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Original Research Article

Association Between Gallstone Chemical Composition and Non-Alcoholic Fatty Liver Disease Diagnosed by Ultrasonography: A Cross-Sectional Comparative Study

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Abstract:

Introduction: Non-alcoholic fatty liver disease (NAFLD) and gallstone disease (GSD) are increasingly prevalent metabolic disorders that frequently coexist and share common risk factors such as obesity, insulin resistance, dyslipidaemia, and type 2 diabetes mellitus. Although several studies have demonstrated an epidemiological association between these conditions, limited data are available regarding the influence of NAFLD on the biochemical composition of gallstones.

Objectives: To evaluate the association between the chemical composition of gallstones and NAFLD diagnosed by ultrasonography, to assess the relationship between serum biochemical parameters and gallstone composition, and to determine whether gallstone constituents vary according to the severity of NAFLD.

Methodology: This cross-sectional observational study was conducted in the Departments of Biochemistry and General Surgery, KPC Medical College and Hospital, Kolkata, over a period of eighteen months. A total of 246 patients with gallstone disease were included, comprising 123 patients with ultrasonographically diagnosed NAFLD and 123 controls without NAFLD. Serum cholesterol and bilirubin levels were estimated, and gallstones obtained after cholecystectomy were analysed for cholesterol and bilirubin content using standard biochemical methods. Statistical analysis was performed using SPSS, and a p-value <0.05 was considered statistically significant.

Results: Low physical activity (67.48% vs 38.21%, p=0.002), oral contraceptive use (23.42% vs 7.14%, p=0.01), type 2 diabetes mellitus (35.77% vs 17.89%, p=0.003), and previous gastrointestinal surgery (19.51% vs 9.76%, p=0.02) were significantly associated with NAFLD. Mean gallstone cholesterol (154.94 ± 120.92 mg/g vs 138.14 ± 106.32 mg/g, p=0.024) and bilirubin concentrations (1.46 ± 0.98 mg/g vs 1.09 ± 0.86 mg/g, p=0.037) were significantly higher among NAFLD patients. A significant positive correlation was observed between serum and gallstone cholesterol and bilirubin levels in the NAFLD group. Gallstone cholesterol and bilirubin concentrations increased progressively with increasing grades of NAFLD (p=0.001).

Conclusion: NAFLD is significantly associated with altered gallstone composition, particularly increased cholesterol and bilirubin content. The progressive rise in gallstone constituents with increasing NAFLD severity suggests that worsening hepatic steatosis contributes to a more lithogenic biliary environment. Early identification and management of metabolic risk factors may help reduce the burden of both NAFLD and gallstone disease.

Keywords: Non-Alcoholic Fatty Liver Disease, Gallstone Disease, Cholesterol Gallstones, Bilirubin, Hepatic Steatosis.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disorder worldwide and represents a major public health challenge due to its rapidly increasing prevalence and close association with obesity, type 2 diabetes mellitus, dyslipidaemia, and metabolic syndrome. It encompasses a spectrum of liver pathology ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), progressive fibrosis, cirrhosis, and hepatocellular carcinoma. Recent epidemiological studies estimate that nearly one-quarter of the global population is affected by NAFLD, making it one of the leading causes of chronic liver disease and liver-related morbidity and mortality worldwide. [1,2]

The pathogenesis of NAFLD is multifactorial and involves complex interactions among insulin resistance, altered lipid metabolism, oxidative stress, inflammatory pathways, genetic susceptibility, and environmental factors. Excess accumulation of triglycerides within hepatocytes results from increased influx of free fatty acids, enhanced de novo lipogenesis, and impaired lipid export from the liver. Progressive hepatic steatosis subsequently promotes inflammatory responses and fibrogenesis, contributing to disease progression. [3-5] In addition to hepatic complications, NAFLD is increasingly recognized as a systemic metabolic disorder associated with several extrahepatic manifestations, including cardiovascular disease, chronic kidney disease, endocrine abnormalities, and gastrointestinal disorders. [1]

Gallstone disease (GSD) is another highly prevalent gastrointestinal disorder affecting millions of individuals worldwide. Cholelithiasis results from the formation of solid concretions within the gallbladder due to abnormalities in the composition of bile. Gallstones are broadly classified into cholesterol stones, pigment stones, and mixed stones, with cholesterol stones accounting for the majority of cases in both Western and Asian populations. [6-8] The development of cholesterol gallstones is influenced by multiple factors, including cholesterol supersaturation of bile, gallbladder hypomotility, nucleation of cholesterol crystals, and impaired enterohepatic circulation of bile acids. [8,9]

Increasing evidence suggests a close relationship between NAFLD and gallstone disease. Both conditions share several common risk factors, including obesity, insulin resistance, type 2 diabetes mellitus, dyslipidaemia, metabolic syndrome, sedentary lifestyle, and advancing age. [1,10] The coexistence of these disorders is therefore frequently observed in clinical practice. Kichloo et al. reported a significant association between NAFLD and gallstone disease among hospitalized patients in the United States, highlighting the possibility of common underlying metabolic mechanisms. [1] Similarly, Ahmed and Ali proposed that NAFLD and cholesterol gallstone disease may represent different manifestations of the same metabolic dysfunction, although the temporal relationship between the two conditions remains unclear. [10]

Several mechanisms have been proposed to explain the association between NAFLD and gallstone formation. Hepatic insulin resistance promotes increased cholesterol synthesis and secretion into bile, resulting in cholesterol supersaturation and a greater tendency for cholesterol crystal formation. Concurrently, abnormalities in bile acid metabolism and impaired gallbladder motility further enhance gallstone formation. [10] Progressive hepatic steatosis may therefore contribute directly to the development of a lithogenic biliary environment. Conversely, gallstone disease and cholecystectomy have also been implicated as potential risk factors for NAFLD, suggesting a bidirectional relationship between these conditions. [11]

Recent studies have highlighted the potential role of fibroblast growth factor-19 (FGF19) in linking gallbladder physiology with hepatic lipid metabolism. FGF19 is produced by the enterobiliary tract and plays a crucial role in regulating bile acid synthesis, lipid metabolism, and glucose homeostasis. The human gallbladder has been identified as an important source of FGF19 secretion into bile. [12] Experimental studies have demonstrated that FGF19 suppresses hepatic fatty acid synthesis and reduces lipid accumulation within hepatocytes. [13] Following cholecystectomy, circulating FGF19 levels decrease while bile acid synthesis increases, potentially contributing to hepatic steatosis and metabolic disturbances. [14] These observations provide further biological plausibility for the close association between gallstone disease and NAFLD.

Despite growing evidence supporting the relationship between these two disorders, most previous studies have primarily focused on epidemiological associations or the prevalence of gallstone disease among patients with NAFLD. [1,10,11] Comparatively few studies have investigated the biochemical composition of gallstones in patients with NAFLD. Understanding whether NAFLD influences the chemical composition of gallstones may provide important insights into the pathophysiological mechanisms linking these disorders. In particular, evaluation of gallstone cholesterol and bilirubin content may help clarify whether hepatic metabolic abnormalities

directly influence biliary composition and stone formation.

Furthermore, the relationship between serum biochemical parameters and the chemical composition of gallstones remains inadequately explored. Correlating serum cholesterol and bilirubin concentrations with their respective concentrations within gallstones may improve understanding of systemic metabolic influences on gallstone formation. Additionally, determining whether gallstone composition varies according to the severity of NAFLD could provide valuable information regarding the impact of progressive hepatic steatosis on biliary pathology.

Therefore, the present study was undertaken to investigate the association between the chemical composition of gallstones and NAFLD diagnosed by ultrasonography. The study aimed to compare the concentrations of cholesterol and bilirubin in gallstones obtained from patients with and without NAFLD, evaluate correlations between serum biochemical parameters and gallstone composition, and examine the relationship between NAFLD severity and biochemical constituents of gallstones. The findings may contribute to a better understanding of the metabolic interplay between NAFLD and gallstone disease and help identify potential targets for prevention and management of these increasingly prevalent disorders.

Materials and Methods

This cross-sectional observational study was conducted in the Department of Biochemistry, KPC Medical College and Hospital, Kolkata, in collaboration with the Department of General Surgery, over a period of eighteen months after obtaining approval from the Institutional Ethics Committee of KPC Medical College and Hospital (IEC No. KPCMCH/IEC/2024/155). Written informed consent was obtained from all participants prior to enrolment in the study.

This cross-sectional observational study was conducted in the Department of Biochemistry, KPC Medical College and Hospital, Kolkata, West Bengal, over a period of eighteen months following approval from the Institutional Ethics Committee (IEC No. KPCMCH/IEC/2024/155). The study was carried out in collaboration with the Department of General Surgery and included patients diagnosed with gallstone disease who were scheduled to undergo cholecystectomy. Written informed consent was obtained from all participants prior to enrolment after explaining the objectives and procedures of the study in a language they could understand.

The study population comprised adult patients aged between 18 and 80 years with ultrasonographically confirmed gallstone disease. A total of 246 participants were included in the study and were divided into two groups. The case group consisted of 123 patients with gallstone disease and concomitant non-alcoholic fatty liver disease (NAFLD) diagnosed by abdominal ultrasonography, whereas the control group comprised 123 patients with gallstone disease without evidence of NAFLD on ultrasonographic examination. Patients with a history of significant alcohol consumption, viral hepatitis, autoimmune liver disease, drug-induced liver disease, hepatobiliary malignancy, pregnancy, or any other known cause of secondary hepatic steatosis were excluded from the study. Individuals who declined to participate or were unable to provide informed consent were also excluded.

All participants underwent detailed clinical evaluation and assessment using a predesigned case record form. Demographic variables including age and sex were recorded. Information regarding lifestyle and metabolic risk factors such as smoking status, tobacco chewing, physical activity level, oral contraceptive pill use, history of diabetes mellitus, hypertension, previous gastrointestinal surgery, and other relevant clinical details was collected through direct patient interview and review of available medical records. A comprehensive physical examination was performed in all cases.

Abdominal ultrasonography was carried out by experienced radiologists using standard imaging protocols. The diagnosis of NAFLD was established based on characteristic ultrasonographic features of hepatic steatosis after exclusion of secondary causes of fatty liver disease. Patients with NAFLD were further categorized into Grade 1, Grade 2, and Grade 3 disease according to the severity of hepatic steatosis observed on ultrasound examination. Gallstone disease was confirmed sonographically prior to surgical intervention.

Venous blood samples were collected from all participants after an overnight fasting period of approximately 12 hours. The blood samples were processed in the Department of Biochemistry laboratory under standard operating procedures. Serum was separated by centrifugation and analysed for relevant biochemical parameters including total cholesterol and total bilirubin using standard enzymatic colorimetric methods. Internal quality control measures were maintained throughout the study period to ensure reliability and reproducibility of laboratory results.

Gallstones obtained during cholecystectomy were collected aseptically and transported to the laboratory for biochemical analysis. Each gallstone specimen was thoroughly washed with distilled water to remove residual bile and surface contaminants. The stones were subsequently dried, weighed, and processed according to standardized laboratory protocols. Gallstone extracts were prepared using established methods, and the

concentrations of total cholesterol and total bilirubin were estimated by validated colorimetric techniques. Biochemical analysis of the gallstones was performed using UV–Visible spectrophotometry under controlled laboratory conditions. The biochemical composition of gallstones was then compared between patients with and without NAFLD.

The primary study variables included gallstone cholesterol concentration and gallstone bilirubin concentration. Secondary variables included serum cholesterol levels, serum bilirubin levels, demographic characteristics, lifestyle factors, metabolic comorbidities, and ultrasonographic grading of NAFLD. Correlations between serum biochemical parameters and corresponding gallstone constituents were evaluated. In addition, the relationship between gallstone composition and the severity of NAFLD was assessed.

All collected data were entered into Microsoft Excel spreadsheets and subsequently analysed using Statistical Package for the Social Sciences (SPSS) software. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Comparisons between cases and controls were performed using appropriate statistical tests depending on the nature and distribution of the variables. Correlation analyses were carried out to determine the relationship between serum biochemical parameters and gallstone composition. Logistic regression analysis was used to identify factors independently associated with NAFLD among patients with gallstone disease. A p-value of less than 0.05 was considered statistically significant for all analyses.

Confidentiality of patient information was strictly maintained throughout the study. Personal identifiers were removed during data entry and analysis to ensure privacy. Participation in the study was entirely voluntary, and participants were free to withdraw at any stage without affecting their medical care. All study procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and institutional research guidelines.

Results

A total of 246 patients with gallstone disease were included in the study, comprising 123 patients with ultrasonographically diagnosed non-alcoholic fatty liver disease (NAFLD) and 123 patients without NAFLD. The demographic profile, lifestyle characteristics, metabolic risk factors, and biochemical composition of gallstones were analyzed and compared between the two groups.

Table 1: Demographic Characteristics of Study Participants (n=246)

Variable	NAFLD + GSD (n=123)	Non-NAFLD + GSD (n=123)	p value
Age (years), Mean ± SD	46.02 ± 11.19	44.95 ± 15.48	0.056
Male	51 (41.46%)	51 (41.46%)	1.000
Female	72 (58.54%)	72 (58.54%)	1.000

The demographic profile of the study population is shown in Table 1. The mean age of patients with NAFLD was 46.02 ± 11.19 years compared with 44.95 ± 15.48 years among controls, and the difference was not statistically significant (p=0.056). Females constituted the majority of participants in both groups (58.54%), indicating a female predominance among patients with gallstone disease irrespective of NAFLD status.

Table 2: Lifestyle and Clinical Risk Factors among Study Participants (n=246)

Variable	NAFLD + GSD (n=123)	Non-NAFLD + GSD (n=123)	p value
Smoking	22 (17.89%)	27 (21.95%)	0.891
Chewing tobacco	31 (25.20%)	27 (21.95%)	0.652
Low physical activity	83 (67.48%)	47 (38.21%)	0.002*
Oral contraceptive use	26 (23.42%)	8 (7.14%)	0.010*
Type 2 Diabetes Mellitus	44 (35.77%)	22 (17.89%)	0.003*
Hypertension	50 (40.65%)	31 (25.20%)	0.030*
Previous GI surgery	24 (19.51%)	12 (9.76%)	0.020*

*Statistically significant

Table 2 demonstrates the distribution of lifestyle and clinical risk factors among cases and controls. Low physical activity was significantly more common among patients with NAFLD than controls (67.48% vs 38.21%, p=0.002). Oral contraceptive use was also significantly higher among cases (23.42% vs 7.14%, p=0.010). Among clinical variables, type 2 diabetes mellitus showed a strong association with NAFLD (35.77% vs 17.89%, p=0.003). Hypertension and previous gastrointestinal surgery were also significantly more frequent among cases,

suggesting a close association between metabolic dysfunction and NAFLD in patients with gallstone disease.

Table 3: Comparison of Gallstone Biochemical Composition between Cases and Controls (n=246)

Parameter	NAFLD + GSD (n=123) Mean ± SD	Non-NAFLD + GSD (n=123) Mean ± SD	p value
Gallstone cholesterol (mg/g)	154.94 ± 120.92	138.14 ± 106.32	0.024*
Gallstone bilirubin (mg/g)	1.46 ± 0.98	1.09 ± 0.86	0.037*

*Statistically significant

The biochemical composition of gallstones differed significantly between the two groups (Table 3). Patients with NAFLD had significantly higher mean gallstone cholesterol concentrations compared with controls (154.94 ± 120.92 mg/g vs 138.14 ± 106.32 mg/g, p=0.024). Similarly, gallstone bilirubin concentration was significantly elevated among NAFLD patients (1.46 ± 0.98 mg/g vs 1.09 ± 0.86 mg/g, p=0.037). These findings indicate that NAFLD is associated with a more cholesterol-rich and bilirubin-rich gallstone composition.

Table 4: Correlation between Serum Biochemical Parameters and Gallstone Composition (n=246)

Parameter	NAFLD Group	Non-NAFLD Group
Serum cholesterol vs Gallstone cholesterol	Significant positive correlation	Weak negative correlation
Serum bilirubin vs Gallstone bilirubin	Significant positive correlation	No significant correlation

Analysis of biochemical correlations revealed a significant positive relationship between serum cholesterol levels and gallstone cholesterol concentration among patients with NAFLD. A similar positive correlation was observed between serum bilirubin and gallstone bilirubin concentration. In contrast, the control group demonstrated a weak inverse relationship between serum and gallstone cholesterol levels and no significant correlation between serum and gallstone bilirubin concentrations. These findings suggest that systemic metabolic abnormalities in NAFLD may directly influence gallstone composition.

Table 5: Association of NAFLD Severity with Gallstone Composition (n=246)

NAFLD Grade	n (%)	Gallstone Cholesterol (mg/g), Mean ± SD	Gallstone Bilirubin (mg/g), Mean ± SD
Grade 1	58 (47.15)	142.36 ± 52.48	1.12 ± 0.25
Grade 2	42 (34.14)	189.72 ± 61.83	1.42 ± 0.30
Grade 3	23 (18.71)	256.44 ± 47.21	1.88 ± 0.35
p value	—	0.001*	0.001*

*Statistically significant

As shown in Table 5, gallstone cholesterol and bilirubin concentrations increased progressively with increasing severity of NAFLD. Mean gallstone cholesterol concentration rose from 142.36 ± 52.48 mg/g in Grade 1 disease to 256.44 ± 47.21 mg/g in Grade 3 disease (p=0.001). Similarly, mean gallstone bilirubin concentration increased from 1.12 ± 0.25 mg/g to 1.88 ± 0.35 mg/g across the same grades (p=0.001). This dose-response relationship suggests that progressive hepatic steatosis contributes to increasing lithogenicity of bile.

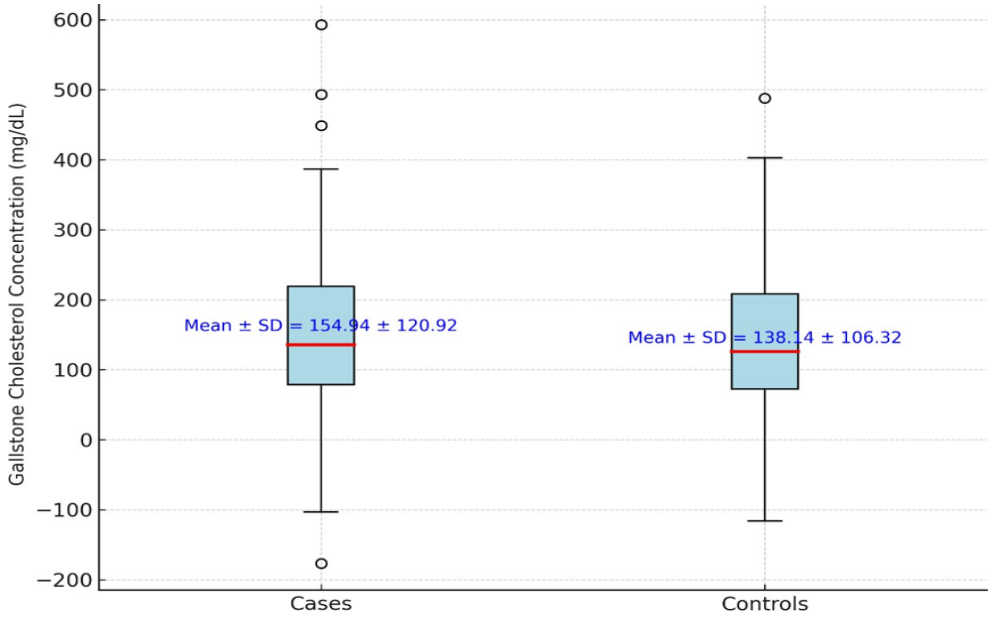

Figure 1: Comparison of mean gallstone cholesterol concentration between patients with and without NAFLD.

The graphical representation of the biochemical characteristics of gallstones is shown in Figures 1–3. As illustrated in Figure 1, patients with NAFLD had a significantly higher mean gallstone cholesterol concentration compared with patients without NAFLD (154.94 ± 120.92 mg/g vs 138.14 ± 106.32 mg/g; p=0.024).

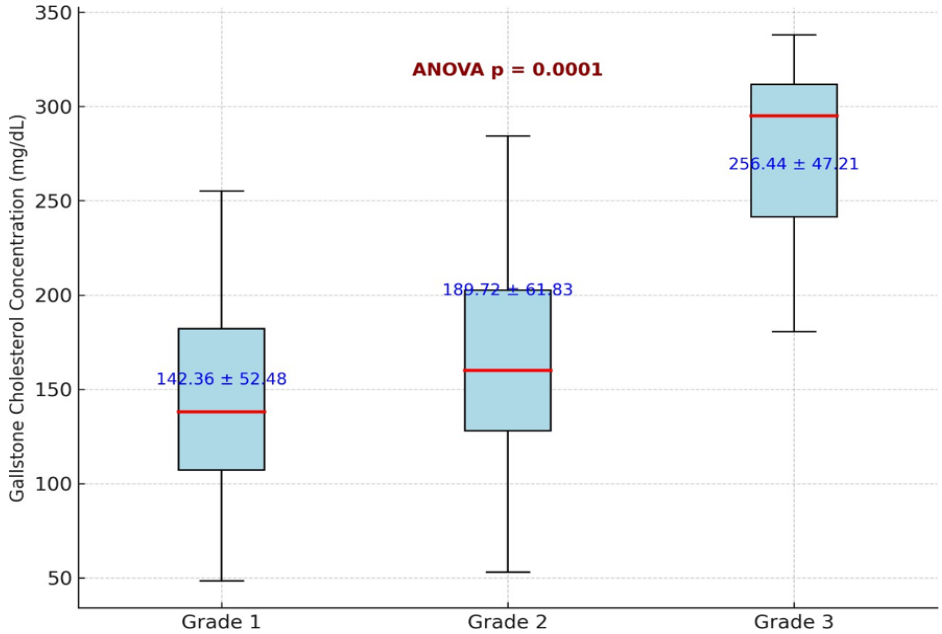

Figure 2: Progressive increase in gallstone cholesterol concentration across different grades of NAFLD.

A progressive increase in gallstone cholesterol concentration was observed with increasing severity of NAFLD (Figure 2). The mean gallstone cholesterol concentration increased from 142.36 ± 52.48 mg/g in Grade 1 NAFLD to 189.72 ± 61.83 mg/g in Grade 2 and 256.44 ± 47.21 mg/g in Grade 3 disease. This trend was statistically significant (p=0.001), indicating a positive association between hepatic steatosis severity and cholesterol enrichment of gallstones.

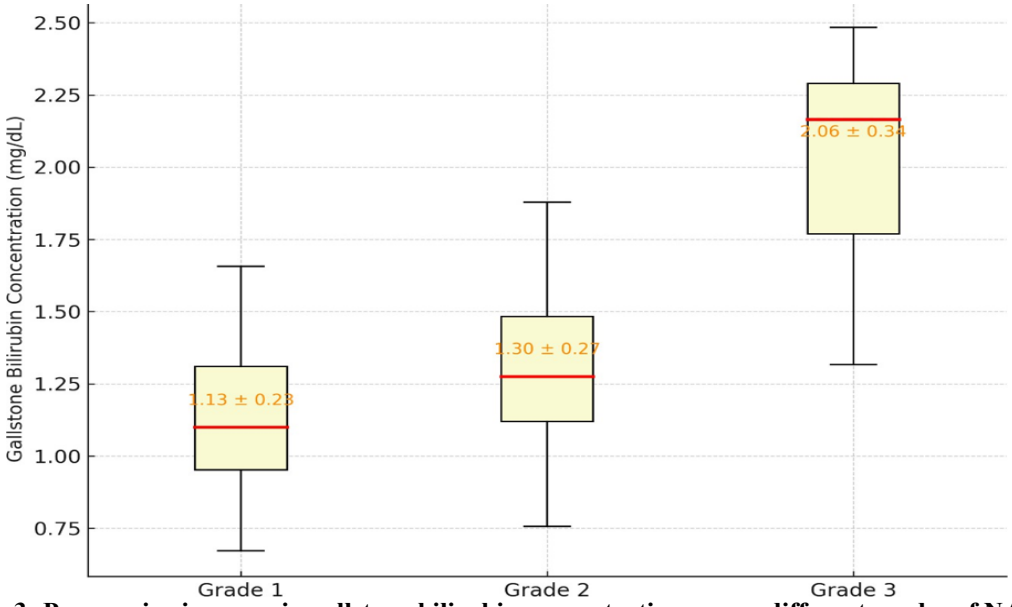

Figure 3: Progressive increase in gallstone bilirubin concentration across different grades of NAFLD.

Similarly, gallstone bilirubin concentration demonstrated a significant upward trend across increasing grades of NAFLD (Figure 3). Mean bilirubin concentration increased from 1.12 ± 0.25 mg/g in Grade 1 disease to 1.42 ± 0.30 mg/g in Grade 2 and 1.88 ± 0.35 mg/g in Grade 3 disease (p=0.001). These findings suggest that progressive NAFLD is associated with increasing bilirubin deposition within gallstones.

Discussion

In the present study, the mean age of patients with NAFLD was 46.02 ± 11.19 years compared with 44.95 ± 15.48 years among controls, with no statistically significant difference between the groups. These findings are consistent with those reported by Kichloo et al. [1], who observed that the coexistence of NAFLD and gallstone disease was most common in middle-aged individuals. Similar observations were reported by Fracanzani et al. [13], who demonstrated that the coexistence of NAFLD and gallstone disease occurred predominantly in individuals between 40 and 60 years of age, suggesting that increasing age contributes to metabolic susceptibility but may not independently determine the occurrence of either condition.

A female predominance was observed in both study groups, with females accounting for 58.54% of participants. This observation is in agreement with the findings of Mukherjee et al. [20], who reported a higher prevalence of gallstones among young females with insulin resistance and NAFLD. Hormonal influences are likely to contribute to this association. In the present study, oral contraceptive use was significantly more common among patients with NAFLD and gallstone disease than among controls (23.42% vs 7.14%, p=0.01). This finding supports previous observations by Pouwels et al. [3], who suggested that estrogen exposure promotes cholesterol supersaturation of bile and increases lithogenicity in women with metabolic disorders. Liu et al. [18] similarly reported a stronger association between NAFLD and gallstones among females than males, emphasizing the importance of hormonal factors in disease pathogenesis.

Among lifestyle-related variables, low physical activity was significantly more prevalent among NAFLD patients than controls (67.48% vs 38.21%, p=0.002). This finding corroborates the observations of Ravisankar et al. [21], who identified sedentary lifestyle as a major modifiable risk factor contributing to both hepatic steatosis and gallstone formation. Physical inactivity contributes to obesity, insulin resistance, and dyslipidaemia, thereby creating a metabolic environment favourable for both NAFLD and cholesterol gallstone formation.

The present study demonstrated a significantly higher prevalence of type 2 diabetes mellitus among patients with NAFLD and gallstone disease compared with controls (35.77% vs 17.89%, p=0.003). Diabetes remained an independent predictor even after adjustment for confounding factors. These findings are strongly supported by Fracanzani et al. [13], who reported a significantly higher prevalence of diabetes among NAFLD patients with gallstone disease compared with those without gallstones. Likewise, Kichloo et al. [1] concluded that diabetes independently predicts the coexistence of NAFLD and gallstone disease. The underlying mechanism is likely related to insulin resistance, which promotes increased hepatic cholesterol synthesis, biliary cholesterol secretion, and gallbladder dysmotility, thereby enhancing lithogenesis.

Although hypertension was significantly more common among NAFLD patients in the univariate analysis, this association did not remain significant after multivariable adjustment. Similar findings were reported in a systematic review by Slouha et al. [22], which demonstrated that while hypertension frequently coexists with NAFLD and gallstone disease, insulin resistance and obesity are the principal independent determinants of this association. These observations suggest that hypertension may represent part of the shared metabolic syndrome rather than an independent causal factor.

Another notable finding of the present study was the significantly higher prevalence of previous gastrointestinal surgery among NAFLD patients. Bansal et al. [24] suggested that alterations in enterohepatic circulation and bile acid metabolism following gastrointestinal surgery may contribute to gallstone formation, particularly in metabolically vulnerable individuals. Such mechanisms may partly explain the observed association in the present study.

A major objective of the study was to evaluate the relationship between serum biochemical parameters and gallstone composition. A significant positive correlation was observed between serum cholesterol and gallstone cholesterol concentrations among patients with NAFLD, whereas controls demonstrated a weak negative correlation. These findings indicate that dysregulated lipid metabolism in NAFLD is directly reflected in biliary composition. Similar observations were reported by Chandran et al. [23], who demonstrated a strong positive correlation between serum cholesterol levels and gallstone cholesterol content in patients with fatty liver disease. In contrast, Steen and Blijenberg [26] observed that pigment stones exhibited minimal correlation with serum cholesterol because of their distinct pathophysiological mechanisms.

The biochemical analysis of gallstones revealed significantly higher cholesterol and bilirubin concentrations among NAFLD patients than controls. Mean gallstone cholesterol concentration was 154.94 ± 120.92 mg/g in cases compared with 138.14 ± 106.32 mg/g in controls (p=0.024), while mean bilirubin concentration was 1.46 ± 0.98 mg/g and 1.09 ± 0.86 mg/g, respectively (p=0.037). Comparable findings were reported by Bansal et al. [24], who demonstrated significantly higher cholesterol concentrations in gallstones obtained from patients with metabolic syndrome. Kalghatgi et al. [25] similarly observed significantly higher cholesterol content in cholesterol and mixed stones than in pigment stones.

The distribution of gallstone types in the present study further supports the metabolic basis of stone formation. Cholesterol-rich stones predominated among NAFLD patients, with 70% demonstrating high cholesterol content. Similar observations were reported by Misra et al. [19], who found that cholesterol-rich stones were the predominant stone type among patients with metabolic liver disease. Their study also highlighted the role of oxidative stress and trace elements in influencing gallstone composition.

One of the most important findings of the present study was the progressive increase in gallstone cholesterol and bilirubin concentrations with increasing severity of NAFLD. Gallstone cholesterol concentration increased from 142.36 ± 52.48 mg/g in Grade 1 disease to 256.44 ± 47.21 mg/g in Grade 3 disease, while bilirubin concentration increased from 1.12 ± 0.25 mg/g to 1.88 ± 0.35 mg/g (p=0.001 for both). These findings demonstrate a clear dose-response relationship between hepatic steatosis severity and gallstone lithogenicity. Fracanzani et al. [15] similarly reported that patients with gallstones had more severe liver disease and greater hepatic lipid accumulation than those without gallstones. Dyson et al. [27] also emphasized the close relationship between NAFLD severity and worsening metabolic dysfunction.

A significant correlation between serum bilirubin and gallstone bilirubin concentration was observed only among NAFLD patients. Chandran et al. [23] reported comparable findings and suggested that impaired bilirubin metabolism associated with hepatic dysfunction may contribute to increased bilirubin precipitation within bile. The mechanism likely involves increased unconjugated bilirubin load and enhanced calcium bilirubinate formation.

The present findings are further supported by the systematic review conducted by Slouha et al. [16], which concluded that NAFLD nearly doubles the risk of gallstone disease and that insulin resistance, oxidative stress, and altered bile composition are central mechanisms linking the two disorders. Collectively, the findings of the present study reinforce the concept that NAFLD and gallstone disease share a common metabolic background characterized by insulin resistance, dyslipidaemia, obesity, hormonal influences, and altered bile composition. The progressive increase in cholesterol and bilirubin concentrations with advancing grades of NAFLD strongly suggests that worsening hepatic steatosis contributes directly to the development of a more lithogenic biliary environment.

Conclusion

The present study demonstrated a significant association between non-alcoholic fatty liver disease (NAFLD) and the biochemical composition of gallstones. Patients with NAFLD exhibited

significantly higher concentrations of cholesterol and bilirubin in gallstones compared with patients without NAFLD, suggesting that hepatic metabolic dysfunction directly influences biliary composition and stone formation. A significant positive correlation was observed between serum biochemical parameters and corresponding gallstone constituents among NAFLD patients, indicating that systemic metabolic abnormalities are reflected in gallstone chemistry.

The study further revealed that metabolic risk factors, particularly type 2 diabetes mellitus, low physical activity, and oral contraceptive use, were significantly associated with the coexistence of NAFLD and gallstone disease. Importantly, a progressive increase in gallstone cholesterol and bilirubin concentrations was observed with increasing grades of NAFLD, demonstrating a clear dose-dependent relationship between hepatic steatosis severity and lithogenicity of bile.

These findings support the concept that NAFLD and gallstone disease are closely interconnected manifestations of a common metabolic disorder characterized by insulin resistance, dyslipidaemia, and altered lipid metabolism. Early identification and management of metabolic risk factors may therefore help reduce both the progression of NAFLD and the development of cholesterol-rich gallstones. Further multicentric prospective studies are warranted to validate these findings and explore the underlying molecular mechanisms linking hepatic steatosis and gallstone formation.

References

1. Kichloo A, Singh J, Albosta M, Wani F, Jamal S, Aljadah M, et al. Association of non-alcoholic fatty liver disease with gallstone disease in the United States hospitalized patient population. World J Gastrointest Pathophysiol. 2021;12(2):14–24. doi:10.4291/wjgp.v12.i2.14.
2. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, Nguyen MH. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One. 2017;12(3):e0173499. doi:10.1371/journal.pone.0173499.
3. Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord. 2022;22(1):63. doi:10.1186/s12902-022-00980-1.
4. Kořínková L, Pražienková V, Černá L, Karnošová A, Železná B, Kuneš J, Maletínská L. Pathophysiology of NAFLD and NASH in experimental models: the role of food intake regulating peptides. Front Endocrinol (Lausanne). 2020;11:597583. doi:10.3389/fendo.2020.597583.
5. Carr RM, Oranu A, Khungar V. Nonalcoholic fatty liver disease: pathophysiology and management. Gastroenterol Clin North Am. 2016;45(4):639–52. doi:10.1016/j.gtc.2016.07.003.
6. Tanaja J, Lopez RA, Meer JM. Cholelithiasis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
7. Njeze GE. Gallstones. Niger J Surg. 2013;19(2):49–55. doi:10.4103/1117-6806.119236.
8. Di Ciaula A, Portincasa P. Recent advances in understanding and managing cholesterol gallstones. F1000Res. 2018;7:F1000 Faculty Rev-1529. doi:10.12688/f1000research.15505.1.
9. Shabanzadeh DM. Incidence of gallstone disease and complications. Curr Opin Gastroenterol. 2018;34(2):81–9. doi:10.1097/MOG.0000000000000418.
10. Ahmed MH, Ali A. Nonalcoholic fatty liver disease and cholesterol gallstones: which comes first? Scand J Gastroenterol. 2014;49(5):521–7. doi:10.3109/00365521.2014.894119.
11. Ruhl CE, Everhart JE. Relationship of non-alcoholic fatty liver disease with cholecystectomy in the US population. Am J Gastroenterol. 2013;108(6):952–8.
12. Zweers SJ, Booij KA, Komuta M, Roskams T, Gouma DJ, Jansen PL, Schaap FG. The human gallbladder secretes fibroblast growth factor 19 into bile: towards defining the role of fibroblast growth factor 19 in the enterobiliary tract. Hepatology. 2012;55(2):575–83.
13. Bhatnagar S, Damron HA, Hillgartner FB. Fibroblast growth factor-19, a novel factor that inhibits hepatic fatty acid synthesis. J Biol Chem. 2009;284(15):10023–33. doi:10.1074/jbc.M808818200.
14. Barrera F, Azócar L, Molina H, Schalper KA, Ocares M, Liberona J, Villarroel L, Pimentel F, Pérez-Ayuso RM, Nervi F, Groen AK, Miquel JF. Effect of cholecystectomy on bile acid synthesis and circulating levels of fibroblast growth factor 19. Ann Hepatol. 2015;14(5):710–21.
15. Fracanzani AL, Valenti L, Russello M, et al. Gallstone disease is associated with more severe liver damage in patients with non-alcoholic fatty liver disease. PLoS One. 2012;7(7):e41183.
16. Slouha E, Biput SJ, Kuteyi A, Kalloo AE, Gorantla VR. Non-alcoholic fatty liver disease and gallstones: a systematic review. Cureus. 2023;15(9):e45027.
17. Damnjanović Z, Jovanović M, Nagorni A, et al. Correlation of inflammation parameters and biochemical markers of cholestasis with the

intensity of lipid peroxidation in patients with choledocholithiasis. Vojnosanit Pregl. 2013;70(2):170–176.
18. Liu J, Lin H, Zhang C, et al. Non-alcoholic fatty liver disease associated with gallstones in females rather than males: a longitudinal cohort study in Chinese urban population. BMC Gastroenterol. 2014;14:213.
19. Misra PK, Dalal S, Kharab S. Role of trace elements in the formation of gallstones. Asian J Biochem. 2014;9:213–220.
20. Mukherjee S, Banerjee AK, Bhattacharyya S, Saha S. Silent gallbladder stone associated with nonalcoholic fatty liver disease in a scenario of insulin resistance in young adults. Indian J Med Biochem. 2019;23(1):226–229.
21. Ravisankar P, et al. Fatty liver disease in-depth analysis. Indo Am J Pharm Res. 2015;5(11):1–7.
22. Slouha E, Biput SJ, Kuteyi A, Kalloo AE, Gorantla VR. Non-alcoholic fatty liver disease and gallstones: a systematic review. Cureus. 2023;15(9):e45027.
23. Chandran P, Kuchhal NK, Garg P, Pundir CS. An extended chemical analysis of gallstones. Indian J Clin Biochem. 2007;22(2):145–150.
24. Bansal A, Akhtar M, Bansal AK. An extended analysis of gallstones: Nagpur, India. J Appl Res. 2015;5(3):1–5.
25. Kalghatgi S, Aravind K, Kulkarni M, Vipin T, Sanganal B. Study of gallstone composition in North Karnataka. Surg Update Int J Surg Orthop. 2017;3(4):93–98.
26. Steen G, Blijenberg BG. Chemical analysis of gallstones. Clin Chim Acta. 1991;29:801–804.
27. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. Frontline Gastroenterol. 2014;5:211–218.