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Gallstone Disease in Females: Predominance, Multiplicity, Gallbladder Stone Formation and Clinical Parameter Analysis in a Prospective Cross- Sectional Observational Study

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ABSTRACT

Background: Cholelithiasis, a prevalent hepatobiliary disorder, involves the formation of cholesterol, pigment, or mixed gallstones within the gallbladder or biliary ducts.

Aim: The aim of the study is to assess the association between liver enzyme levels and cholelithiasis clinical parameters.

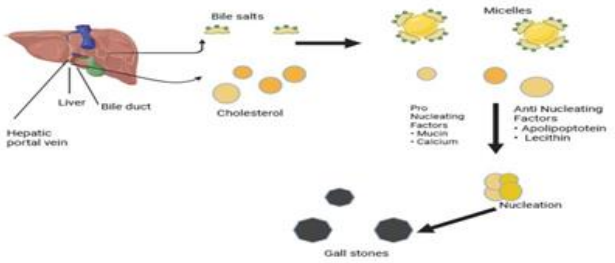
Methods: In this study, we selected a population to collect data for identifying or associating the relationship of cholelithiasis with liver enzymes. Therefore, a cross-sectional study is used to gather information that can derive a relationship between cholelithiasis and liver enzymes. The total population size was 60 cholelithiasis patients. The calculated value 52. Then the considered sample size was also 52 cholelithiasis patients. Total number of patients who participated in this study was 57.

Result: Indirect bilirubin was the liver enzyme indicator that showed the biggest difference between men and women ($p = .001$). Men had much higher levels ($M = 3.21$) than women ($M = 1.11$). The effect size was big (Cohen's $d = 0.998$), which shows that the difference was important and clinically relevant. However, other enzymes, such as total bilirubin, direct bilirubin, SGOT, SGPT, and ALP, did not show any statistically significant differences between men and women ($p > .05$), and the effect sizes were mostly small to moderate.

Conclusion: The study's findings demonstrate the important role that liver enzymes, specifically SGPT and ALP, play in determining the size of gallstones in cholelithiasis patients, with smaller stones being linked to greater enzyme levels. Interestingly, male patients had considerably higher indirect bilirubin levels, which may indicate that the illness process has metabolic differences specific to gender.

KEYWORDS: Cholelithiasis, Liver Enzymes, Cross sectional Observational Study, Gall stone formation, Ursodeoxycholic acid.

Graphical Abstract:



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INTRODUCTION

Cholelithiasis, commonly known as gallstone disease, is among the most common hepatobiliary disorders, which is characterized by the formation of solid stones in the gallbladder or biliary system. The stones typically consist of either cholesterol, bilirubin, or a combination of both [1,2]. It involves the formation of solid concretions within the gallbladder or biliary ducts due to disturbances in bile composition, supersaturation with cholesterol, reduced gallbladder motion, and stagnation of the bile. It is broadly classified into cholesterol stones, which are composed of cholesterol crystals and appear yellow in color, pigment stones, which are brown or black in color mainly consist of calcium bilirubinate and mixed types, which include varying proportions of cholesterol and pigment components [3,4,5]. The study of cholelithiasis has advanced greatly over time. The pathophysiology process of gallstone formation involves phases of cholesterol supersaturation, nucleation of microcrystals facilitated by mucin glycoproteins, and the growth of these crystals into macroscopic stones under conditions of gallbladder hypomotility and chronicity [6,7]. The prevalence of gallstone disease varies worldwide across populations. Epidemiological data as demonstrated by systematic reviews and meta-analyses including more than 32 million individuals between 2000 and 2023 indicate that approximately 6.1% of the global population may develop gallstones during their lifetime, with a higher prevalence among females compared to males (7.6% versus 5.4%) [8]. The disease shows a clear female predominance, which has been primarily attributed to hormonal influences such as estrogen, which increases biliary cholesterol saturation and alters gallbladder function. Gallstone formation is influenced by various other multiple factors such as genetic mutations in transport proteins, defects in bilirubin metabolism, and biochemical imbalances. Other risk factors include age, diabetes mellitus, family history, sedentary lifestyle, and specific dietary patterns, which further contribute to the increased risk among women [9]. Clinically, gallstones can remain asymptomatic for a long time or present with a range of complications, including biliary colic (discomfort in the upper right abdomen), acute cholecystitis (gallbladder inflammation), choledocholithiasis (infection of the biliary tree), and pancreatitis (inflammation of the pancreas, particularly when stones obstruct the biliary ducts) [10,11]. The number and anatomical location of gallstones are important factors in determining the severity of symptoms and risk of complications. Multiple stones are frequently associated with recurrent pain and a higher risk of obstruction. Management strategies depend on the presence and severity of symptoms. A combination of non-pharmacological, pharmacological, and surgical approaches is used to manage gallstone disease. Lifestyle measures such as balanced fat intake, increased fiber consumption, and weight loss help lower risk [12,13]. Non-surgical options for preventing gallstone formation include bile acid therapy (with ursodeoxycholic acid), NSAIDs (for pain relief), and statins (to lower bile cholesterol saturation) [14,15,16]. Laparoscopic cholecystectomy is the most widely used surgical treatment option, while endoscopic procedures, percutaneous drainage, and other interventions like shock wave lithotripsy or herbal remedies are considered in selected cases, as these are associated with limited supporting evidence [17,18,19]. Although limited region-specific data focusing on the patterns of gallstone disease in female patients is present, especially regarding the occurrence of multiple stones and their anatomical localization. This study was designed to assess the prevalence, determine the frequency of multiple stones and location of gallstones in women diagnosed with cholelithiasis in order to provide a better understanding of disease characteristics in this population. Although the formation of gallstone is not new but changes in our life style in current scenario, problem aggravated specially to women where proposed ratio was 1:3 between male and female. This study proposed hypothesis to evaluate that ratio among different age of women (less than 18 years of age; between 18-40 years and above 40 years of age) due to level of sex hormones especially estrogen level variation in female. Additionally, fluctuation of liver enzyme with respect to gallstone characteristics (size of gall stone, number of gallstones etc.) to determine the involvement of metabolic disorder. The objective and hypothesis of study were first, to analyse the fluctuations of liver enzymes (e.g., Bilirubin, ALT, AST, ALP,) in patients diagnosed with cholelithiasis; second, to find out the effect of liver enzymes on gallstones characteristics. Third, to investigate the effect of patient demographics (age, gender) on the cholelithiasis. The current study aims to assess the association between liver enzyme levels and cholelithiasis clinical parameters. Cholelithiasis, or gallstone formation, is a common hepatobiliary disorder with a high morbidity rate in the world, especially in the developing world. It is characterized by calculi in the gallbladder that may result in biliary obstruction, inflammation, or impaired liver function. Bilirubin, ALT, AST, and ALP are significant laboratory measures that indicate the function of the liver and biliary system. Changes in the level of these enzymes are commonly seen in patients who are gallstone disease, specifically, when biliary obstruction or hepatocellular injury is present. Despite its clinical importance, little is known about how these differences in liver enzyme activities are associated with characteristics of the gallstones, including size, number, and location, or how these laboratory measurements are influenced by the patient demographics or clinical presentations.

A greater appreciation of these relationships may improve diagnostic accuracy, guide therapeutic interventions, and could potentially allow prediction of disease severity or complications. Through understanding patterns and associations in this context, the study will seek to generate new information that would potentially inform more individualistic and efficacious clinical decision-making in the management of gallstone disease.

MATERIAL AND METHODS

This prospective cross-sectional observational study included 57 patients admitted to the surgical ward (IPD) who were confirmed cases of cholelithiasis via ultrasonography. Inclusion criteria required patients to be 18 years or older, have a confirmed diagnosis of cholelithiasis, and provide informed consent to participate in the study. Patients suffering from any pre- existing liver disease or those unwilling to participate were excluded. The sample size was calculated using the Raosoft sample size calculator, considering a total population of 60 cholelithiasis patients, with a 95% confidence level and a 5% margin of error, yielding a

required sample size of 52. However, 57 eligible participants were ultimately enrolled. Participants were briefed in detail about the study through a participant information sheet, and written informed consent was obtained prior to data collection in accordance with Good Clinical Practice guidelines. Data collection involved the use of structured forms to record demographic details, gallstone characteristics (size, number, and location as observed via ultrasonography), and liver enzyme values (ALT, AST, ALP, and bilirubin), which were obtained from hospital laboratory records. The study did not involve any intervention, and all patient data were collected from existing clinical records.

RESULT

The first goal of the study was to look at how the levels of liver enzymes, such as bilirubin (total, direct, indirect), ALT (SGPT), AST (SGOT), and ALP changed in patients with cholelithiasis, based on their gender. The results, which came from an independent samples t- test, looked at the biochemical markers and gallstone characteristics of male (n = 16) and female (n = 41) patients. The average size of gallstones was a little bigger in men (M = 3.69) than in women (M = 3.61), but this difference was not statistically significant (p = .673, Cohen's d = 0.125), which means that there was very little difference between the two genders. In the same way, males had a higher average number of stones (M = 1.88) than females (M = 1.66), but this difference was close to being significant (p = .106) with a moderate effect size (d = 0.485), which suggests that this trend may become significant in larger samples. Indirect bilirubin was the liver enzyme indicator that showed the biggest difference between men and women (p = .001). Men had much higher levels (M = 3.21) than women (M = 1.11). The effect size was big (Cohen's d = 0.998), which shows that the difference was important and clinically relevant. However, other enzymes, such as total bilirubin, direct bilirubin, SGOT, SGPT, and ALP, did not show any statistically significant differences between men and women (p > .05), and the effect sizes were mostly small to moderate.

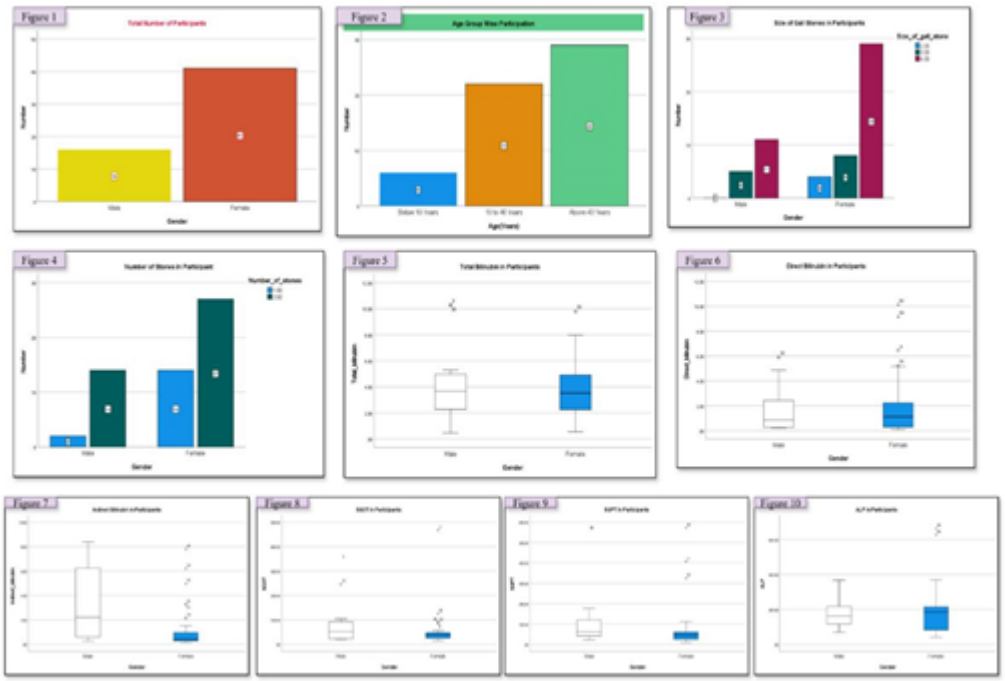

Figure illustration: Figure 1: Total Number of Participants This bar graph illustrates gender distribution among study participants. It shows a significant gender imbalance, with females (n=44) comprising a much larger portion of the sample than males (n=14). Figure 2: Age Group Wise Participation This figure displays participant distribution across three age groups: below 18, 18–40, and above 40 years. The majority fall in the “Above 40 Years” group (n=30), followed by “18 to 40 Years” (n=23), with the fewest under 18 (n=5). Figure 3: Size of Gall Stones in Participants This grouped bar chart compares gallstone sizes among males and females. Most females have large gallstones (size 4.00), whereas males exhibit smaller sizes, primarily 1.00 and 2.00. This indicates a gender-linked difference in gallstone severity, with larger stones more prevalent in females.

Figure 4: Number of Stones in Participants This graph shows the distribution of single versus multiple gallstones by gender. Females are more likely to have multiple stones (n=27), while males show lower frequency for both categories. Figure 5: Total Bilirubin in Participants The boxplot compares total bilirubin levels between genders. Males show a slightly wider spread and higher outliers, while females have a tighter interquartile range but some elevated values. Figure 6: Direct Bilirubin in Participants This figure shows direct bilirubin values, with females presenting more outliers and a higher median. Males have lower dispersion and fewer abnormal values. Figure 7: Indirect Bilirubin in Participants Here, males exhibit a wide range in indirect bilirubin, while females have tighter values with multiple outliers. The suggests greater variability in bilirubin metabolism among males, whereas females show clustering with sporadic elevations. Figure 8: SGOT in Participants This boxplot presents SGOT enzyme levels across genders. Males show a narrow range and lower values, whereas females exhibit numerous high outliers, suggesting elevated liver enzyme levels in some female participants. Figure 9: SGPT in Participants Similar to SGOT, SGPT levels are notably higher in females, with multiple outliers beyond the normal range. Males exhibit lower and less variable SGPT levels. Figure 10: ALP in Participants This figure compares ALP levels between males and females. Median ALP is higher in females, who also display more outliers above the upper quartile, including one extreme value. Males show a compact distribution. The

analysis shows that male cholelithiasis patients have much higher levels of indirect bilirubin. This could mean that their metabolism or disease progression patterns are different. Other enzyme changes were not statistically significant, but the trends that were seen (especially for SGOT and the number of stones) need to be looked into more with bigger sample sizes to get a better idea of how gallstone disease affects men and women differently.

DISCUSSION

This study examined the effects of liver enzymes and demographic variables on the size and quantity of gallstones in individuals with cholelithiasis. The study investigated gender disparities, biochemical predictors, and the combined impact of demographic and biochemical determinants. It was organized around three main goals. The initial goal focused on variations in liver enzyme levels and gallstone characteristics by gender. Indirect bilirubin levels were found to be significantly higher in males ($p = 0.001$), with a large effect size (Cohen's $d = 0.998$), even though the majority of liver enzyme levels and gallstone characteristics (number and size) did not differ significantly between males and females. This result could be the result of underlying variations in bilirubin metabolism or hemolytic activity that are related to gender. Despite approaching significance, the variations in SGOT and the number of stones were equivocal and should be investigated further with larger sample samples. For the second goal, multiple linear regression demonstrated that liver enzymes, notably SGPT and ALP, were significant negative predictors of gallstone size. This suggests that higher SGPT and ALP levels are related to smaller gallstones, a surprising but intriguing discovery. These enzymes might have a function in the metabolic environment, preventing or limiting stone enlargement. Adding demographic factors (age and gender) to the model did not improve predictive power, as demonstrated by a decrease in adjusted R^2 and loss of statistical significance. These findings suggest that biochemical markers have a stronger relationship with gallstone size than patient demographics. The third goal investigated predictors of the number of stones. Both models one with simply liver enzymes and the other with demographic factors—were statistically insignificant. The low R^2 values and high p-values across models suggest that neither liver enzymes, age, nor gender is a significant predictor of gallstones. These data suggest that additional, unmeasured factors such as gallbladder motility, bile composition, or genetic susceptibility might influence the frequency of stones. The use of parametric tests was validated by the Shapiro-Wilk and Kolmogorov-Smirnov tests, which verified the assumption of normality for all variables. The study's conclusions are more credible because of this statistical rigor. Even while the sample size ($N = 57$) is sufficient for initial investigation, it could restrict how broadly the findings can be applied, particularly when it comes to subgroup comparisons.

CONCLUSION

The study's findings demonstrate the important role that liver enzymes, specifically SGPT and ALP, play in determining the size of gallstones in cholelithiasis patients, with smaller stones being linked to greater enzyme levels. Interestingly, male patients had considerably higher indirect bilirubin levels, which may indicate that the illness process has metabolic differences specific to gender. Gallstone size and quantity, however, were not substantially predicted by demographic variables like age and gender or by other liver functions like total and direct bilirubin. Furthermore, the regression models showed that the variance in the quantity of stones could not be adequately explained by either demographic characteristics or biochemical markers. According to these results, gallstone production and count may be influenced by other unknown factors, including heredity, dietary practices, or gallbladder motility, even if specific biochemical markers can provide information about gallstone size. To have a more thorough grasp of the causes and development of gallstone disease, new studies with bigger sample sizes and more varied clinical characteristics are advised.

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