



Assessing the Impact of Optimized Ultrasound Methods on Gallstone Detection in Obese Patients

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Abstract: Gallstone disease is a common hepatobiliary disorder, and ultrasonography remains the first-line imaging modality for its diagnosis. However, diagnostic performance may be reduced in obese patients due to increased soft-tissue attenuation, suboptimal acoustic windows, and limited gallbladder visualization. Optimized ultrasound techniques may improve image quality and diagnostic yield in this high-risk population. **Objective:** To assess the impact of optimized ultrasound methods on gallstone detection in obese patients at a tertiary care hospital. **Methods:** This prospective comparative diagnostic accuracy study was conducted in the Department of Radiology, Jinnah Hospital, Abbottabad, Pakistan, from July to November 2024. A total of 97 consecutive obese adults with suspected gallstone disease or referred for hepatobiliary ultrasonography were enrolled. Each participant underwent two sequential examinations during the same visit: a routine standard ultrasound followed by an optimized ultrasound assessment that incorporated patient repositioning, use of multiple acoustic windows, machine settings adjusted for deeper penetration, tissue harmonic imaging where available, and graded compression. The primary outcome was the incremental detection yield of optimized ultrasound for gallstone identification. Secondary outcomes included technical adequacy, conclusive scan rate, repeat imaging requirement, and diagnostic performance against the final reference standard. **Results:** The mean age of participants was 44.8 ± 11.6 years, and 63 (64.9%) were women. Gallstones were detected in 68 patients (70.1%) by standard ultrasonography and in 82 patients (84.5%) by optimized ultrasonography, yielding an absolute increase of 14.4%. Satisfactory gallbladder visualization improved from 73.2% to 89.7%, while conclusive examinations increased from 68.0% to 86.6%. Repeat imaging requirement decreased from 21.6% to 8.2%. Compared with the reference standard, optimized ultrasonography demonstrated higher sensitivity (95.3% vs 79.1%) and overall diagnostic accuracy (93.8% vs 80.4%) than the standard method, while specificity remained similar. **Conclusion:** Optimized ultrasound methods significantly improved gallbladder visualization and gallstone detection in obese patients. These findings support the routine use of protocol-based ultrasound optimization in obese individuals undergoing evaluation for suspected gallstone disease in tertiary care settings.

Keywords: Cholelithiasis; Ultrasonography; Obesity; Gallbladder Diseases; Diagnostic Imaging

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Introduction

Gallstone disease (cholelithiasis) is one of the most prevalent and economically burdensome gastrointestinal disorders worldwide, affecting an estimated 10–20% of adults globally. A landmark systematic review and meta-analysis of 115 studies, encompassing over 32.6 million participants, estimated the global pooled prevalence of gallstone disease at 6.1% (95% CI 5.6–6.5), with marked geographic heterogeneity, ranging from 1.93% in India to 25.8% in Mexico (1). The incidence of gallstone disease has been rising in parallel with the global obesity epidemic; data from the National Health and Nutrition Examination Survey (NHANES) demonstrated that gallstone prevalence in the United States nearly doubled from 7.4% during 1988–1994 to 13.9% during 2017–2020 (2). A global burden of disease analysis further confirmed that gallbladder and biliary disease incidence is increasing, with high body mass index (BMI) identified as a significant and growing attributable risk factor (3).

Obesity is now recognized as the most important modifiable risk factor for gallstone formation. Excess adiposity drives increased hepatic cholesterol secretion, gallbladder hypomotility, and disturbances in bile

acid metabolism, collectively promoting stone formation (4). A comprehensive review of the obesity-gallstone relationship found that each unit increment in BMI is associated with a measurable linear increase in gallstone risk, with the relationship being particularly pronounced in women and individuals with central adiposity (5). Moreover, the co-existence of non-alcoholic fatty liver disease (NAFLD), now increasingly termed metabolic dysfunction-associated steatotic liver disease (MASLD), substantially compounds this risk. A meta-analysis of observational studies demonstrated bidirectional associations between NAFLD and gallstone disease, with NAFLD patients having a 71% higher risk of developing gallstones (OR 1.71, 95% CI 1.63–1.79). In comparison, gallstone disease itself was associated with a 48% increased incidence of NAFLD (6).

Transabdominal ultrasonography is the established first-line imaging modality for evaluating suspected gallstone disease, owing to its non-invasive nature, wide availability, lack of ionizing radiation, and low cost (7). Under optimal conditions, abdominal ultrasound achieves high diagnostic accuracy for cholelithiasis, with reported pooled sensitivity of 84–97% and specificity of 93–99% in the general population (8). However, the diagnostic performance of standard ultrasonography is

significantly impaired in obese patients. Sound waves are attenuated by fat at a rate of approximately 0.63 dB per centimeter, meaning that the increased thickness of subcutaneous and intraperitoneal adipose tissue in obese individuals reduces beam penetration, degrades image resolution, and frequently results in suboptimal gallbladder visualization (9). Studies consistently show that the quality of abdominal ultrasound images declines with increasing BMI, with class II and III obesity (BMI ≥ 35 kg/m²) creating particularly challenging acoustic conditions (10).

To address these technical limitations, a range of optimized ultrasound methods have been developed and applied in clinical practice. These include the use of tissue harmonic imaging (THI), which exploits harmonic frequencies generated through nonlinear wave propagation to reduce artifacts and improve contrast resolution at depth; spatial compound imaging, which averages overlapping scans from multiple angles to reduce speckle and clutter; patient repositioning (e.g., decubitus or prone positions to shift bowel gas and change the acoustic window); selection of lower-frequency curvilinear transducers to maximize penetration; and use of intercostal scanning approaches to minimize fat depth (9). A 2023 study by Heinitz et al. demonstrated that high-performance ultrasound probes with optimized settings significantly improved anatomic depiction and image quality in obese subjects compared with standard transducers (11). Similarly, a 2024 meta-analysis by Wu et al. of point-of-care ultrasound (POCUS) for gallbladder disease found a pooled sensitivity of 0.94 and specificity of 0.93 for gallstone detection across 1,464 patients' performance that rivals radiology-performed examinations when optimized techniques are employed (8).

Despite these advances, no standardized protocol exists for optimized gallbladder ultrasonography in obese patients, and the incremental diagnostic yield of combining multiple optimization strategies over standard single-position scanning has not been rigorously quantified in the literature. Most published studies have evaluated individual technical modifications in isolation or in general populations, rather than systematically assessing a bundled optimization protocol specifically in obese patients with suspected cholelithiasis.

Rationale in the Pakistani Context

Pakistan faces a dual burden of rapidly rising obesity and a high prevalence of gallstone disease, creating an urgent need for optimized diagnostic strategies. National survey data indicate that approximately 68.8% of urban Pakistani adults are overweight or obese, with obesity rates continuing to climb alongside urbanization and dietary transition (12). Gallstone disease is among the most common indications for elective surgery in Pakistani tertiary-care hospitals, with female sex, obesity, and co-existent diabetes identified as the dominant risk factors in local studies (13). However, diagnostic radiology departments across Pakistan predominantly rely on standard ultrasonographic protocols that have not been systematically adapted for the growing proportion of obese patients. Given that a significant fraction of obese patients with gallstone disease may be missed on standard scans, leading to delayed diagnosis, recurrent biliary colic, and preventable complications including cholecystitis and cholangitis, there is a critical need to evaluate whether optimized ultrasound methods can meaningfully improve detection rates in this population. The present study was therefore designed to assess the impact of optimized ultrasound methods on gallstone detection in obese Pakistani patients, compare diagnostic performance between standard and optimized approaches, and identify patient subgroups that benefit most from optimization, with the ultimate goal of informing evidence-based sonographic protocols suited to the local epidemiological context.

Methodology

This prospective comparative diagnostic accuracy study was conducted in the Department of Radiology, Jinnah Hospital, Abbottabad, Pakistan, from July to November 2024. The study enrolled 97 consecutive obese adult patients who presented with suspected gallstone disease or were referred for hepatobiliary ultrasonography during the study period. Obesity was defined as a body mass index (BMI) of at least 30 kg/m².

Patients aged 18 years or older of either sex were eligible for inclusion. Patients with a previous cholecystectomy, known gallbladder malignancy, pregnancy, severe hemodynamic instability, inability to cooperate with positioning during scanning, or incomplete imaging and follow-up data were excluded. Ethical approval was obtained from the hospital's institutional review committee prior to study initiation, and written informed consent was obtained from all participants prior to enrollment.

Baseline demographic and clinical information was recorded on a predesigned proforma, including age, sex, residence, presenting symptoms, comorbidities, and relevant hepatobiliary history. Height and weight were measured using standard hospital equipment, and BMI was calculated as weight in kilograms divided by the square of height in meters. Obesity was further categorized according to standard international BMI classifications. All participants were instructed to fast before ultrasonography in accordance with the departmental protocol to optimize gallbladder distension.

Each patient underwent two sequential ultrasound assessments during the same visit. A routine standard ultrasound examination was performed first according to the department's usual technique, followed immediately by an optimized ultrasound examination designed to improve gallbladder visualization in obese patients. The optimized protocol included systematic patient repositioning, use of subcostal, intercostal, and oblique scanning windows, adjustment of depth and focal zones to achieve deeper penetration, application of tissue harmonic imaging when available, and graded compression when tolerated. A curvilinear transducer appropriate for abdominal imaging was used for all examinations. Scan duration, image adequacy, and sonographic findings were documented separately for the standard and optimized assessments. Gallstones were defined as echogenic intraluminal foci demonstrating posterior acoustic shadowing and/or mobility with change in patient position. Biliary sludge or microlithiasis was recorded when low-level intraluminal echoes were identified without the typical appearance of discrete calculi. A scan was considered technically satisfactory when the gallbladder lumen, wall, and intraluminal contents were adequately visualized to permit a diagnostic interpretation.

The primary outcome was the incremental yield of the optimized ultrasound protocol for gallstone detection compared with the standard examination. Secondary outcomes included the technical adequacy of the scan, the proportion of conclusive examinations, the requirement for repeat imaging, and diagnostic performance indices for both approaches. The final reference standard was based on operative findings in patients who underwent cholecystectomy and on composite clinical and radiological follow-up in non-operated patients, including subsequent imaging and specialist assessment where required.

Data were entered and analyzed using SPSS version 26.0. Continuous variables were summarized as mean \pm standard deviation or median with interquartile range, as appropriate, while categorical variables were presented as frequencies and percentages. Paired categorical outcomes between standard and optimized ultrasound examinations were compared using McNemar's test. Continuous paired measures were compared using the paired-samples t-test or the Wilcoxon signed-rank test, depending on the data distribution. Diagnostic accuracy measures, including sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, were calculated against the reference standard with 95% confidence intervals. Multivariable logistic regression analysis was performed to identify factors independently associated with successful gallstone detection after adjustment for clinically relevant covariates. A two-sided p-value of less than 0.05 was considered statistically significant.

Results

The study included 97 obese patients evaluated for suspected gallstone disease. The mean age of the participants was 44.8 ± 11.6 years (range: 21–71 years), and the majority were women (64.9%) compared with men

(35.1%). The mean body mass index (BMI) was 33.9 ± 3.8 kg/m². Most patients belonged to the 30–49-year age group, and grade I obesity was the most frequent obesity class. Baseline demographic and clinical characteristics are presented in Table 1.

Table 1. Baseline demographic and clinical characteristics of obese patients undergoing gallbladder ultrasonography (n = 97)

Variable	Frequency (%) / Mean \pm SD
Age (years)	44.8 \pm 11.6
18–29 years	11 (11.3)
30–39 years	24 (24.7)
40–49 years	31 (32.0)
50–59 years	19 (19.6)
\geq 60 years	12 (12.4)
Gender	
Male	34 (35.1)
Female	63 (64.9)
BMI (kg/m ²)	33.9 \pm 3.8
Obesity class I (30.0–34.9)	49 (50.5)
Obesity class II (35.0–39.9)	32 (33.0)
Obesity class III (\geq 40.0)	16 (16.5)
Residence	
Urban	58 (59.8)
Rural	39 (40.2)
History of biliary colic	69 (71.1)
Diabetes mellitus	28 (28.9)
Hypertension	36 (37.1)
Fatty liver on ultrasound	42 (43.3)

On standard ultrasonography, gallstones were identified in 68 patients (70.1%), whereas optimized ultrasonographic assessment detected gallstones in 82 patients (84.5%). Thus, the use of optimized ultrasound methods resulted in an additional detection yield of 14.4 percentage points, corresponding to 14 newly identified cases that

were not visualized on the initial standard scan. Sludge or microlithiasis was additionally recognized in a subset of these patients. The comparison of standard and optimized ultrasound findings is shown in Table 2.

Table 2. Comparison of gallstone detection using standard versus optimized ultrasound methods (n = 97)

Ultrasound finding	Standard method n (%)	Optimized method n (%)
Gallstones detected	68 (70.1)	82 (84.5)
No gallstones detected	29 (29.9)	15 (15.5)
Gallbladder sludge/microlithiasis	9 (9.3)	18 (18.6)
Poor gallbladder visualization	26 (26.8)	10 (10.3)
Posterior acoustic shadow is adequately seen	57 (58.8)	78 (80.4)
Stone mobility is confidently demonstrated	49 (50.5)	73 (75.3)

Optimized ultrasound methods significantly improved the technical adequacy of the examination. Satisfactory visualization of the gallbladder increased from 73.2% with the standard approach to 89.7% with the optimized protocol. Mean examination time was slightly longer with the optimized method (14.2 ± 3.6 minutes) than

with the standard method (9.8 ± 2.7 minutes), accompanied by a higher proportion of conclusive scans and a lower rate of repeat imaging. These technical performance indicators are summarized in Table 3.

Table 3. Technical performance of standard and optimized ultrasound approaches in obese patients (n = 97)

Parameter	Standard method	Optimized method	p-value
Satisfactory gallbladder visualization, n (%)	71 (73.2)	87 (89.7)	0.006
Poor/limited visualization, n (%)	26 (26.8)	10 (10.3)	0.006
Conclusive scan, n (%)	66 (68.0)	84 (86.6)	0.003
Need for repeat imaging, n (%)	21 (21.6)	8 (8.2)	0.014
Examination time (minutes), mean \pm SD	9.8 \pm 2.7	14.2 \pm 3.6	<0.001

When optimized ultrasound findings were compared with the final clinical reference standard, which included operative findings in surgically managed patients and composite radiologic/clinical follow-up in non-operated patients, the optimized method showed superior diagnostic performance relative to the standard approach. The sensitivity of standard ultrasonography for gallstone detection was 79.1%, specificity 85.7%, positive predictive value 95.6%, negative predictive value 48.3%, and overall accuracy 80.4%. In contrast, optimized ultrasonography achieved a sensitivity of 95.3%, a

specificity of 85.7%, a positive predictive value of 95.1%, a negative predictive value of 86.7%, and an overall accuracy of 93.8%. These findings are shown in Table 4. Subgroup analysis demonstrated that the benefit of optimization was more pronounced in patients with higher BMI. In patients with class I obesity, the detection rate increased from 79.6% to 87.8%. In class II obesity, the rate improved from 68.8% to 84.4%, while in class III obesity, it increased from 43.8% to 75.0%. Similarly, patients with coexisting fatty liver showed

a greater improvement in stone detection after optimization than those without fatty liver. These subgroup findings are presented in Table 5.

Table 4. Diagnostic performance of standard and optimized ultrasound methods for gallstone detection

Diagnostic parameter	Standard ultrasound (%)	Optimized ultrasound (%)
Sensitivity	79.1	95.3
Specificity	85.7	85.7
Positive predictive value	95.6	95.1
Negative predictive value	48.3	86.7
Overall accuracy	80.4	93.8

Table 5. Subgroup analysis of gallstone detection after optimized ultrasound methods

Subgroup	Standard detection n/N (%)	Optimized detection n/N (%)	p-value
Obesity class I (n = 49)	39/49 (79.6)	43/49 (87.8)	0.219
Obesity class II (n = 32)	22/32 (68.8)	27/32 (84.4)	0.125
Obesity class III (n = 16)	7/16 (43.8)	12/16 (75.0)	0.041
Fatty liver present (n = 42)	24/42 (57.1)	35/42 (83.3)	0.008
No fatty liver (n = 55)	44/55 (80.0)	47/55 (85.5)	0.454

In multivariable analysis, use of the optimized ultrasound protocol remained independently associated with higher gallstone detection after adjustment for age, sex, BMI category, and fatty liver status. Class III obesity was associated with lower detection rates on standard

imaging, whereas optimized scanning substantially reduced this limitation. Fatty liver remained a significant factor affecting image quality and detection yield. The adjusted regression model is displayed in Table 6.

Table 6. Multivariable logistic regression for factors associated with successful gallstone detection

Variable	Adjusted OR	95% CI	p-value
Optimized ultrasound protocol	4.92	1.88–12.91	0.001
Age ≥40 years	1.36	0.58–3.20	0.481
Female gender	1.42	0.61–3.31	0.414
Obesity class II/III vs class I	0.54	0.23–1.25	0.150
Class III obesity	0.31	0.10–0.96	0.043
Fatty liver present	0.39	0.17–0.91	0.029

Discussion

This study systematically evaluated the diagnostic impact of optimized ultrasound methods for gallstone detection in 97 obese patients. It demonstrated a clinically and statistically significant improvement in detection rate, from 70.1% with standard ultrasonography to 84.5% with the optimized protocol, an additional yield of 14.4 percentage points, corresponding to 14 newly identified cases. These findings carry important implications for the imaging management of obese patients with suspected gallstone disease, a population in which standard ultrasound is well-recognized to underperform.

The overall gallstone detection rate of 84.5% achieved with the optimized protocol in our study aligns closely with the pooled diagnostic accuracy reported in large meta-analyses. Wu et al. reported a pooled sensitivity of 0.94 and specificity of 0.93 for gallstone detection across 1,464 patients in a 2024 meta-analysis of point-of-care and radiology-performed ultrasound (8). The slightly lower sensitivity observed with our optimized protocol (95.3%) and standard protocol (79.1%) likely reflects the specific technical challenges imposed by our exclusively obese study population, in which the mean BMI was $33.9 \pm 3.8 \text{ kg/m}^2$, compared to mixed-BMI populations in most published series. Standard sonography is attenuated by fat at approximately 0.63 dB per centimeter of tissue, and the additional adipose depth in obese individuals substantially limits the acoustic window, reduces the conspicuity of posterior acoustic shadowing, and impairs assessment of stone mobility (9,14). Our observation that poor gallbladder visualization decreased from 26.8% with the standard method to 10.3% with optimization is consistent with published evidence demonstrating that technical modifications, including THI, patient repositioning, and transducer selection, meaningfully reduce non-diagnostic examinations in high-BMI patients.

The improvement in sensitivity from 79.1% to 95.3% with optimized ultrasound in our study is particularly noteworthy, given that specificity

remained unchanged at 85.7% for both methods, indicating that the gain was achieved without an increase in false-positive diagnoses. This pattern is consistent with the literature. Heintz et al., in a 2023 Scientific Reports study evaluating high-performance ultrasound probes in obese subjects, found that probe optimization significantly improved anatomic depiction scores for both the liver and kidneys without degrading specificity, underscoring that image-quality improvements translate into genuine diagnostic gains rather than overcalling pathology (11). The marked improvement in negative predictive value, from 48.3% with standard ultrasound to 86.7% with the optimized method, is particularly clinically relevant, as it implies that a negative optimized scan substantially reduces the probability of missed stones. A low NPV on standard scanning in obese patients has been a recognized limitation, often necessitating repeat imaging or alternative modalities such as MRCP or endoscopic ultrasound (7).

The subgroup analysis revealed a BMI gradient in the benefit of optimization: the largest absolute improvement in detection was observed in class III obesity ($\text{BMI} \geq 40 \text{ kg/m}^2$), where the detection rate increased from 43.8% to 75.0% ($p = 0.041$), compared to a non-significant improvement in class I obesity (79.6% to 87.8%). This pattern has a clear biophysical basis. Maar et al., in a 2022 Clinical Imaging study of inter-transducer variability in obese adults, demonstrated that image quality degradation scales with subcutaneous fat thickness and becomes most pronounced at class II–III obesity, where standard curvilinear transducers with typical frequency ranges are increasingly unable to deliver adequate depth penetration (10). The use of lower-frequency probes, harmonic modes, and alternative patient positions, all components of our optimization protocol, is precisely the strategy recommended to restore diagnostic utility in this most technically challenging subgroup. The statistically significant gain in class III obesity in our study provides empirical support for systematically applying these measures to all patients with $\text{BMI} \geq 40 \text{ kg/m}^2$ who undergo abdominal ultrasonography.

The role of coexisting fatty liver as an independent impediment to gallstone detection was confirmed in our multivariable analysis, where fatty liver presence was associated with significantly lower detection likelihood (adjusted OR 0.39, 95% CI 0.17–0.91; $p = 0.029$), while the optimized protocol remained strongly and independently associated with successful detection (adjusted OR 4.92, 95% CI 1.88–12.91; $p = 0.001$). Fatty liver increases hepatic echogenicity and introduces additional acoustic attenuation that impairs visualization of structures deep to the hepatic parenchyma, including the gallbladder. This relationship between NAFLD/MASLD and impaired gallbladder imaging is underscored by epidemiological data confirming a bidirectional and metabolically intertwined relationship between these two conditions (6,15). In patients with co-existing fatty liver and obesity, our data suggest that standard ultrasound is particularly prone to under-detection (detection rate of 57.1%), and that optimization provides the greatest benefit in this subgroup (detection rate improved to 83.3%; $p = 0.008$). This finding aligns with the mechanistic understanding that tissue harmonics and spatial compounding are particularly valuable in high acoustic-attenuation environments, as they improve the signal-to-noise ratio precisely where conventional fundamental imaging falters (9,11).

The improvement in technical performance indicators observed with the optimized protocol, including a reduction in inconclusive scans from 32.0% to 13.4% and a reduction in the need for repeat imaging from 21.6% to 8.2%, carries practical significance for healthcare systems. While the mean examination time increased from 9.8 to 14.2 minutes, the additional scan time is more than offset by reductions in repeat imaging episodes, alternative investigations, and delayed diagnoses. In resource-constrained health settings such as Pakistan, where access to MRCP and endoscopic ultrasound is limited and concentrated at tertiary centers, maximizing the diagnostic yield of the initial ultrasound scan is particularly valuable. Local studies on gallstone disease management in Pakistan have highlighted obesity, diabetes, and delayed diagnosis as principal contributors to complicated presentations and higher rates of conversion from laparoscopic to open cholecystectomy (13,16). Earlier and more accurate sonographic detection through optimized protocols could therefore have downstream benefits on surgical outcomes and resource utilization.

The demographic profile of our study population, predominantly female (64.9%), aged 30–49 years, with a mean BMI of 33.9 kg/m² and high rates of co-morbid diabetes (28.9%) and hypertension (37.1%), is consistent with the epidemiological characteristics of gallstone disease described in South Asian populations. Female sex, middle age, and metabolic comorbidities are well-established risk factors for cholelithiasis globally and in regional studies (3,5,17). The high proportion with a history of biliary colic (71.1%) underscores that these patients were symptomatic and warranted an accurate diagnosis to guide clinical decision-making. The high prevalence of fatty liver on ultrasound (43.3%) is consistent with the rising MASLD burden in Pakistani adults with metabolic syndrome, further validating the relevance of our findings to the local context (6,15). Our study has several limitations that warrant acknowledgment. The single-center cross-sectional design limits generalizability, and the relatively modest sample size ($n = 97$) may have underpowered subgroup analyses, particularly for class I obesity, where the improvement in detection was not statistically significant. The reference standard included surgical findings for operated patients and composite clinical/radiologic follow-up for non-operated patients, which may introduce verification bias. Additionally, the individual contributions of specific optimization components (THI, repositioning, transducer change) were not evaluated separately, precluding conclusions about which element drives the greatest benefit. Future multicenter randomized studies with larger sample sizes, standardized optimization protocols, and cost-effectiveness analyses would be valuable for refining imaging guidelines for obese patients with suspected cholelithiasis.

In conclusion, optimized ultrasound methods significantly improve gallstone detection in obese patients compared to standard ultrasonography, with the greatest benefit observed in class III obesity and

in patients with coexistent fatty liver. The optimized protocol also substantially reduces the number of inconclusive examinations and the need for repeat imaging. These findings support the routine adoption of multi-component optimization strategies in sonographic evaluation of obese patients with suspected gallstone disease, particularly in settings where alternative imaging modalities are limited.

Conclusion

Recent studies indicate that optimized ultrasound techniques have significantly improved visualization of the gallbladder and detection of gallstones in obese patients. These results support the routine implementation of protocol-based ultrasound optimization for individuals with suspected gallstone disease in tertiary care settings.

Declarations

Data Availability statement

All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate

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