.426*	39.75± 5.123	40.00± 16.269*	237.25± 40.227*
Female	26	277.96±35.143	184.42± 66.219	44.46± 7.936	36.58± 13.276	188.00± 39.754

*** Significant difference** at P< 0.05
****Highly significant difference** at P< 0.01

(Table- 3) The concentraction of lipid profile in two age groups in patient with gallstone

Age Group	No.	Cholesterol [Mean ±SD]	TG [Mean ±SD] .	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
25-40	7	273± 37.278*	190.91± 68.773*	42.00± 44.39	34.29± 13.073*	191.17± 43.385*
41-55	23	282.00± 32.60	172.57± 64.678	44.39± 8.239	37.87± 13.729	205.71± 41.290

***Significant difference** at P< 0.05
****Highly significant difference** at P< 0.01

(Table- 4) the concentraction of lipid profile in sera of obese and non obese patients

Group	No.	Cholesterol [Mean ±SD]	TG [Mean ±SD] .	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
Non-obese	18	211.00± 97.905**	148.11 ± 71.139**	35.44 ±16.702*	34.50±12.420*	254.67±93.672*
obese	12	307.42± 156.986	203.50 ±72.604	45.33 ±8.669	46.25±17.741	265.56±324.600

*** Significant difference** at P< 0.05
****Highly significant difference** at P< 0.01

(Table-5) The concentraction of lipid profile in sera of smokers and non smokers patients

Group	No.	Cholesterol [Mean ±SD]	TG [Mean ±SD] .	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
Smokers	3	297.00± 46.87*	129.33± 17.92*	38.00±1.73**	25.67±3.78**	233.33±41.63
Non-smokers	27	273.04± 34.75	193.00±67.75	44.48±7.87	38.30±13.56	190.26±41.27

*** Significant difference** at P< 0.05
****Highly significant difference** at P< 0.01

(Table-6) The concentraction of lipid profile in sera of patient with other disease and without disease

Group	No.	Cholesterol [Mean ±SD]	TG [Mean ±SD] .	HDL [Mean ±SD]	VLLD [Mean ±SD]	LDL [Mean ±SD]
With disease	7	282.00 ±32.604*	172.57±64.678*	42.00±5.859*	34.29±13.073*	205.71±41.290
Without disease	23	273.43±37.278	190.91 ±68.773	44.39±8.239	37.87±13.729	209.17±43.385

*** Significant difference** at P< 0.05
****Highly significant difference** at P< 0.05

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