Risk factors of Gallbladder stone formation
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Abstract
Gallbladder stone (GBS) formation is one of the most prevalent and costly gastrointestinal tract disorders in the world; to find the risk factors of GBS formation; 166 patients were admitted to Sohag University Hospital (63 cases had GBS, while the other 103 had no GBS) were included in the study. Their ages ranged from 15-85 ±3 years; The possible risk factors for GBS formation are family history of GBS, excessive fatty meal intake, higher body weight, higher body height, increased abdominal circumference, higher body mass index (BMI), chronic liver disease (CLD), presence of hepatic focal lesions, splenic enlargement and positive hepatitis C virus antibodies (HCV Abs). Multivariate regression analysis showed that the independent risk factors for GBS formation were positive family history and excessive fatty meal intake; Positive family history of GBS and excessive fatty meal intake were the independent risk factors for GBS formation.

Keywords: GBS; CLD; HCV; HBV; BMI; weight; height.

1. Introduction
Gallbladder disease (GBD) is a significant health burden in the developed world and its prevalence has risen by more than 20% in the past three decades (Shaffer, 2005). In the United States, 20-25 million individuals are affected by GBD, resulting in an average annual treatment cost of $6.2 billion (Ibrahim et al., 2018). Obesity, diet, rapid weight loss, metabolic syndrome, and conditions such as CLD, Crohn’s disease, irritable bowel syndrome, and gallbladder (GB) stasis increase the risk of GBS formation (Housset et al., 2016). Surgical removal of the GB, cholecystectomy, is the most recommended treatment for GBD; however, the procedure increases the risk of non-alcoholic fatty liver disease, cirrhosis, and metabolic effects (Soonawalla, 2018). Oral dissolution therapy using ursodeoxycholic acid is currently one of the leading non-invasive alternative treatments for GBS. Ursodeoxycholic acid dissolves small cholesterol GBS by decreasing the hepatic secretion of cholesterol and lowering the chances of unsaturated GB bile formation (Portincasa et al., 2012). Although oral dissolution therapy has proven to be a safe alternative to cholecystectomy, the recurrence rate of GBS was observed in 86 subjects after 96 confirmed post-dissolution treatments over a 12-yr follow-up period (Villanova et al., 1989). The high incidence rate and treatment cost associated with GBD has encouraged the search for preventative
strategies. In approximately 75% of cases, impaired GB motility results from the accumulation of cholesterol GB. Therefore, agents that reduce lipid concentrations or improve GB motility may reduce the need for surgical interventions (Martínez Castaño et al., 2014).

2. Materials and Methods

This study was conducted on 166 patients admitted to Tropical Medicine and gastroenterology Department, Sohag University Hospital (63 cases had GBS, while the other 103 had no GBS). Their ages ranged from 15-85 ±3 years. Patients Less than 15 years old were excluded from the study. Patients were divided into 2 groups:

Group 1: 103 patients without GB stone(s) (59 males, 44 females).
Group 2: 63 patients with GB stone(s) (28 males, 35 females).

The study was a cross-sectional study and was conducted from April 2021 to March 2022 after being authorized by the Scientific Ethics Committee of our faculty. All patients signed informed written consent before starting the data collection with an explanation of the purpose of the study.

All patients will be subjected to

3.1. Complete history taking stressing on age, sex, and excessive fatty meal intake.
   - Comorbid conditions (diabetes and hypertension).
   - History of taking lipid-lowering drugs.
   - History of oral contraceptives.
   - History of liver disease (history of hematemesis, history of encephalopathy)
   - Family history.

3.2. Thorough clinical examination: General examination, vital signs, height, weight, BMI.

3.3. Laboratory Investigation: 1- Lipid profile (total cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL)). 2- Thyroid function test. 3- Blood sugar. 4- Complete blood picture 5- Liver function (serum bilirubin, serum albumin, prothrombin time (PT), prothrombin concentration (PC) and international normalized ratio (INR)) 6- Serology (Hepatitis B surface antigen (HBS Ag), HCV Abs) 7- Serum creatinine.

3.4. Abdominal ultrasound is to evaluate liver, spleen and GB (presence or absence of stone(s), their size and number).

Statistical analysis:

Data were analyzed using the SPSS (statistical package software for social sciences). Quantitative data were represented as mean, standard deviation, median and range. Data were analyzed using a student t-test to compare the means of the two groups. When the data were not normally distributed Mann-Whitney test was used. Qualitative data were presented as numbers and percentages and compared using the Chi-square test. Graphs were produced by using Excel or SPSS programs. The P-value was considered significant if it was less than 0.05.

3. Results

The study population included 166 cases. Out of them; 63 cases (38%) had GBS, while the other 62% had no GBS. The mean age of patients with GBS was 55.67±16.64 years compared to 56.29±16.16 years among cases without GBS, with an insignificant difference. Around half of the study cases were males (52.4%). Females showed a higher prevalence of stone formation (55.6%) compared to only 44.4% among males, but the difference was not significant. Nearly two-thirds of the cases with GBS had multiple stones (61.9%), while the other 38.1% had a single stone. The majority of stones were small-sized (66.7%); with large-sized stones seen in only 21 cases (33.3%).

Positive family history was seen in more than half of the GBS cases (55.6%) compared to only 12.6% among cases without stones; with a highly significant difference. Excessive fatty meal intake was seen in more than half of the GBS cases (60.3%) compared to only less than one-third (30.1%) among cases without stones; with a highly significant difference. Cases with GBS had significantly higher body weight and abdominal circumference, and higher BMI compared to those without stones.

Less than one-fifth of the study population were diabetics. Diabetes was slightly higher among cases with GBS (25%) compared to those without stones (15.5%); with an insignificant difference. Also, less than one-fifth of the study population was hypertensive. Hypertension was
slightly higher among cases with GBS (22.2%) compared to those without stones (17.5%); with an insignificant difference.

More than 60% of the study population had CLD (63.3%). Cases with CLD showed more liability for GBS compared to cases without CLD, with an insignificant difference. There were statistically insignificant differences between patients with GBS and those without GBS regarding a history of hematemesis, encephalopathy, and jaundice. Cases with GBS had slightly higher total cholesterol and TG levels, but with statistically insignificant differences.

Females taking hormonal contraceptive pills, patients with hepatic focal lesions, and patients with splenic enlargement had a statistically higher risk for developing GBS (p-value were 0.001, 0.003, 0.001 respectively).

Ascites was found in one-third of the cases with statistically insignificant difference between the group of GBS and those without GBS. Most of the study cases had mild degree anemia; with a mean hemoglobin level of around 11 gm/dL. White blood cells and platelet count were within normal values among the vast majority of the cases. Regarding the blood picture, there were insignificant differences between the group who have GBS and those without GBS. Our study population had slightly elevated Alanine transaminase levels and more elevated Aspartate Transaminase levels. The vast majority of our cases had elevated levels of both total and direct bilirubin. Also, our cases had mildly elevated PT, low PC, and a mean INR of around 1.2. The vast majority of the cases with GBS had either hepatitis B or C infection or both; with only 30% of the study cases having negative HBS Ag and HCV Abs infection. GBS was more common in patients with hepatitis C virus (HCV) (41.27%) than in patients with hepatitis B virus (HBV) (3.17%). Creatinine was mildly elevated among the majority of our cases. The PT was significantly lower among cases with GBS compared to those without.

Univariate binary logistic regression analysis for the possible risk factors for GBS formation showed that family history of GBS, excessive fatty meal intake, higher body height, higher body weight, increased abdominal circumference, higher BMI, CLD, presence of hepatic focal lesions, splenic enlargement and positive HCV Abs were the possible risk factors for GBS formation. The multivariate regression analysis showed that the independent risk factors for GBS formation were positive family history and excessive fatty meal intake.

Table 1. Univariate binary logistic regression analysis for the possible risk factors for GB stones among the study population.

<table>
<thead>
<tr>
<th>Item</th>
<th>Odd's ratio</th>
<th>CI of Odd's ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.998</td>
<td>0.979-1.017</td>
<td>0.810</td>
</tr>
<tr>
<td>Sex</td>
<td>1.676</td>
<td>0.891-3.153</td>
<td>0.109</td>
</tr>
<tr>
<td>Family history</td>
<td>8.654</td>
<td>4.027-18.596</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fatty meal</td>
<td>3.530</td>
<td>1.830-6.812</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height</td>
<td>1.062</td>
<td>1.025-1.101</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight</td>
<td>1.042</td>
<td>1.019-1.065</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal circumference</td>
<td>1.091</td>
<td>1.058-1.126</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.057</td>
<td>1.001-1.117</td>
<td>0.046</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.540</td>
<td>0.248-1.177</td>
<td>0.121</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.741</td>
<td>0.339-1.620</td>
<td>0.453</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>3.702</td>
<td>1.900-7.211</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hematemesis</td>
<td>1.647</td>
<td>0.811-3.346</td>
<td>0.168</td>
</tr>
<tr>
<td>History of encephalopathy</td>
<td>0.870</td>
<td>0.396-1.912</td>
<td>0.729</td>
</tr>
<tr>
<td>History of jaundice</td>
<td>1.372</td>
<td>0.659-2.855</td>
<td>0.398</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.003</td>
<td>0.997-1.009</td>
<td>0.292</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.002</td>
<td>0.996-1.008</td>
<td>0.563</td>
</tr>
</tbody>
</table>
Use of contraceptive pills  0.997  0.692-1.435  0.985  
Hepatic focal lesions  3.364  1.493-7.579  0.003  
Splenic enlargement  1.726  1.249-2.387  0.001  
Ascites  0.884  0.663-1.179  0.403  
Haemoglobin  1.030  0.910-1.167  0.639  
Mean corpuscular volume  0.996  0.961-1.034  0.849  
White Blood Cell  1.045  0.980-1.115  0.178  
Platelets  1.001  0.998-1.004  0.364  
Alanine transaminase  1.000  0.996-1.003  0.846  
Aspartate Transaminase  0.999  0.995-1.002  0.488  
Total bilirubin  0.974  0.908-1.046  0.474  
Direct bilirubin  0.938  0.804-1.094  0.414  
Albumin  1.082  0.917-1.276  0.350  
Prothrombin time  0.936  0.831-1.054  0.277  
Prothrombin concentration  1.013  0.997-1.029  0.113  
International Normalized Ratio  0.714  0.259-1.968  0.515  
Serology  2.289  1.284-4.081  0.005  
Creatinine  1.096  0.799-1.503  0.571  

4. Discussion

GBS is found in 10-20% of the general adult population (Barker et al., 1979; Gidrey, 1990). The study population included 166 cases. Out of them; 63 cases (38%) had GBS, while the other 62% had no GBS. The mean age of the study population was 55-56 ±16 years. Our results agree with Chen et al. (1998) who found that the incidence of gallstones increases with age across all ethnic groups, becoming 4 to 10 times more likely in individuals older than 40 years, with a very low rate among infants and children. Some specific populations, such as American Indians (Pima), have an increased incidence of GBS (up to 70% by 30 years of age), implicating hereditary metabolic factors (Wesdorp et al., 2000).

Our study demonstrates that around half of the study cases were males (52.4%). Females showed a higher prevalence of stone formation (55.6%) compared to only 44.4% among males. Nearly two-thirds of the cases had multiple stones (62%), while the other 38% had a single stone. The majority of stones were small-sized (66.7%); with large-sized stones seen in only 21 cases (33.3%).

These results agreed with Saldinger et al. (2016) who found that female gender is a risk factor for developing GBS, surpassing males in the incidence of GBS. This can be in part explained by a hormonal effect; estrogen decreases bile salts secretion and increases cholesterol, whereas progestins act by impairing GB emptying, causing stasis. Pregnancy is associated with up to 30% risk of developing biliary sludge.

The present study showed that positive family history was seen in more than half of the GBS cases (55.6%) compared to only 12.6% among cases without stones; with a highly significant difference. Gilat et al. (1983) found that GBS is more frequent by a ratio of 3:1 in siblings and other family members of affected persons than in spouses or unrelated controls. They found a 21% prevalence rate in first-degree relatives compared with 9% in matched controls. Sarin et al. (1995) also observed a prevalence that was 5 times higher in relatives than in controls. Furthermore, cholesterol supersaturation is higher in fasting duodenal bile of older sisters of patients with cholesterol GBS than in controls. Cholesterol synthesis rates, bile saturation levels, and GBS prevalence rates are also significantly higher on pair-wise correlations in monozygotic than in dizygotic male twins (Kesaniemi et al., 1989). Despite these observations, a mode of inheritance that fits a Mendelian pattern cannot be shown in most cases. Evidence for a genetic component of cholesterol GBS in humans is mostly indirect and based on geographic and ethnic differences.
and family and twin studies (Kesaniemi et al., 1989). Although some independent risk factors (e.g., aging, gender, parity, obesity, insulin resistance, some drugs, rapid weight loss) for GBS formation have been found (Everson et al., 1991), none can explain the striking differences in the prevalence of GBS among different populations, thereby suggesting a genetic contribution to the etiology of the disease (Wang et al., 2010).

In our study, excessive fatty meal intake was seen in more than half of the GBS cases (60.3%) compared to only less than one-third (30.1%) among cases without stones; with a highly significant difference. Cases with GBS were significantly longer, had significantly higher body weight and abdominal circumference, and had higher BMI compared to those without stones. Our results agree with Harman (1968), who found the importance of high caloric and carbohydrate intake as risk factors for the development of GBS in modern society. Obesity is a well-established major risk factor for the development of GBS, with an incidence of 25% in morbidly obese patients (Li et al., 2009). The association with simple obesity is important in females, whereas in males it is mostly associated with intraabdominal (central) obesity and metabolic syndrome (Tsai et al., 2004). A large prospective study of obese women demonstrated a strong linear association between BMI and the prevalence of cholelithiasis. In this study, the risk of GBS was 7-fold higher in women with the highest BMI (>45 kg/m2) than in non-obese control women. Obesity is associated with increased hepatic secretion of cholesterol into bile, possibly because of higher enzymatic activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase and increased cholesterol synthesis in the liver. As a result, GB bile is more lithogenic in obese than in nonobese persons, and a higher ratio of cholesterol to solubilizing lipids (bile acids and phospholipids) is observed in the former group. GB motility is often impaired in obese persons, thereby promoting mucin secretion and accumulation, as well as cholesterol crystallization (Nervi et al., 2006).

The present study showed that less than one-fifth of the study population were diabetics. Diabetes was slightly higher among cases with GBS (25%) compared to those without stones (15.5%). Our results agree with Ruhl and Everhart (2000), who found that patients with diabetes mellitus have long been considered to be at increased risk of developing GBS because hypertriglyceridermia and obesity are associated with diabetes mellitus and because GB motility is often impaired in patients with diabetes mellitus. Mice with hepatic insulin resistance induced by liver-specific disruption of the insulin receptor are markedly predisposed to the formation of cholesterol GBS (Biddinger et al., 2008).

Our study shows that more than 60% of the study population had CLD. Cases with CLD showed more liability for GBS compared to cases without CLD. The frequency of GBS in patients with CLD ranges from 3.6% to 46%, with a 1.2- to 5-fold increase compared with the general population (Bouchier, 1969; Barker et al., 1979; Sheen et al., 1989; Gidrey, 1990). Most case-control studies found that the prevalence of cholelithiasis was significantly higher in patients with CLD than in controls (Fornari et al., 1990). A recent prospective ultrasound study of 500 patients with various forms of CLD found the prevalence of GBS to be 29.4% (Conte et al., 1991). Similarly, Acalovschi et al. (2014), found that CLD patients but not those with cirrhosis had a higher prevalence of GBS than healthy people.

The present study showed that cases with GBS had slightly higher total cholesterol and TG levels. Our results agree with Attili et al. (1997), who found that plasma HDL cholesterol levels are inversely correlated with the prevalence of cholesterol GBS. Hypertriglyceridermia is positively associated with an increased prevalence of GBS. These seemingly independent variables are interrelated because high plasma TG levels tend to increase with increasing body mass and are inversely correlated with plasma HDL levels.

Females taking hormonal contraceptive pills had a statistically significant higher level of GBS formation (50%). These results agreed with Saldinger et al. (2016), who found that oral contraceptive use and low-dose estrogen therapy in postmenopausal women also increase the risk of GBS formation. Estrogen has been proposed to be an important risk factor for the formation of cholesterol GBS. In mice, the hepatic estrogen receptor α, but not β, plays a crucial role in cholesterol GBS formation in response to estrogen (DeLeon et al., 2020).
The vast majority of the cases with GBS had either hepatitis B or C infection or both; with only 30% of the study cases having negative HBS Ag and HCV Abs. GBS was more common in patients with HCV (41.27%) than in patients with HBV (3.17%). Our results agree with Bini et al. (2005), who found that chronic HCV infection was strongly associated with GBD among men. Lee et al. (2014), showed that HBV and HCV were associated with GBS in the elderly. The direct infection of the GB by HCV may also play an important role in the development of GBS. Loriot et al. (1999), found HCV RNA concentration to be the same in serum, bile, and cultures of GB epithelial cells. Other investigators have also detected HCV RNA in GB specimens obtained from HCV-infected patients at the time of autopsy (Yan, 2000).

Sheen and Liaw (1989), reported that increased prevalence of GBS was associated with the duration and severity of HBV-related liver disease, not HCV-related liver disease. This means that the risk of cholelithiasis increases over time in patients with HBV.

5. Conclusions

The incidence of GBS increases with age, female gender, obesity, diabetes mellitus, hypertriglyceridemia, hormonal contraceptive pills, HCV, family history of GBS, excessive fatty meal intake, higher body height, higher body weight, increased abdominal circumference, high BMI, chronic liver disease, presence of a hepatic focal lesion and positive HCV Ab.

The independent risk factors for GBS formation are positive family history and excessive fatty meal intake.

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مقال

عوامل الخطر لتكوين حصوات المرارة

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يدعوت مرض تكوين حصوات المرارة عبئًا صحياً كبيرًا في العالم المتقدم وقد ارتفع انتشاره بتراوح في العقود الثلاثة الماضية.

يتائر حوالي 25 مليون فرد في الولايات المتحدة بمرض حصوات المرارة، مما يؤدي إلى متوسط تكلفة عالج سنوية تبلغ 2.6 مليار دولار. شجع معدل الإصابة المرتفع وتكلفة العلاج المرتفعة بمرض حصوات المرارة على البحث عن استراتيجيات وقائية.

يتباث مرض حصوة المرارة عن تفاعل معقد بين العوامل الوراثية والبيئية. تشمل الأسباب الشائعة لتكوين حصوات المرارة فرط إفراز الكوليسترول، وتغير في العصارة الصفراوية، وامتصاص الكوليسترول، ونقص حركة المرارة. تلعب العدوى البكتيرية وزائدة حمل البيليروبيان دوراً في تكوين حصوات المرارة. هدف هذه الدراسة هو إيجاد عوامل الخطر لتكوين حصوات المرارة.

أجريت هذه الدراسة على 176 تم حجزهم في قسم طب المناطق الحارة والجهاز الهضمي بمستشفى جامعة سوهاج.

الأسماء الرئيسية: حصوات المرارة، تليف كبري، التهاب فيروسي كبري